Clinical Intervention Study
TAI-CHI-CHI vs. Health Education in Geriatric Depression

FULL PROTOCOL TITLE

Brain connectivity and response to Tai Chi in geriatric depression

Principal Investigator:

Helen Lavretsky, M.D.

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STUDY TEAM ROSTER

Research Team	Role
Helen Lavretsky, M.D.	*Principal Investigator*
Stephen Chen, M.D.	*Co-Investigator*
Jason Jalil, M.D.	*Co-Investigator*
Sarah A. Nguyen, M.D.	*Co-Investigator*
Pauline Wu, D.O.	*Co-Investigator*
Katherine Narr, Ph.D.	*Co-Investigator*
Linda Ercoli, Ph.D.	*Co-Investigator*
Prabha Siddarth, Ph.D.	*Co-Investigator/Statistician*
Damla Senturk, Ph.D.	*Statistician*
Beatrix Krause, Ph.D.	*MRI Technician/Data Analyst*
Lisa Kilpatrick, Ph.D.	*Data Analyst*
Kitikan Thana-Udom, M.D.	*Clinical Research Associate*
Michaela M. Milillo	*Study Coordinator/ Health and Wellness Instructor*
Ashlyn Applegate	*Study Coordinator*
Raquel Hernandez Sotomayor	*Research Assistant*
Katie Cho	*Research Assistant*
Kate Hollister	*Tai Chi Instructor*
Emiko Madison	*Tai Chi Instructor*
Gevork Salmastyan	*Research Assistant*

PARTICIPATING STUDY SITES

UCLA Semel Institute

Motion Picture Television Fund (MPTF)

PRÉCIS

Brain connectivity and response to Tai Chi in geriatric depression.

The study will focus on clinical outcomes and biomarkers of treatment response of Tai Chi Chih use compared to Health Education in geriatric depression. Biomarkers of response will include multimodal MRI and exploratory measures of gene expression and inflammatory markers.

Objectives

We will examine the efficacy of Tai Chi Chih (TCC) compared to health and wellness education

seminar (HEW) and will examine neural mechanisms of brain connectivity in response to interventions using fMRI biomarkers in 220 older adults with major depression who have been on stable treatment for at least 4 months and still meet criteria for major depression. They will be randomized to adjunctive TCC or HEW. We will establish whether variations in emotional regulation and brain connectivity moderate or predict mood and functional improvement linked with TCC using fMRI markers over the course of the 12 weeks, and will follow maintenance of response in follow-ups at 6 and 12 months (or 24 months if 12 months have passed). 110 subjects will have multimodal MRI evaluation. We anticipate that improvement in depression and cognition will be accompanied by the changes in brain connectivity using fMRI measures of connectivity.

This study incorporates a multidisciplinary investigative team from several centers at UCLA including the Semel Institute (Dr. Lavretsky, PI), and Ahmanson Lovelace Brain Mapping Center (Dr. Narr), as well as benefits from health+wellness education (Dr. Ercoli and statistical expertise (Dr. Senturk, SI-STAT and Dr. Siddarth), and the availability of the cohorts for recruitment from 2 sites*. We plan to assess the ability of the TCC to induce faster and durable remission, and lasting benefits in mood and cognition compared to those in the control group (HEW) as well as the mechanisms (predictors/moderators) of treatment response to each of the elements of combined intervention.*

1. STUDY OBJECTIVES

1.1 Primary Objective

Aim 1: *Evaluate the effects of **TCC** versus **HEW** on reduction of depressive symptoms and improvement in the secondary outcomes during the 12-week treatment trial, in addition to 6 and 12 month follow-up (or 24 months if 12 months have passed).*

Hypothesis 1: Compared to ***HEW, TCC*** will result in a) greater reduction of depressive symptoms and b) improvement in the cognitive domains of executive function and memory at 12 weeks, 6 months, and 12-month follow-up (or 24 months if 12 months have passed).

1.2 Secondary Objectives

Aim 2. *To test whether 12 weeks of **TCC** compared to **HEW** training alter neural regulation of affect during an fMRI affect labeling task and functional connectivity in older adults with depressive symptoms. Imaging biomarkers will predict/moderate treatment response.*

Hypothesis 2a: Compared to HEW, **TCC** will lead to improved functional connectivity in the Default Mode Network as measured with resting state (rs-) fMRI;

Hypothesis 2b: Compared to **HEW, TCC** will result in greater activation in the anterior cingulate;

Hypothesis 2c: Imaging biomarkers (hippocampal and anterior cingulate volume, white matter hyperintensity and DMN connectivity) will moderate and predict 1) rate of improvement in symptoms and function; and 2) improvement in cognitive performance.

Aim 3. *To examine whether older depressed participants with MCI (LLD+MCI) differ in their cognitive trajectory and neural circuitry compared to older depressed participants without MCI (LLD-MCI) at extended follow-up of 6 and 12 months.*

Hypothesis 3a: We hypothesize that changes in episodic memory and executive function will be greater in those with LLD+MCI than in those with LLD-MCI.

Hypothesis 3b: We hypothesize that changes in the default mode/hippocampal-frontal network and in the executive-control and cortico-limbic circuitry will be greater in those with LLD+MCI than in those with LLD-MCI.

Aim 4. *To examine whether greater degradation of neural circuitry is associated with greater cognitive decline.*

Hypothesis 4: We hypothesize that changes in default mode/hippocampal-frontal network and executive-control and cortico-limbic circuitry will be associated with decline in episodic memory and executive function.

Exploratory Aim 1: We will explore the role of treatment-resistance or poor response to the intervention in predicting cognitive outcomes.

Exploratory Aim 2: We will follow a subset of participants (those recruited within 2 years of the initial evaluation) to assess clinical trajectory of mood and cognitive symptoms with 24-month follow-up.

Exploratory aim 3: We will explore the difference in changes in gene expression and peripheral pro-inflammatory cytokines associated with clinical response to the interventions. To use peripheral blood to conduct transcriptome profiling with the same R01 protocols to identify gene expression and peripheral inflammatory biomarkers correlates of clinical response to TCC versus HEW.

Exploratory Hypothesis 3: Treatment response to TCC or HEW will be associated with changes in expression of inflammation, neuroplasticity-related genes and peripheral biomarkers (e.g. cytokines, telomerase), and baseline levels of these genes and cytokines will predict future clinical response.

2. BACKGROUND AND RATIONALE

2.1 Background on Condition, Disease, or Other Primary Study Focus

Later-life major depression and depressive symptoms (LLD) have a prevalence of 5-15% in community-dwelling elderly associated with a significant risk of high rates of relapse, morbidity, mortality, and suicide.[1-3] Over 60% of the depressed elderly fail to achieve symptomatic remission and functional recovery with pharmacotherapy,[4,5] accompanied by unremitting cognitive dysfunction and poor health function.[6-8] Finding new treatments that enhance functioning of the "aging tsunami" is thus a national imperative.[9,10] In a recent meta-analysis, Tai Chi appeared to have a significant impact on reducing depressive symptoms compared with the waiting list control groups in older adults (-0.27 (95% CI -0.52 to -0.02, P=0.03).11 In another recent pilot study of older adults with heart failure,12 Tai Chi practice reduced somatic symptoms of depression. Our preliminary data[13] demonstrates that Tai Chi Chih (TCC), a "movement meditation," enhances treatment with the antidepressant, escitalopram, in LLD. As compared to depressed elderly randomized to escitalopram and health education and wellness training (HEW) (n=37), those receiving escitalopram and TCC (n=36) exhibited greater rates of remission, greater clinical, health and cognitive improvement and reduced markers of inflammation, which also prospectively predict depressive symptoms.[7,14,15] TCC is a brief standardized version of Tai Chi that offers consistent training and practice schedules;[16,17] posing an important advantage over prior relaxation response-based therapies. Other studies have established that TCC decreases sympathetic output, improves viral immunity and vaccine response, and augments health

functioning in older adults.[18,19] To date, no studies have addressed the neural mechanisms of response to Tai Chi by investigating emotional and working memory processing, and network connectivity and plasticity in associated brain systems with fMRI. With this proposal, we aim to extend our preliminary findings to evaluate the underlying neural mechanisms of brain plasticity and connectivity in response to TCC compared to HEW during a 12-week acute trial with 6 month and 12 month follow-up (or 24 months if 12 months have passed). We will overcome limitations of our pilot studies by using interventions in older adults with major depression who have been on a stable form of treatment for at least 4 months, but have not achieved remission and still meet criteria for an episode of major depression in order to explore the underlying neural mechanisms of treatment response. We will also compare TCC to the HEW control gr***oup to control for non-specific attention and social support effects (HEW) (active attention control group). This will allow us to estimate the effect sizes of each intervention for future studies, potentially leading to the development of preventive interventions for major depression in older adults.***

Cognitive and neuroimaging biomarkers of LLD and treatment response – what do we know and what can we learn? The identification of state and trait biomarkers of brain function have provided additional knowledge about the mechanisms of geriatric depression.[20,21] For example, memory or executive function may reflect impairments in 'top down' cognitive control [22-25] with *reduced* neural activity in prefrontal cortex (PFC).[26] However, *increased* activity in cortical/subcortical regions critical to memory,[27] planning[28] and attention tasks,[29-31] may be due to compensatory mechanisms relating to cognitive impairments.[32-34] These brain activation patterns; as well as reports of decreased striatal response during sequence learning in normal aging,[35] but increased activity in the depressed elderly[36] may also distinguish normal aging from LLD. *Conversely, tasks of emotional regulation recruit* 'limbic' emotional regions such as the amygdala and striatum[37,38] with a more 'bottom up' pattern of altered processing.[22,39-44]*Assessment of the neural correlates of emotional processing and affect labeling will complement our other primary (mood) and secondary outcomes (cognitive, health functioning, and fMRI biomarkers of executive function and working memory – Aim 1) to address both "bottom-up" and "top-down" altered processing models.* Systems-level functional connectivity may also act as a biomarker of treatment response in major depression as has been demonstrated for an emotional face-matching task specifically in OFC networks following a 4-week antidepressant trial.[45] Brain activation at rest is not impeded by the subject's cognitive abilities as can limit the investigative power of task-related fMRI and may thus *further reveal which brain systems account for (and predict) depressive symptoms in LLD and vary with respect to treatment outcome*. rs-fMRI allows the study of brain network activity by determining blood-oxygen-level dependent (BOLD)-related synchronous neural activity in spatially distinct brain regions while a person is at rest. Altered connectivity in "default mode" (DMN) network nodes[46,47] has been reported in major depression[48-51] and in LLD specifically. DMN connectivity has been shown to link with white-matter hyperintensities (WMH); supporting the hypotheses that disconnection in functionally distinct networks might contribute to depression.[52] Recent evidence also demonstrates treatment-related changes in resting state connectivity in depression in fronto-limbic regions following antidepressant[48] and electroconvulsive therapy (ECT).[53,54] Further, previous work has shown that DMN activity relates to engagement of 'task positive' networks involved in effortful cognition, and that these relationships differ in major depression and associate with maladaptive rumination.[55] Functional imaging has proven an important tool for visualizing the mechanisms of antidepressant action[40,56-59-80]; the proposed research will newly address the mechanisms of treatment response to TCC in LLD.[60] LLD is also linked with *structural brain abnormalities* including global and regional brain tissue reductions, particularly in PFC/ACC, striatal and hippocampal regions,[61-64] and WMHs, which appear to be associated with *gait imbalance and psychomotor retardation*.[65-68] Cross-sectional studies support protective effects of treatment against volume loss in the

hippocampus, amygdala and VLPFC,[62,63,66,69-71] including in LLD.[70,71 94,95,91-94] Early evidence also suggests gray matter variations in fronto-limbic circuits may be predictive of treatment outcome.[58,72] To complement our fMRI outcome measures (Aims 1 and 2), we will address whether *changes in brain structure in fronto-limbic regions and WMHs, will change or differ as a consequence of TCC or HEW interventions. We will predict future response/relapse, and will relate the results to our other outcome measures. Since longitudinal evidence suggests that 1) antidepressants affect functional connectivity, including in LLD,[48,53,54,73] and 2) short-term integrative body-mind training or meditation alters functional as well as structural connectivity in the ACC[74,75] and in auditory/salience and medial visual networks[76] over short time frames (4-8 wks), it is reasonable to predict that TCC will lead to more pronounced changes in functional connectivity and structural plasticity than HEW (Aims 1-2).*

2.2 Study Rationale

Rationale for duration of interventions and follow-up, and masking procedures: 1) The proposed study uses some elements of the design used in our R21 pilot with novel features of not using psychotropic drugs, double-blind assignment and masking procedures to treatment groups, and the 2 active control conditions to assess the effects of the interventions on the improvement in primary and secondary clinical outcomes, and biomarkers. Based on our pilot study, participants needed 12 weeks to learn and master TCC and thus a 12-week duration is necessary for the active treatment trial.

The post-intervention follow-ups *will include all participants including those who could show worsening of symptoms and discontinue the study* to follow and compare the effect of TCC versus HEW to explore the role of biomarkers as predictors and moderators of the response. Those who agree to follow-up and do not demonstrate response or show worsening will be encouraged to seek treatment alternatives that will be documented in the chart and controlled for in analyses (e.g. types of treatments, dose, and duration). Most participants will be treated in UCLA clinics and their treatment records will be available for review of response patterns. *Follow-up of 6 and 12 months (or 24 months if 12 months have passed) will allow us to document maintenance of symptoms improvement, relapse of major depressive episodes, and compare the rates of maintained remission between the groups, as well as predictor/moderator effects of the biomarkers.*

New knowledge – establishing biomarkers of treatment efficacy: This project will address fundamental gaps in scientific knowledge in identifying biomarkers of response to TCC compared to 2 control conditions HEW. The use of a control group is innovative and will allow us to control for nonspecific support elements in the HEW group. The proposed research extends our pilot work to minimize the use of psychotropic drugs that will allow us examine the mechanism of response to TCC more directly. We will also expand longitudinal assessments up to 24 months to monitor possible relapse into major depressive episode, and worsening of depressive symptoms, that will also integrate response with cognitive performance and with the fMRI probes of emotional processing and working memory and rs-fMRI measures of functional connectivity. Moreover, this study will "zero in" on the mechanisms TCC effect on emotional regulation thus testing relevant stress-responsivity pathways. **Clinical and scientific impact of biomarkers:** Inclusion of biomarkers in studies of clinical response may help *identify the subgroups of older adults who are more likely to benefit from TCC, or those at increased risk for relapse, which would be important for clinical practice.*

Innovation.

Clinical translation. This project will be the first randomized clinical trial to test the CAM mind-body strategy of TCC versus HEW in order to characterize the underlying neurobiological mechanisms of response to TCC. Our treatment model also offers benefits of limiting exposure to multiple drugs, improved physical health, physical strength, agility, and a sense of wellbeing. We have found the TCC program to be very well received by our subjects.

Tai Chi Chih: TCC and other mind-body interventions are directed toward combining aerobic exercise with stress-reduction and structured mindful cognitive approaches that add to improvement in mood and self-esteem. TCC is also known as a "CHI- generating mindful exercise. [77] The search for the mechanisms of change has focused on two components, relaxation and exercise. In a meta-analysis on the effects of relaxation training, Hyman et al. found that various relaxation response-based interventions led to a reduction of somatic symptoms with additional effects on symptoms of anxiety and depression,[78] blood pressure,[79] and recovery from immune-mediated diseases.[78,80] *As observed in our pilot,[81] TCC in combination escitalopram, appears beneficial for enhancing treatment in LLD, an approach that may readily incorporated into future clinical practice*. Comparing a Chi-generating exercise (TCC) to a comparable non-chi generating exercise is innovative and may shed light on the difference between the two in clinical outcomes (mood, health functioning and quality of life), and the underlying mechanism.

Longitudinal design and long-term follow-up: The goals of behavioral and drug therapies for depression are to improve depressive symptoms and induce remission, thereby preventing relapse and recurrence.[5,82,83] Remission rates in clinical trials of antidepressant drugs in older adults are around 30-35%.[84] Extended treatment trials are necessary to optimize interventions for depressive symptoms in older adults.[85,86] Hence, we propose to use a 12-week acute treatment trial to also satisfy the TCC training objectives. Follow-ups at 6 and 12 months will evaluate maintenance of response/remission or depression relapse (or 24 months if 12 months have passed). Notably, very few studies designed to establish the mechanisms of treatment response in the elderly have included extended follow-up.

Primary and secondary outcome measures: Using fMRI biomarkers of emotional responsivity and affect labeling, and working memory, in addition to clinical measures, during therapeutic manipulation at two time periods is highly innovative. More importantly, this study will examine neural regulation of affect as a putative biological mechanism for the effects of the interventions, thereby advancing psychobiological models of emotional responsivity as predictors of outcomes in geriatric depression. *If our hypotheses are correct, our approach will inform public and clinical practice, and will provide currently missing information about the underlying mechanisms of response to TCC versus controls in geriatric depression.*

Image acquisition and analysis: Multimodal imaging data obtained at 3T will include sequences designed for optimal trade-offs between acquisition time, spatial and temporal resolution, where each modality, not widely applied for the study of LLD, provides unique and complementary information to better evaluate the neurobiological bases of treatment response. Further, we will apply advanced and innovative computational image analysis approaches for integrating data that are noticeably absent in the published literature, focusing on brain aging, connectivity and plasticity and associations with cognition and other health outcomes. *The application of a comprehensive imaging protocol for evaluating treatment response may advance understanding of the mechanisms underlying LLD and treatment-induced brain plasticity and impact clinical practice by providing guidance for delineating biomarkers of treatment response.*

Timeliness: The use of mind-body interventions will be shortly "in-demand" and highly beneficial for the rapidly growing large cohort of aging US "baby-boomers" who have higher rates of depression, and are more likely to use such treatments to prevent or treat the symptoms. Demonstrating the efficacy of such interventions and underlying mechanisms in older adults is becoming a research priority.

Design and outcomes

Interventions and Duration *We anticipate screening about 500 subjects to recruit 220 older depressed adults (≥60 years old, all of whom will have a range of depressive symptoms and will be on stable treatment (antidepressant or psychotherapy) for at least 4 months. All will meet criteria for major depression and will be randomized to receive 12 weeks of TCC vs HEW used as an adjunct therapy to the stable antidepressant regimen. Follow-ups will confirm clinical endpoints of stable remission and the overall improvement in depression and cognition at Months 6 and 12 (or Month 24 if 12 months have already passed).*

Interventions and Duration

Intervention 1 - Tai Chi Chih (TCC): *TCC employs "meditation through movement" as a means of helping older adults cope with fatigue, perceived physical limitations, and negative emotional states, which are commonly associated with insomnia.*

Intervention 2 - Health and Wellness Education (HEW) control group: *This condition will serve as an active control for nonspecific treatment elements such as attention and group support that pose rival explanations for the effectiveness of TCC. Participants will be informed that this intervention is designed to help reduce the severity of depressive symptoms, and all participants will be offered a choice of TCC in the end of the interventions, to balance expectations for the TCC intervention; therefore, reducing the placebo effect.*

Duration of Interventions: *Each class will be held for 60 minute per day/week. Each TCC class will allow 10 minutes of warm-up (e.g., stretching, breathing), 5 minutes of cool down. Each HEW class will allow 5 min recap from the previous week and 10 min question and answer in the end of the lecture. Homework assignments will be equal in all groups and ask for at least 20 minutes of either TCC, or computer searches on the topics of wellness at home. Participants will be asked to keep diaries of daily exercises, time spent and adherence to the tasks will be compared between the groups, and will be used in the analyses of response.*

Sample Size and Population

Subjects: *We will recruit 220 older depressed adults aged 60 years old or older, all of whom will have a range of depressive symptoms, meet criteria for an episode of major depression, and will be stable on anti-depressant treatment for at least 4 months. Subjects will be recruited, assessed and randomized according to the scientific protocol and the consent procedures approved by the UCLA Institutional Review Board and the Office of Protection of Research Subjects, as well as the analogous units at participating institutions.* We anticipate a maximum of 10% dropout in the first 12 weeks; hence, we will have **99 completers per group at 12 weeks**; and an additional 20% dropout at the end of the follow-up period of 12 months (or 24 months if 12 months have passed) (**88 completers per group**).

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

SOP: Patients will be assessed at screening and at baseline prior to the initiation of the intervention and biweekly thereafter until 12 weeks, and monthly between 3 and 6 month follow-up. There will also be a final Month 12 follow-up. If unable to schedule this final follow-up, participant will follow-up at Month 24 instead. Those who meet criteria after a telephone screening, will be invited for an in-person screening interview that may be split into 1-3 visits to accommodate clinical, neuropsychological, MRI and laboratory procedures. Baseline assessment ("Baseline 2") will be performed within 2 months of the start of the interventions. The informed consent form will be reviewed with the participant, and signed prior to initiation of the assessments. Inclusion and exclusion criteria will be reviewed using the inclusion/exclusion

criteria form completed as a source document at screening, baseline, and prior to the first day of TCC and HEW classes.

4.1 Inclusion Criteria

Inclusion criteria: All 220 participants will have major depression and will 1) be on stable treatment for at least 4 months and still meet criteria for major depression; 2) Have a 24-item Hamilton Rating Scale for Depression (HAMD) score of > 14 at baseline consistent with moderate-severe depression; and 3) Have a Mini-Mental State Exam (MMSE) score > 24.

4.2 Exclusion Criteria

Exclusion criteria: subjects will be excluded if they had any current other psychiatric disorders (except for frequently comorbid anxiety symptoms or insomnia), are receiving effective antidepressant and psychotropic medications or psychotherapy; or have had recent unstable medical or neurological disorders (myocardial infarction within past 6 month, surgery within last 3 months); any disabilities preventing their participation in TCC exercise per PI's discretion; prior experience with practicing a mind-body intervention (defined as Tai Chi, Tai Chi Chih, yoga, meditation) or have practiced it at least weekly within the past 12 months; diagnosis of dementia; those with metal implants that contraindicate brain imaging. Please see Tables 1 and 3 for detailed *assessment schedul*es.

Exclusion criteria for fMRI: 1) acute medical and neurological illness 2) evidence of metal in the body; 3) claustrophobia; 4) decline consent. Subjects that are only excluded from the fMRI portion of the study are allowed to remain in the study.

4.3 Study Enrollment Procedures

Recruitment of Subjects. Subjects will be recruited, assessed and randomized according to the scientific protocol and the consent procedures approved by the UCLA Institutional Review Board and the Office of Protection of Research Subjects, as well as the analogous units at participating institutions (e.g., Motion Picture and Television Fund). We project a recruitment period of 4.4 years to achieve a study group of **220 subjects**. With the anticipated average of about 4 subjects/month. The incentive to participate appears sufficient since the proposed project offers free TCC and HEW classes to every enrolled patient. In addition, two additional avenues are being developed, such as collaboration with the LA County Department of Mental Health.

We project our minority recruitment at 30%. If the rate of recruitment is not sufficient through the UCLA clinical and research services, advertising in local newspapers will be used. Recruitment through local support groups for depressed patients will also be attempted. Dr. Lavretsky also has links to the local community of care providers for the elderly and to mental health professionals, who could serve as a source of referrals. She serves as a Consultant to the LA County Department of Mental Health Services for older adults, an outreach program which should help recruit minority participants. The anticipated rate of recruitment is 39 subjects in year 1 and 40 in year 5 and **47 subjects per year in years 2-4** to accommodate 12/24-month follow-up, a realistic rate for our study. Data analysis will be performed in year 5. We anticipate the dropout to be about **10% in the first 12 weeks, and additional 20 % at 12/24 months**.

Retention of subjects. We have used successful strategies for retention of elderly subjects with depression. These include an ongoing and continuous process of consent to study procedures, involvement of family members whenever possible, and use of concerned and empathic clinicians and staff. However, it is important to note that our data analytic approach is robust to attrition.

Cohort maintenance. The comprehensive evaluation of subjects prior to randomization and entry into the treatment trial is designed to minimize the risk of attrition. The evaluation of weekly compliance questionnaires will also alert the PI to potential attrition problems.

Table 1.Timeline.

Years of the project	ACTIVITIES
Year 1 (1-3 months)	Review assessment instruments with research assistant, research nurse; Inter-rater reliability sessions. Develop patient schedule with the UCLA CTSI. Develop patient database and databooks. Start subject recruitment at month 4.
Year 1 (4-12 months)	Review and maintain accuracy of databooks. Develop database (with research assistant and P. Siddarth) (recruit 39 subjects). DSMC meets every 6 months after recruitment starts.
Year 2	Continue recruitment (47 subjects). Conduct inter-rater reliability sessions. DSMC meets every 6 months.
Year 3	Recruit 47 subjects. Conduct inter-rater reliability sessions. DSMC meets every 6 months.
Year 4	Recruit 47 subjects. Conduct inter-rater reliability sessions. DSMC meets every 6 months
Year 5	Recruit 40 subjects in the first 6 months. Continue 6 month follow-up. Anticipate completion of final subject by month 10. DSMC meets once. Complete data analysis (months: 10-12).

Table 2. TARGETED / PLANNED ENROLLMENT: Number of Subjects 220

Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	10	6	16
Not Hispanic or Latino	130	74	204
Ethnic Category Total of All Subjects*	140	80	220
Racial Categories			
American Indian/Alaska Native	1	1	2
Asian	10	8	18
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	10	6	16
White	118	64	182
Racial Categories: Total of All Subjects *	140	80	220

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Each class will be held for 60 minute per day/week. Homework assignments will be equal in all groups and ask for at least 20 minutes of either TCC, or computer searches on the topics of wellness at home. Participants will be asked to keep diaries of daily exercises, time spent and adherence to the tasks will be compared between the groups, and used as a predictor/mediator in the primary analyses of response. All classes will be offered once a month as booster session for the remainder of the study between weeks 12 and 24. Subjects will be given a DVD of the training course and encouraged to continue practice at home. Subjects in the HEW group will be asked not to join any of the TCC classes until the end of the study. At the end of the study, participants will be offered an option of participating in the two alternative classes.

Tai Chi Chih (TCC): TCC employs "meditation through movement" as a means of helping older adults cope with fatigue, perceived physical limitations, and negative emotional states, which are commonly associated with insomnia. Patients will be informed that TCC constitutes a health management intervention, which incorporates meditation and physical activity to promote a sense of well-being and control over negative symptoms associated with depression. The standard detailed protocol for TCC uses an adapted protocol from the "Tai-Chi-Chih Joy through movement" by Justine F. Stone[141] and has been used in several studies by our research group, and others (i.e., R21-AT002307). TCC sessions will be held for 60 min a day once a week. Each class will allow 10 minutes of warm-up (e.g., stretching, breathing), 5 minutes of cool down. Participants will be instructed to practice at home for 20 minutes per day using handouts and documenting their practice in their daily diaries.

Health and Wellness Education (HEW) control group: This condition will serve as an active control for nonspecific treatment elements such as attention and group support that pose rival explanations for the effectiveness of TCC. Participants will be informed that this intervention is designed to help reduce the severity of depressive symptoms, and all participants will be offered a choice of TCC in the end of the interventions, to balance expectations for the TCC intervention; therefore, reducing the placebo effect. The PI and trained study staff will implement HEW using a manual that will present educational information and describe learning objectives and patient activities to promote integration of material. The detailed protocol for Health Education has been developed and used in the pilot project. The HEW sessions will be held once a week for 60 minutes over the **12**-week treatment period. We will follow a didactic format, inviting experts to lecture on key topics, followed by focused group discussion, and post-discussion self-help quizzes to assess patient learning. This novel use of a non-exercise control intervention, which matches the exercise intervention in duration, frequency and social contact, represents an important methodological advance.[143] Participants will be instructed to practice at home in computer searches addressing the health topics discussed in the session for 20 minutes per day. Weekly supervision by Dr. Ercoli with project health education instructors will be used to monitor instructors' adherence to the educational protocol. Tai Chi Chih trainers will follow the same manual. We will contact Tai Chi Chih Association for an opportunity to monitor TCC trainers' adherence to the manual protocol. Participants will be asked to keep diaries of exercise or computer searches and the adherence will be compared between the groups.

5.2 Handling of Study Interventions

Treatment credibility. It is essential that subjects perceive both interventions as equally credible for promoting a similar degree of expectation for improvement. We will evaluate treatment

credibility, expectation for change, and satisfaction after the 2nd session of treatment and at post-intervention, according to procedures using a 10-point Likert scale, as we have done in our pilot.

5.3 Concomitant Interventions

Alternate treatments. All subjects will be medically stable prior to entry into the study protocol and will be stable on psychotropic medications or ongoing psychotherapy for at least 4 months. However, if subjects engage in alternate treatments (other types of mind-body (e.g. yoga), integrative (e.g., acupuncture), psychotherapy, switching to different antidepressant treatment, or have an elective surgery or hospitalization), they will be advised to inform the PI or a designated staff at every visit. All assessments will be done blindly. If the alternate treatment is judged to affect outcomes of the study, the subject will be withdrawn from the interventions but followed naturalistically for 6 months. This decision will be made by the PI on the basis of blinded assessments. Subjects will be informed about an option of pursuing clinical care in the community.

The concomitant medication document and any changes will be recorded at each visit. The database will have a variable identifying the type of antidepressants or any other psychotropic drugs or other prescription or over-the –counter medications/supplements that participants are taking. Classes of drugs will be recorded as well.

5.4 Adherence Assessment

SOP: Weekly supervision by PI- Lavretsky with designated study staff will be used to monitor adherence to the intervention and protocol during each group and clinical visit and during weekly staff meetings. Participants will be asked to keep diaries of exercise or computer searches and the adherence will be compared between the groups. Subjects will not be removed from the study due to poor adherence to retain sufficient sample for the analyses. If they decide to drop out from the intervention arms for any reasons, they will be followed naturalistically. Subjects who miss three or more intervention sessions will be identified as deviation from the protocol. Adherence will be calculated and used in the analyses as a covariate.

5.5 COVID-19 Restrictions – Enacting March 2020

As of March 2020, UCLA has enacted several restrictions on our campus to prevent COVID-19 contraction and spread. In order to align with these standards, we are giving participants the option to participate virtually through web-based and telephone meetings. This will be offered for any appointment that is able to be completed remotely, as well as for both interventions the study is testing. This will include teleneuropsychological assessments as well. All remaining portions that are not possible to obtain remotely (e.g., MRI, venipuncture) will be obtained as close to the proposed visit date as possible.

We will also instill a COVID-19 Questionnaire to all active participants to ensure optimal safety reporting and to correlate with current psychiatric measures. This will be administered as a survey, with the responses coming verbatim from the participant. The survey will be completed at one time point, but we will continue to track participants' well-being through our administration of UKU.

6. STUDY PROCEDURES

All study assessments will be conducted in a private examination room at either at UCLA Semel Institute or the UCLA Motion Picture Television Fund Campus.

Screening Procedures (Screening / Visits 1a-c): The informed consent form will be reviewed with the participant during the first in-person visit. There will be general medical and psychiatric history taken, vital signs (blood pressure, pulse) (5 minutes duration), and an ECG (electrocardiogram) will be performed (25 minutes). The questionnaires and screening procedures are performed to determine eligibility. The initial visit will last approximately 2 hours.

Clinical Assessments will be performed in 2-3 visits due to participant's burden: (Tables 1;3). *Diagnosis.* The Structured Clinical Interview DSM-IVTR/-5 (SCID)[121] will be used to make a diagnosis of major depression or other mood disorders or rule out other diagnoses (e.g., psychosis and dementia) at screening. Inter-rater reliability for all instruments will be established at the beginning of the trial across both sites and all staff members on the first 5 recruited patients, it will be rechecked routinely twice a year for consistency throughout the study. Master level research associates supervised by the PI will be trained to reliability for the diagnostic interview (SCID) and other assessments. The current inter-rater correlation coefficient (ICC) for SCID ratings among SCID interviewers is ≥ 0.9. A second level of reliability check is afforded by a bi-weekly diagnostic consensus conference during which diagnosis is also confirmed.

Blood Samples. During screening procedures between 1st in-person visit and visit 2, blood will be drawn by needlestick for routine laboratory testing and genetic analysis (15 minutes). The blood samples will be processed at the UCLA Clinical and Translational Research Center (CTRC). Approximately 8 tablespoons of blood will be drawn. This visit can be combined with either the visits for MRI or Cognitive testing visit.

Screening diagnostic / Neuropsychological Evaluation Visit (Visit 2):
Dr. Lavretsky or a designated member from the study staff will conduct a neuropsychiatric evaluation using cognitive assessments during Visit 2 that takes about 2-3 hours.

Visit 3. Magnetic Resonance Imaging (MRI) Scan. One MRI brain scan will be performed. The scan will occur at the UCLA Brain Mapping Center. MRI Safety metal screening will be performed. Only 55 pts/arm will undergo the fMRI procedure.

Visit 4. Baseline visit will be performed within 2 months prior to initiation of the interventions with assessment of mood and vital signs (30 min)

Follow-Up Visits (Visits 4-9 and 12-13): Follow-up assessment will occur bi-weekly (every 2 weeks) for 12 weeks and then monthly in order to ensure safety. Each visit will last approximately 30 minutes. Interviews and questionnaires will be used to determine if the treatment is working and useful. Adherence will be monitored at each visit. At each follow-up visit, vital signs will be measured (5 minutes), practice and homework log will be reviewed, and questionnaires will be administered (25 minutes).

Weekly Tai Chi or Health and Wellness Education (12 classes): Weekly Tai Chi or health and wellness education training will occur every week for 60 minutes for 12 weeks. The weekly sessions will last approximately 60 minutes per class. Homework adherence and diaries will be monitored at each class.

Visits 10/11 and 14-19- Outcomes and Endpoints. 12 week follow-up visit (Visit 10/11): During the final visit, a physical, psychiatric examination, and a follow-up blood draw for genetic analysis will be repeated. MRI will be repeated at 12 weeks. The follow-up visit 12 will be split in 2 visits (for clinical, cognitive, laboratory, and MRI) will last 4 hours (1 hour MRI). The 6-month follow-up

visit (14/15) will be approximately 1.5 hours. Both the 12- or 24-month follow-up visit will be split in 2 visits (for clinical, cognitive, laboratory, and MRI) will last 4 hours (1 hour MRI). The Month 12/24 visit will be completed by only those participating in the MRI portion of the study. Since this visit was added onto the original study, some of the participants may have passed their Month 12/24 time point. We will allow this visit to occur any time succeeding the Month 12 mark, and will note the visit type by proximity of their timeline (ie: Participant completes visit at Month 17, would be assigned to Month 12 Visit).

6.1 Schedule of Evaluations

Assessment schedule.

6.2 Description of Evaluations

Primary outcome measures will include efficacy evaluations using the 24-item HAMD[110] to quantify mood symptoms. ***Secondary outcome measures*** will include the Clinical Global Impression-Severity and Improvement scale (CGI)[122] to quantify overall severity and clinical improvement over time (2 min). In addition, we will administer the Montgomery Asberg Depression Rating Scale (MADRS)- commonly administered in geriatric depression that is easily comparable to other trials, and the Geriatric Depression Scale (GDS),[123] a self-assessment scale often used in geriatric depression trials (10 min).

Measures of comorbid neuropsychiatric symptoms will include the Hamilton Anxiety Scale,[113] a widely used measure of anxiety symptoms (10 min), the Apathy Evaluation Scale (AES),[111] a measure of the severity of apathy (15 min), and the Unified Parkinson's Disease Rating Scale (UPDRS)[124] used for the assessment of psychomotor slowing and extrapyramidal symptoms (10 min). Pittsburgh Sleep Quality Index (PSQI) is an 18 item self-report measure of subjective sleep quality (5 min).[125]

Measures of medical comorbidity will include the Stroke Risk Factor Prediction Chart (SRF)[126,127] of the American Heart Association for rating cerebrovascular risk factors (5 min) and the Cumulative Illness Rating Scale-Geriatric (CIRS-G)[128] used for rating the severity of chronic medical illness in 14 organ-systems (15 min). *Health-Related quality of life and physical functioning* will be determined using the Medical Outcomes Study Short Form 36-Item Health Survey (SF-36),[129] that records health-related quality-of-life, mental, physical, and social functioning (15 min), the Connor-Davidson Resilience scale (CD-RISC), as a measure of stress coping ability[130](5 min), the Quality of Life Enjoyment Scale (Q-LESS-Q),[131] a brief assessment instrument of life satisfaction commonly used in clinical trials of psychiatric populations including the elderly (5 min). Pain assessment has been also included as a measure of self-rated pain to monitor any changes in the pain severity over time (5 min) We will also include a self-reported physical activity record for monitoring physical activity in both groups (3 min).[132]

Neuropsychological Assessments: Neuropsychological tests will be administered **at baseline, week 12, and at 6, and 12 or 24 months**. Our approach to cognitive assessment and analyses is based on our prior work and benefits from the neuropsychological expertise of Dr. Ercoli who will train research staff on cognitive assessment. We will use a focused test battery developed by Dr. Ercoli sensitive to assessing domains shown as impaired in geriatric depression through prior research by our group and others.[25,133-135] Domains of cognition will be assessed which are likely to show impairment in geriatric depression (1) Executive functioning, which will be separated into the two areas of primary interest—cognitive set-shifting and inhibitory control; (2) working memory; (3) information processing speed and attention, including an indices of complex and

sustained attention); (4) episodic memory/delayed recall. Most of the measures have 2 or 4 alternate forms so that unique forms may be administered to subjects during repeat testing. In addition to the cognitive outcome measures, we will include a brief word reading test, the Wechsler Test of Adult Reading (WTAR),[136] to estimate premorbid cognitive functioning. We will conduct a confirmatory factor analysis to assess the goodness of fit of our *a priori* domain assignments. Cognitive domain scores will be obtained as follows: for each cognitive test, raw scores will be converted to standardized (Z) scores with a mean of 0 and a SD of 1, combining all subjects. For each of the tests in a domain, the Z scores will be averaged to create a single Z score. The primary focus will be on executive functioning and working memory

Neuropsychological assessment by specific composite domain.

COMPOSITE DOMAIN	NEUROPSYCHOLOGICAL TESTS AND VARIABLES DERIVED FROM EACH
Language	Boston Naming Test (total correct); Category Fluency (total animals named in 60 seconds); Letter Fluency (total words generated for F, A, and S in 60 seconds each)
Executive Function	Trailmaking B:Trailmaking A ratio; Stroop Color-Word Interference Test Part C (Kaplan version; seconds to complete)
Attention and Processing Speed	Trailmaking A (seconds to complete); WAIS-IV Digit Symbol Substitution Test Stroop Color Naming and Word Reading (average of seconds to complete)
Visuospatial Ability	WAIS-IV Block Design; Rey-Osterreith Complex Figure (total score for copy using Taylor's criteria)
Episodic Memory	California Verbal Learning Test II (trial 5 vs. Long Delay Free Recall)); Rey-Osterreith Complex Figure (total score 30-minute delayed recall)

6.2.1 Screening Evaluation

Consenting Procedure

Prior to enrollment, during in-person screening visit (#1) the PI or Co-Is will obtain IRB-approved informed consent from subjects. The consent form will be reviewed with each subject prior to signing this consent form. All subjects will be offered an opportunity to discuss the study with their primary physicians and family members prior to signing the consent form.

This will be followed by neuropsychiatric and physical examinations, and laboratory tests (2 1/2 hours). Though eligibility will be assessed again at baseline, participation may be terminated if the subject stops meeting entry criteria. No subject will be asked to discontinue effective antidepressant medications or psychotherapy.

Screening (Visits 1-3)

All subjects will receive an initial *medical evaluation* including a complete physical examination with neurological and neuropsychiatric examinations, electrocardiogram (ECG) (for potential cardiac complaints), and laboratory testing to rule out new-onset medical illnesses that could account for behavioral and cognitive symptoms. *All abnormal physical or laboratory findings will be reported to subjects' primary physicians with subject's consent. If abnormal physical or laboratory results are considered responsible for depression, the subject will be excluded from participation.* All subjects will be interviewed about their recent history of psychiatric and medical illnesses, psychosocial stressors, current medications and health status. In addition, vital signs including pulse rate, systolic blood pressure, and body weight will be obtained at each visit. The UKU Side Effect Rating Scale,[109] a comprehensive rating scale for monitoring adverse events in

clinical trials, will also be completed at all visits except screening.

Dementia. We will screen for possible incipient dementia to exclude participants. This will include reviewing an extensive history and mental status exam together with corroborating information with regard to functional skills. A MMSE score of ≤ 24, the clinical dementia rating score of CDR score >0.5 or an established dementia diagnosis will mandate exclusion. The evaluation for dementia includes: 1) an interview by a psychiatric nurse to identify physical and cognitive limitations; 2) a standard battery of hematologic studies; 3) neurological examination (UPDRS); 3) neuropsychological examination (detailed below); and 4) psychiatric evaluation (SCID), as detailed above. Adjudication of dementia is based on DSM-5 criteria.[110,111] At the consensus conference, additional information will be reviewed (e.g., family history, drug use).[112] *We will use performances between -1.5 and -2 (SD) below age and education norms on one of two memory tests from our cognitive battery to establish cut-offs according to a widely accepted practice for the diagnosis of dementia.*

Blood Sample. Blood samples will be collected for the RNA and telomerase analyses at screening (visits 1-3) and 12 weeks post-randomization (or early termination). A sample of 10ml of blood will be collected and it will be processed by the Core Laboratories of UCLA Department of Human Genetics.

Neuropsychological Assessments: Neuropsychological tests will be administered **at screening (visits 1-3), week 12 (visits 10-11), and 6, 12, and 14 months (visits 14-19)** (See 6.2 for descriptions of evaluations).

MRI Imaging Biomarkers: Imaging data will be acquired from 110 subjects (55 per group) at baseline, at 12-weeks post-randomization (or for early termination), and at 12-months post-randomization on a Siemens Prisma system using a 32-channel head coil. Each scanning session will last 60 minutes and include 1) a T1-weighted multi-echo MPRAGE (MEMPR) sequence with real time motion tracking and correction for the examination of brain structure; 2) a bandwidth matched T2-space sequence for detection and quantification of WMHs and CSF; and 3) three separate BOLD EPI sequences for the acquisition of rs- and task-related fMRI data used for the examination of emotional and working memory processing and functional connectivity. In addition, a 3-plane localizer and a non-BOLD EPI sequence co-planar to the fMRI data for cross-modal image registration will be acquired. The morphometry sequences are optimized for determining brain tissue contrast with reduced distortion.[113]

Table 5. FMRI sequences

3D T1 MEMPAGE	TEs/TR= 1.74, 3.6, 5.46, 7.32/2530 ms, TI=1260 ms, FA=7, FOV=256 mm, matrix=256x192, voxel size=1 mm^3, run time: 8:14 min.
3D T2 SPACE	TE/TR= 425/3200 ms, FA='T2 var', FOV=256 mm, matrix=256x192, voxel size=1 mm^3, ETL=769, 2xGRAPPA. run time: 4:43 min.
fMRI (emotion and working memory)	TE/TR=25/2500 ms, FA=80°, matrix 64x64, FOV=20 cm, 34 axial slices, slice thickness 3 mm, .75-mm gap, run time: ~6 min each.
Resting state fMRI	TE/TR=30/2000 ms, FA=80°, matrix 64x64, FOV=20 cm, 34 axial slices, slice thickness 3 mm, .75-mm gap, run time: 8 min.

Functional Imaging: To address our hypotheses that TCC versus HEW will result in greater activation in right VLPFC and the amygdala during emotional processing (Hypothesis 2a) and greater working memory-related activation in the DLPFC and other network regions (Hypothesis 2b) we will employ two widely-validated brain activation tasks that probe emotional processing and working memory function (see preliminary studies). Similarly, analysis of rs-fMRI data will

determine whether TCC associates with plasticity in brain network activity. **Faces Affect Labeling Task.** We will use a previously detailed Faces/Shapes brain activation task shown to produce robust and reliable activations in amygdala and PFC regions during emotion processing, making this paradigm particularly appropriate for longitudinal investigation.[114,115,116] In brief, during this task participants are required to match emotions (activating the amygdala and VLPFC), label emotions (activating the VLPFC) or match forms (a control condition) while presented with faces showing different emotional expressions or geometric shapes [**Fig.1**]. Since subjects are not asked to attend or regulate their affect, brain activation is more closely linked with affective reactivity, rather than with affect perception.[116] The task includes 5 blocks of the control shape-matching task interleaved with 4 blocks of the experimental faces condition. Button press responses monitor accuracy and reaction time. Though some mixed findings exist, previous studies, and our pilot data suggest that implicated VLPFC hypoactivity, as well as task-related activation of the amygdala may vary with response to treatment and clinical state.[117] **Working Memory Task.** We will use a well-established 2-back version of the n-back task detailed in Townsend et al.,[118] to elicit neural activation linked with working memory. This task consists of two blocked conditions with a 20 s resting condition interleaved between conditions. In the experimental 2-back condition subjects track letter sequences and press a button when they detect a letter that appeared 2 positions back. In the control 0-back condition, subjects will press a button every time the letter "X" appears on-screen. Button presses record accuracy and response time. This version of the n-back has been employed in our laboratory to show significant reductions in activation of the DLPFC and posterior parietal cortex across mood state in bipolar subjects compared to controls[118] as well increased activation in association with clinical response in LLD (see preliminary studies). **Resting State-fMRI:** During acquisition of BOLD rs-fMRI data, subjects will be asked to remain awake with their eyes closed. Though resting state connectivity is highly reproducible over time in healthy subjects[119], experience-based neural plasticity in resting state connectivity has been shown to occur over periods as short as 2-9 days.[120] Recent research points to significant changes in DLPFC and cingulate connectivity in association with ECT.[53,54]

Preprocessing and Analysis: *Preprocessing of all fMRI data* will follow similar workflows executed in the LONI pipeline environment, where standard and state-of-the-art image analysis programs interact seamlessly in a parallelized supercomputing environment. Specifically, preprocessing steps incorporating FSL (www.fmrib.ox.ac.uk/fsl) and custom processing modules, will include: a) removal of non-brain tissue; b) rigid-body head motion correction; c) spatial smoothing (5-6 mm FWHM); d) denoising and high pass filtering using FSL's MELODIC; and d) co-registration of the BOLD and T1 images using the matched bandwidth EPI images for intermediate registration. First-level analysis will include the modeling of activation between task conditions (e.g. match emotion vs. shapes; or 0-back versus 2-back). Temporal derivatives and the 6 motion parameters will be included as covariates of no interest to improve statistical sensitivity. Second-level (fixed effects) and third-level (mixed effects) analyses will average runs within subjects and across groups respectively for subsequent comparisons between the treatment groups. *Longitudinal analysis:* In addition to subtracting changes in activation between baseline and follow-up scans within individuals for subsequent group comparisons, we will represent fMRI maps as statistical change maps in time, with each voxel representing a smooth change in magnitude (positive or negative) from the baseline dataset. These maps will be presented against the structural template, to determine the effects of structural change, functional change, and their interaction. One sample t-tests will determine if activation for each individual changes significantly over time. *Task-related connectivity analysis:* To investigate effective "functional" connectivity, we will perform a psychophysiological interaction (PPI) analysis,[121] which determines differences in task-dependent functional connectivity between regions-of-interest (ROIs). We will investigate the effect of the emotion-labeling task relative to the shape control, using voxels in the amygdala as "seeds" and voxels in the PFC as the "target." Group-level FSL FEAT analyses will determine voxels throughout the brain showing significantly correlated task-

dependent activity with the ROI. Regressors include the physiological variable (e.g., amygdala activity), the psychological variable (emotion vs. shape), and their interaction. Higher-level analyses will determine the degree of functional coupling among treatment groups. *Resting state analysis:* After applying preprocessing steps similar to those described above, widely documented independent components analysis (ICA),[51,122] using FSL's MELODIC, will estimate the optimal number of components for each subject and will remove components representing artifacts. After low pass filtering (0.1-0.01 Hz) and transformation into atlas space, the best-fit DMN component for a subject will be selected for higher-level group analysis. We will explore components of other networks. We will also examine voxel correlation within particular networks in follow-up analyses of ROIs[49,51,123] Changes in functional connectivity will be quantified by comparing Fisher z-transformed correlation coefficients between ROIs. **Structural Imaging**. Structural image analysis will incorporate methods refined and validated by project co-investigators and include: 1) volumetric analysis using automated and manual segmentation, 2) refined shape/surface structure analyses and 3) tensor based morphometry (TBM) analysis using Jacobian determinates to quantitatively map voxel-level morphometric change throughout the brain. In brief, widely used Freesurfer http://surfer.nmr.mgh.harvard.edu/) processing streams that include a) correction for magnetic field inhomogeneities; b) removal of non-brain tissue; c) tissue segmentation; d) separation of the hemispheres and subcortical structures; e) extraction of the white/gray and pial cortical surface and f) initial segmentation of cortical and subcortical ROIs (with manual correction of errors) will be used to estimate regional tissue volumes for comparison across treatment groups and time. These analyses will be followed by more refined morphometric analysis to reveal local changes in the shape/surface structure of subcortical ROIs as informed by the volumetric and TBM results (see below). Procedures include the use of surface-based mesh modeling and skeletonization methods that have been used to assess extremely local changes in the morphology of the amygdala, striatum and hippocampus in several clinical groups including LLD and across time in our prior studies.[124-126] Finally, we will employ TBM methods that are shown as highly sensitive for detecting subtle changes in brain morphometry associated with maturation/aging or disease,[117,126-131] to examine both global and local changes in brain tissue structure across treatments and time. **WM Hyperintensities (WMH).** The 3D T2-space images will be used to visualize WMHs by employing a semi-automated WMH identifier.[132] Briefly, WMH burden will be assessed based on the signal intensities of co-registered 3D MEMPR and T2-SPACE images, and population statistics on the spatial distribution and neighborhood structure of WM lesions. With every unit increase in WM burden, we will estimate % reduction in local brain tissue volume. GLM and multiple regression analyses will determine links between WM burden and clinical measures in each group and may be controlled.

Blood samples and peripheral biomarkers.

Genetic testing. Participation in the genetic portion of the study is voluntary. If subjects are deemed ineligible, they will not undergo genetic testing.

DNA and gene expression: Genomic DNA will be extracted from whole blood and isolated using a Wizard Genomics DNA Purification Kit (Promega Corporation, Madison, WI, USA), and determined with 0.7 % agarose gel (Beutler et al., 1990) according to the standard protocol.

RNA and microarray analyses: RNA will be extracted from whole blood and isolated using a Wizard Genomics DNA Purification Kit (Promega Corporation, Madison, WI, USA), and determined with 0.7 % agarose gel (Beutler et al., 1990) according to the standard protocol.

Telomerase and NFkappa B. peripheral cytokine collection: Standard Operating Procedures (SOP): The procedures that will be taken to protect the confidentiality of the subjects who donate samples. Telomerase and NFkB samples will be obtained once at baseline and once at week 12 to evaluate telomerase activity in response to the antidepressant intervention. Telomerase and NFkB samples will be collected concurrently with the other investigational labs, so no additional needlestick is necessary for this test. Subjects will not be asked to fast before the draw and will

not receive any additional monetary compensation for this portion of the research. 10ml of blood will be drawn into one green Heparin tube yielding 50-150 micrograms of the specimen. After collection, telomerase samples will be labeled with the study code, date and time of collection, and an identification number assigned to each subject by the PI or the study coordinator. This identification number will be the only link between the samples and the subjects and will only be accessible to the PI and to certified members of the research team. This information will be stored in an electronic log that is password protected. No other personal identifiers will be listed on the specimen. The collection will be performed at the UCLA Clinical and Translational Research Center (CTRC).

6.2.2 Enrollment, Randomization and Baseline assessment

Clinical Assessments: *will be repeated as per the Assessment table (attached).* Those who continue to meet the inclusion criteria at screening, and agree to participate in the study, will be scheduled for a baseline assessment (baseline 2) that will include assessment of depression severity that will be scheduled within 2 months of starting the interventions.

Randomization

After all screening and baseline test results are reviewed and eligibility criteria are confirmed, subjects will be randomized to two groups if patients continue to meet eligibility criteria and sign the informed consent form. The anticipated waiting time from screening to randomization to the initiation of the interventions is up to 3-4 months.[NCCIH1] All eligible subjects will be randomized to the TCC, or HEW control group using a computer-generated random assignment scheme, which assigns subjects in a 1:1 ratio to each group using a customized module built into the centralized data system. Randomization will be stratified by site and by whether the subject will receive imaging or not. Specifically, there will be four strata for randomization defined by the 2 x 2 combinations of site (UCLA or MPTF), with approximately 60-70% of the subjects being recruited at UCLA and the remainder at MPTF, and imaging (yes or no). Within these strata, class sizes of 6 or 7 will be randomized to TCC or HEW.[NCCIH4] Access to the data system, including the randomization program, is controlled via a hierarchical system of password protected logins, ensuring that study personnel can view only the parts of the system that are appropriate for their role. This helps to maintain blinding and ensure subject confidentiality. Careful security protocols will also be maintained prior to the entry of data into the online system. Specifically, paper copies of patient study forms will be stored in locked file cabinets and will be accessible only to authorized personnel. A shredder will be used to discard all unwanted study documents.

6.2.3 Blinding

We have considered different options for blinding/masking procedures. We will provide treatment groups that both incorporate “exercise and wellness education,” which we believe will generate equal expectations for benefit. Both groups will be informed that they will receive exercise and education upon consent and entry into the study. Each group will have an option of trying and switching to the two other interventions after up to 24 months of follow up, which facilitates blinding and controls for the exercise effect. For ethical reasons, we cannot have a “usual care” group in which older depressed subjects at high risk for worsening of depressive symptoms in the absence of behavioral treatment. All subjects will be blind to the fact that symptomatic, functional and cognitive improvement are the outcomes of interest, and the differences in the interventions, since all participants will be recruited to receive a “wellness and exercise treatment program.”
Blind Assignment: The trained behavioral raters will also be “blind” to the treatment assignment.

Both statisticians and the PI will be blind to the group assignment. Only trainers and study coordinators will be unblinded, but they will not have access to the data monitoring or analyses. Database staff entering the data or managing the data will be blind to the treatment assignment. Groups in the documentation will be identified as 1 and 2. If unblinding occurs in the process of assessment, we will assign different raters who will continue to be unaware of the treatment group, and will re-establish reliability on all assessment instruments. We will minimize potential "placebo effects" by balancing expectations by introducing two "wellness and exercise" interventions for depression. We will measure and compare the expectancy for the outcomes at baseline, Week 12, Month 6 and Month 12 as specified below (or Month 24 if 12 months have passed). Both classes will be taught by different instructors. We will control for the "instructor" effect if several instructors teach classes.

6.2.4 Follow-up Visits

Follow-up Assessments: Assessments of efficacy and safety will take place approximately *every 2 weeks* for the remaining weeks of the 12 week trial, followed by naturalistic monthly follow-up from **4-6 months**. All follow-up visits, except those at 3, 6, and 12 months- will last for about 30 min. Due to the nature of our population of older adults who may forget their appointments or have competing medical appointments some flexibility in scheduling will be allowed with the interval (+/-) 1 full week of the expected week throughout the study.

Similarly, longer assessments at months 3, 6, and 12 may be split into to 2 visits to accommodate clinical, neuropsychological, MRI and laboratory assessment (at 3 and 12 months). The MADRS, HAM-D, and GDS are to be obtained once between the baseline and screening visits, but re-administered at baseline if the baseline visit occurred significantly after the screening visit for 2 months or later. If participant is unable to complete at Month 12, this follow-up will be completed at Month 24.

We will assess home practice and adherence, safety and medications at each assessment visit. The visit frequency will serve as an additional safeguard to monitor worsening of depression, and the emergence of side-effects or suicidal ideations. All behavioral outcome assessments will be identical in both groups and performed by the trained raters blind to the treatment assignment *(Table 2)*.

6.2.5 Completion/Final Evaluation (will be scheduled within a window of +/- 1 week in regards to the week of the final class)

The follow-up visits at 3, 6, and 12 months will last 1.5-3 hours for outcome measures.

Visit 10-11 at week 12: A physical, psychiatric examination, and a follow-up blood draw and MRI for peripheral biomarker analysis will be repeated.

Visit 14-17 at 6 and 12 months - will reassess primary and secondary outcomes and the remission/relapse status.

Remission at 12 weeks / 6 and 12/24 month follow-up: Subjects who achieve remission by week 12 will be followed monthly to detect relapse of major depressive episodes. The goal of continuation treatment is to ensure stability of response/remission, and to optimize coping, cognition, physical functioning and life satisfaction. We will explore the predictors and biomarkers of relapse for future studies. We will continue to monitor safety monthly by phone and in-person at 6 and 12 months, when relapse of depression will be documented. If participant is unable to complete within the Month 12 timeframe, this will instead be followed during the Month 24 timeframe.

6.2.6 COVID-19 Questionnaire and Assessment

We will also instill a COVID-19 Questionnaire to all active participants to ensure optimal safety reporting and to correlate with current psychiatric measures. This will be administered as a survey, with the responses coming verbatim from the participant. We will also remotely administer HAMD, HAMA, and MADRS at the same time point. This visit will only be administered once, but will happen as soon as possible once approved, and will be a visit separate from the rest of the scheduled portions of the study.

7. SAFETY ASSESSMENTS

7.1 Specification of Safety Parameters

Data and Safety Monitoring plan.
We will assess safety in each individual case during each visit and during telephone contact with the subject or with their family members, if they are available, between the visits. Assessment of side-effects will be performed by the PI and Co-Is. They will participate in the review of any side-effects rated 3 or greater on the UKU scale. These subjects will be discontinued from the study. We have established guidelines for discontinuation from the study in the proposed protocol listed above. All severe side-effects will be reported to the UCLA IRB/Human Subjects Protection Committee within 1 week and to the NIH staff within two weeks.

Potential problems and solutions.
Assessment and monitoring of suicidal risk will occur **at each visit and by phone between visits.** We have applied the standard procedures that are required and approved by the UCLA Institutional Review Board. We have employed the same procedures in our ongoing studies of late-life depression since 1995, and have had no suicide attempts among our subjects. We will monitor suicidal ideations using the third item of the Hamilton Depression Scale during each visit to the site. Monitoring for SI will be done at each visit independent of the outcome assessment. The blind rater will be instructed to inform the PI of scores ≥ 3. Drs. Lavretsky and Wu will be available for "on call" consultations to address concerns of worsening of suicidal ideations. They will also participate in the review of the dropouts due to suicidal ideations and other causes during DSMP meetings. Subjects with active suicidal ideations with or without a plan, HAMD-24 (3) score ≥ 3, will be excluded from participation. In the presence of persistent suicidal ideations, subjects will be discontinued from the study and referred to clinical services or to the community for treatment.

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

Safety Evaluations: Safety will be assessed at each visit. All subjects will receive an initial *medical evaluation* including a complete physical examination with neurological and neuropsychiatric examinations, electrocardiogram (ECG) (for potential cardiac complaints), and laboratory testing to rule out new-onset medical illnesses that could account for behavioral and cognitive symptoms. *All abnormal physical or laboratory findings will be reported to subjects' primary physicians with subject's consent. If abnormal physical or laboratory results are considered responsible for depression, the subject will be excluded from participation.* All subjects will be interviewed about their recent history of psychiatric and medical illnesses, psychosocial stressors, current

medications and health status. In addition, vital signs including pulse rate, systolic blood pressure, and body weight will be obtained at each visit. The UKU Side Effect Rating Scale,[137] a comprehensive rating scale for monitoring adverse events in clinical trials, will also be completed at all follow-up visits.

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at screening/ baseline, appears to worsen. Adverse events are to be recording regardless of their relationship to the study intervention.

NOTE: Changes in the primary and secondary outcome measures (e.g. depression) will not be considered side-effects but will be monitored as outcome variables.

A **serious adverse event (SAE)** is generally defined as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly.

All severe adverse events will be reported to the subjects and to their primary physicians and the UCLA IRB/ DSMB committee within one week of PI's awareness, and within two weeks to the NIH.

- If a patient demonstrates worsening of their depressive symptoms by week 12 (i.e., CGI-I score > 4) of treatment, they will be discontinued from the study and referred for appropriate clinical services.
- Suicidal ideations with the HAMD item 3 score of 3 or greater

All dropouts will be analyzed by the reason for termination and the reasons will be classified as:

- a) Lack of efficacy; b) Side-effects; c) Lost-to-follow up; d) Hospitalization; e) Death; f) Other
- Relation to the study interventions: a) likely; b) probable; c) unlikely.

7.4 Reporting Procedures

We will arrange for **Data Safety Monitoring Committee** meetings at approximately six month intervals. The DSMC will consist of one external and one internal member, Dr. Aaron Kaufman, an expert geriatric psychiatrist, Dr. Perry Hu- a geriatrician, and a statistician- methodologist Dr. Christina Kitchen. All DSMC members have considerable experience in geriatric clinical issues. The DSMC will meet at least once every six months to review study progress. Members of the committee agree to keep all information confidential. Those members of the investigative group that are involved with the treatment and assessment of patients within the study will not review data that involve the comparison of outcomes (i.e., adverse events, treatment response, remission, etc.).

The Board will also issue a report annually to the PI and the IRB. The board will review summaries of the study progress to ensure that consent documentation is properly obtained and stored. They will also review progress in filling subject recruitment ethnic requirements. They will also determine whether study coordinators and investigators are collecting and organizing data properly. Key personnel will provide progress reports to facilitate this review. The board will also review any recent research relevant to the study. The summary reports will provide data on enrollment and adverse events. Adverse events will be monitored using the UCLA Adverse Event and/or Incident Reporting forms (Forms HS-5 & HS-6). Additional support for these activities will be provided by the CTSI of the UCLA School of Medicine, which appointed Associate Dean,

Stanley Korenman, and a full-time staff member, Laurie Shaker-Irwin, Ph.D., to assist in data safety monitoring in all protocols administered through the CTSI. A committee consisting of at least four medical school faculty members will review the findings of the DSMC and make recommendations for additional action as needed.

Assessment and monitoring of suicidal risk will occur **at each visit and by phone between visits.** We have applied the standard procedures that are required and approved by the UCLA Institutional Review Board. We have employed the same procedures in our ongoing studies of late-life depression since 1995, and have had no suicide attempts among our subjects. We will monitor suicidal ideations using the third item of the Hamilton Depression Scale during each visit to the site. Monitoring for SI will be done at each visit independent of the outcome assessment. The blind rater will be instructed to inform the PI of scores ≥ 3. Dr. Lavretsky will be available for "on call" consultations to address concerns of worsening of suicidal ideations. He will also participate in the review of the dropouts due to suicidal ideations and other causes during DSMB meetings. Subjects with active suicidal ideations with or without a plan, HAMD-24 score ≥ 3, will be excluded from participation. We will call subjects between visits to monitor suicidal ideations. We will encourage participation of their family members in the study who will be encouraged to call the patient and the study staff in case suicidal ideations emerge during the study. In the presence of persistent suicidal ideations, subjects will be discontinued from the study and referred to clinical services or to the community for treatment.

7.5 Follow-up for Adverse Events

All subjects will be interviewed about their recent history of psychiatric and medical illnesses, psychosocial stressors, current medications and health status. In addition, vital signs including pulse rate, systolic blood pressure, and body weight will be obtained at each visit. The UKU Side Effect Rating Scale,[137] a comprehensive rating scale for monitoring adverse events (AE) in clinical trials, will also be completed at all follow-up visits.

7.6 Safety Monitoring

We will arrange for **Data Safety Monitoring Committee** meetings at approximately six month intervals. The DSMC will consist of one external and one internal member, Dr. Aaron Kaufman, an expert geriatric psychiatrist, Dr. Perry Hu- a geriatrician, and a statistician- methodologist Dr. Christina Kitchen. Both DSMC members have considerable experience in geriatric clinical issues. The DSMC will meet at least once every six months to review study progress. Members of the committee agree to keep all information confidential. Those members of the investigative group that are involved with the treatment and assessment of patients within the study will not review data that involve the comparison of outcomes (i.e., adverse events, treatment response, remission, etc.).

The Board will also issue a report annually to the PI and the IRB. The board will review summaries of the study progress to ensure that consent documentation is properly obtained and stored. They will also review progress in filling subject recruitment ethnic requirements. They will also determine whether study coordinators and investigators are collecting and organizing data properly. Key personnel will provide progress reports to facilitate this review. The board will also review any recent research relevant to the study. The summary reports will provide data on enrollment and adverse events. Additional support for these activities will be provided by the CTSI of the UCLA School of Medicine, which appointed Associate Dean, Stanley Korenman, and a full-time staff member, Laurie Shaker-Irwin, Ph.D., to assist in data safety monitoring in all protocols administered through the CTSI. A committee consisting of at least four medical school faculty

members will review the findings of the DSMC and make recommendations for additional action as needed.

8. INTERVENTION DISCONTINUATION

Subjects have the right to refuse to participate or to withdraw from the research study and discontinue participation at any time. Subjects will continue to be followed with their permission if study intervention is discontinued.

All subjects will be medically stable prior to entry into the study protocol and will be stable on psychotropic medications or ongoing psychotherapy for at least 4 months. However, if subjects engage in alternate treatments, they will be advised to inform the PI at every visit. If the alternate treatment is judged to interfere significantly with the intervention, the subject will be withdrawn from the interventions but followed naturalistically for 12 months. Subjects will be informed about an option of pursuing clinical care in the community.

Subjects who do not improve or get worse during acute intervention phase or relapse during the continuation phase will be offered treatment outside the protocol in the Geriatric Psychiatry Clinics, or other community clinics. They will also be invited to come for the 6 and 12 month follow-up to determine their progress and the patterns of response to treatment while controlling for any changes in treatment.

9. STATISTICAL CONSIDERATIONS

Statistical Analysis Plan. To clarify, for our primary analysis of assessing treatment effects, the GLMM model proposed will not include treatment adherence or time in practice. Once treatment effects are interpreted from our primary analysis, as a secondary analysis we will include these variables (i.e., treatment adherence or time in practice) as well as their interactions with treatment into the model, to assess whether the confirmed treatment effects depend on (or vary as a function of) time in practice. In addition this dependence is allowed to vary between treatment groups. This is equivalent to correlating a continuous time in practice measure with the treatment effects within each group. Hence, this secondary analysis will lead to a separate interpretation than our primary analysis assessing treatment effects.

9.1 General Design Issues

We have tested all of the procedures in the pilot study, and are hopeful that the majority of participants will be able to tolerate the duration and complexity of the tests. All estimates of dropouts are based on our pilot experiences in several studies. If we find our estimates of adherence being different, we will factor those in the analyses. Other potential pitfalls are discussed in the Human Subject section.

9.2 Sample Size and Randomization

Power and sample size estimate: We assume that dropout will be at 10% and 20% at the end of 12 week treatment phase and 6 and 12 month follow-up, respectively. For Aim 1, if the treatment main effects are linear over the 12 week treatment phase on HAMD, our design with 7 time points (baseline, 2, 4, 6, 8, 10, 12 weeks) and with complete data on 99 subjects within each arm (10% dropout) will have 80% power at significance level α=0.05 for detecting a treatment and time interaction corresponding to no difference at baseline and a difference of d = 0.32 SDs between any two arm at the end follow-up. Our R21 project found an effect size of 0.65 in HAMD between TCC and HEW at 12 weeks. If treatment effects continue to grow linearly over the 6 and

12 month follow-up, our design with complete data on 88 subjects within each arm (20% dropout) will have 80% power at significance level α=0.05 for detecting a group difference between any two arms of d = 0.36 SDs at 6 and 12 month follow-up. The reported power is based on number of subjects with complete data, and hence is a conservative estimate of the actual power of our design, since the proposed GLMM framework utilizes all observed data collected from all subjects. In addition, the reported power analysis is based on a correlation of 0.5 between measurements obtained at different time points. Selection of the covariance structures within the GLMM model will be based on likelihood ratio test and/or AIC (Akaike Information Criterion). If we find either significant interactions or main effects, follow-up contrasts will be used to determine at which time points there has been significant change and/or differences in treatment effects relative to baseline.

For Aim 2 of detecting group differences in imaging biomarkers, our design with complete data on 55 subjects within each arm at the end of treatment will have 80% power at significance level α=0.05 for detecting a group difference between any two arms of d = 0.53 SDs. Our preliminary studies reported in Section 1.1.2 for antidepressant treatment response, found effect sizes of d = 0.57 for changes in BOLD response in bilateral occipital cortex and right VLPFC (faces affective reactivity task), in BOLD response in left DLPFC, premotor and bilateral posterior parietal regions (N-Back task) between baseline and end of treatment at 16 weeks. If TCC changes in bran function resemble those induced by psychopharmacological agents, our design will have enough power to detect group differences in imaging biomarkers.

9.3 Definition of Populations

Study population includes older adults (age 60 y.o. and older) with the current diagnosis of major depression without dementia who have been on stable treatment for at least 4 months, but still have moderate – severe depressive symptoms. ITT analyses will be on all the subjects who were randomized and who had baseline assessments. Per protocol population will be the subset of the ITT subjects who attended at least 80% of the sessions and who had no major protocol violations such as hospitalization, adverse events and stopping of medication.

9.4 Interim Analyses and Stopping Rules

We will not conduct interim analyses on primary outcomes.

- If a patient demonstrates worsening of their depressive symptoms by week 12 (i.e., CGI-I score > 4) of treatment, they will be discontinued from the study and referred for appropriate clinical services.
- Suicidal ideations with the HAMD item 3 score of 3 or greater
- Other SAEs.
- Engagement in alternative treatments interfering with the primary outcomes of the interventions

All dropouts will be analyzed by the reason for termination and the reasons will be classified as:

- a) Lack of efficacy; b) Side-effects; c) Lost-to-follow up; d) Hospitalization; e) Death; f) Other
- Relation to the study interventions: a) likely; b) probable; c) unlikely.

9.5 Outcomes

Measures and Outcomes: All instruments provide a comprehensive assessment of the severity of depression, health related quality of life, medical comorbidity, cognitive and functional

impairment, life satisfaction and quality of life and will enable the PI to compare results of the study to other investigations. Diagnosis will be determined on study entry. *All outcome measures will be administered at baseline, week 12, 6 months, and 12 months or upon early termination*. *Mood, anxiety, and safety measures will be administered at all visi*ts.

9.5.1 Primary Outcome

Primary outcomes include measures of depressive symptom severity. Primary outcomes will be measured and defined as follows: 1) continuous outcome of change in HAMD scores;

9.5.2 Secondary Outcomes

Secondary outcomes *include remission status, global improvement, cognition and memory domains, and fMRI neural correlates of working memory, and brain structure. Worsening of depressive symptoms and relapse to major depressive episode will be determined during 6 and 12 month follow-up visits. We will investigate whether variations in emotional regulation will moderate or predict emotional and functional improvement linked with TCC compared to the control condition*

Exploratory outcomes and biomarkers – multimodal MRI analyses, changes in gene expression and peripheral pro-inflammatory cytokines associated with clinical response to the interventions.

9.6 Data Analyses

Statistical Analysis Plan.

The study will include 220 subjects assigned to TCC/HEW in a 1:1 ratio, within each treatment arm. Prior to performing the primary analyses, descriptive statistics and graphical summaries will be obtained for the primary outcomes (HAMD-24 (Aim 1), connectivity in DMN in rs-fMRI and BOLD activation during the mood task in the anterior cingulate (Aim 2)) to check for outliers and violations of model assumptions and to assess the need for transformations or non-parametric methods. We will also plot each of the primary measures as a function of time to examine the form of the longitudinal trajectories. (HAMD-24 scores will be recorded every 2 weeks throughout the intervention.) Longitudinal analysis proposed will rely on generalized linear mixed models (GLMMs). GLMMs properly account for correlations induced by repeated measurements within subjects, allow for both fixed and time-varying covariates and automatically handle missing data, producing unbiased estimates as long as observations are missing at random. This allows the use of all available data from all subjects, thereby minimizing the effects of loss to follow-up. To make the most efficient use of our data we will fit a single model using all time points for each outcome. Our specific aims and hypotheses correspond to specific contrasts within those models. Demographic variables (e.g., age, gender, etc.) and variables characterizing the course of depressive symptoms (e.g., age of onset, chronicity, number of episodes, length of the current episode, etc.) will be compared by treatment group and examined in relation to outcome variables; this would define one possible set of appropriate covariates, if needed.

Aim 1: Primary analysis: Our primary analysis will consist of a GLMM for HAMD-24 with treatment group effects and time main effects, along with two-way interactions of time and group. The primary analysis will assess treatment effects at the end of treatment at 12 weeks. Our primary analysis is valid under the assumption of missing-at-random. To study missingness patterns, we will compare mean response trajectories of the primary outcome measure HAMD for subjects who dropped out and those who did not. We will further compare treatment groups for drop-out rates, time to drop-out and drop-out reasons. Since it is theoretically impossible to rule out data missing-not-at-random, we will also perform sensitivity analysis taking a pattern mixture approach. Our pattern mixture approach will make assumptions on how the outcome trajectory

for subjects who dropped out will differ from those for subjects who did not. These differences will be allowed to be vary across treatment arms and the amount of difference will be varied in a plausible range to obtain a full sensitivity analysis.

Secondary analysis: Treatment maintenance will be assessed via the GLMM at follow-up as part of the secondary analysis. Secondary analysis will also include comparison of remission status (binary outcome) at the end of treatment between treatment groups via a logistic regression. Analyses for secondary outcomes clinical global improvement (CGI) and cognitive domain scores in the executive function and memory domains will be similar to those above for the continuous HAMD scores. As noted in the Neuropsychological Assessment section, there are 4 cognitive domains that are being assessed. Of these, the two primary cognitive domains of interest for this proposal are executive function and memory. A separate GLMM as above will be estimated using these 2 domain scores as dependent variables. In addition treatment adherence and time in practice will be considered as moderators of treatment effects in the longitudinal modeling of primary depression and imaging outcomes in the first two aims.

Aim 2. Hypothesis 2a, b: Primary analysis: Similar to Aim 1, the primary outcomes of DMN connectivity in rs-fMRI and BOLD activation during the mood task in the anterior cingulate will be modeled with a GLMM model with treatment group and time as main effects, along with their two-way interaction. Significant interactions would suggest differential trajectories over time for activation and connectivity outcomes in the two treatment groups. Primary analysis will assess treatment effects at 12 weeks in the primary outcomes. Sensitivity to missing data will be studied as explained in detail in Aim 1. The outcomes of DMN connectivity in rs-fMRI and BOLD activation will be treated as co-primary endpoints and further we will carefully examine the effect size of each co-primary endpoint to ensure clinically meaningful treatment effect.

Secondary analysis: Secondary measures for Aim 2 will be BOLD activation during the cognitive task in hippocampus. Secondary analysis will include assessment of treatment maintenance effects on the primary and secondary outcomes and of treatment effects during treatment in the secondary outcome of BOLD activation during the cognitive task in hippocampus.

Exploratory Hypothesis 2c: Exploratory analysis: Exploratory variables will include hippocampal and anterior cingulate volume and white matter high intensity. To test hypotheses about the relationships between the imaging metrics and the primary outcomes in mood and cognition, we will derive domain summary scores for the 2 cognitive domains of interest, memory, and executive function. We will assess treatment moderator effects of baseline hippocampal and anterior cingulate volume and white matter high intensity on HAMD and memory and executive function domain scores. Statistical evaluation of moderators will be done by including the posited moderator variable as an independent variable and evaluating the moderator x treatment group interaction term in the GLMM models proposed in Aim 1. In addition we will assess the treatment mediator effects of change in DMN connectivity on change in HAMD and memory and executive function domain scores. Mediation effects will be assessed via multiple regression models, where the posited mediator variable is regressed on treatment, outcome is regressed on treatment and the outcome is regressed on the mediator and the treatment. For confirmation of the mediation effect, treatment effects need to be found significant on the outcome and the mediator as well as a decreased treatment effect size on the outcome in the presence of the mediator.

Exploratory aim 1: We will explore the difference in changes in gene expression and peripheral pro-inflammatory cytokines associated with clinical response to the interventions, by comparing the change in these markers between the two intervention groups and by examining how the change in these markers correlate with changes in depression and cognitive scores in the two groups.

9.7 Data Sharing & Future Research

Data from this study will be used for other ongoing or future research projects in the following ways:

- Share it with researchers in the U.S. or other countries for data analyses
- Use it to improve future protocol
- May be shared with the Sponsor

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

A blinded observer will perform outcome assessments. All key personnel involved in any aspect of data access and query must complete training on HIPAA and other good research practice modules as required by UCLA. Backup files archived on removable media and paper forms are stored in locked cabinets accessible only by appropriate personnel. We maintain confidentiality of all research subjects and follow the guidelines of the UCLA OPRS.
We will maintain the subjects' privacy in our data collection methods. The interviews will be conducted in a private interview room where conversations cannot be overheard by others in the Semel Institute building. All medical procedures, including fMRI will be conducted in a private examination room at the UCLA Semel Institute, CTRC and MPTF outpatient facilities, or the Brain Mapping Center.

The risk of breach of confidentiality is reduced by: 1) storing records in a locked file, with access available only to the PI and designated project staff; 2) removing identifying information from all data during the data analysis phase of the project; and 3) removing identifying information from all data presented publicly in lectures, seminars, or publications.

The Data Collections Forms are described in Appendix B: Table 3. Assessment Schedule. Additional NCCIH forms are listed below:

1. Visit Checklist
2. Documenting the Consent Process
3. Inclusion/Exclusion Core Form
4. Demographics Form
5. Medical History Form
6. Vital Signs
7. Physical Exam
8. Randomization and Enrollment Form
9. Concomitant Medication Form

10.2 Data Management

Data management: Data management and statistical support for this project, other than storage and processing of raw imaging files, will be provided by SIStat, the biostatistics core in UCLA's Semel Institute for Neuroscience and Human Behavior, (project co-investigator, Dr. Senturk). Dr. Siddarth will collaborate with Dr. Senturk on data analysis. SIStat staff will construct a secure customized web-based data collection and management system for this study incorporating the

subject registry, administrative tools and protocols, and a centralized database which will accommodate both direct data entry and electronic upload of summary files. The fMRI data will be stored in the computing cluster in the UCLA Brain Mapping Center (see Facilities) and processed by members of the Narr/ Lavretsky labs, who have specific expertise in the study paradigms; the resulting derived measures will be uploaded to the centralized database. Moreover, access to the data system, including the randomization program, is controlled via a hierarchical system of password protected logins, ensuring that study personnel can view only the parts of the system that are appropriate for their role. This helps to maintain blinding and ensure subject confidentiality. Careful security protocols will also be maintained prior to the entry of data into the online system. Specifically, paper copies of patient study forms will be stored in locked file cabinets and will be accessible only to authorized personnel. A shredder will be used to discard all unwanted study documents.

10.3 Quality Assurance

10.3.1a. Training in assessments

SOP: **Clinical Assessments.** All behavioral outcome assessments will be identical in all groups and performed by the trained raters blind to the treatment assignment. Inter-rater reliability for all instruments will be established at the beginning of the trial. All research associates will be trained by the PI, Dr. Ercoli, and Ms St Cyr on administration of all clinical study assessment including DSM-5R SCID interview. Inter-rater reliability will be established on all assessment instruments on the first 5 recruited subjects and rechecked every year for reliability to maintain reliability index high.

Neuropsychological Assessment. Psychometrists in the study will be taught how to administer the test battery by Dr. Ercoli or a designated post-doctoral geriatric neuropsychology fellow under Dr. Ercoli's supervision. The psychometrist will be observed in testing and scoring, and the work product will be reviewed. Each psychometrist is given feedback on areas of strengths and weaknesses, and a final overall rating as to readiness to start testing subjects.

10.3.1b. Training in administering interventions.

SOP:
Health and wellness education (HEW). Dr. Ercoli and trained study staff at both sites will implement HEW using a manual that will present educational information and describe learning objectives and patient activities to promote integration of material. The detailed protocol for Health Education has been developed and used in the pilot project. The HEW sessions will be held once a week for 60 minutes over the 12-week treatment period. We will follow a didactic format, inviting experts to lecture on key topics, followed by focused group discussion, and post-discussion self-help quizzes to assess patient learning.

SOP: Dr. Ercoli will provide weekly supervision for health education instructors to monitor consistency and adherence to the educational protocol.
Fidelity of Training: Our group will take the following steps to facilitate and ensure the fidelity of training. For the HEW condition, the co-investigator (Dr. Ercoli) meets with staff to learn the HEW training manual. During this meeting, the group will go over the material. Standard background readings are assigned to trainers on each topic in the training manual. Standard Readings on weekly HEW topics assigned for each session to teachers. Trainers receive education on other factors important to instilling belief in the effectiveness of the HEW training in subjects, including:

- How to develop rapport
- Using an effective presentation style (speech rate, volume, eye contact, how to facilitate subject participation in the groups)

- Addressing subjects' expectations for benefit—educating subjects so that they develop realistic expectations about how HEW condition can be beneficial.

Adherence and Quality control: After the training meeting, Dr. Ercoli will implement quality control assessment to determine each trainers' readiness for implementation of HEW. This will include mock presentations of the material on which trainers present to each other, and Dr. Ercoli, and are then rated (pass, needs improvement, and comments) on several dimensions including the material in the manual as well as the quality of presentation style. Presenters are given positive feedback and constructive criticism on their presentation. If trainers do not meet the 'pass' standard, will be given more time to practice and learn the material and will be re-reviewed. Finally, trainers will receive weekly supervision by Dr. Lavretsky used to monitor trainers' adherence to the intervention and protocol.

Tai Chi Chih. The Tai Chi Chih instructors will follow the same manual for this interventions. All instructors have been trained and certified by the Tai Chi Chih Association and its founder David Stone. We will contact Tai Chi Chih Association to send monitors or provide other ways of monitoring the TCC instructor's adherence to the manual protocol.

10.3.2 Quality Control Committee

Quality control will be conducted by the Data Safety and Monitoring Committee (DSMC) (see Monitoring 10.3.5).

10.3.3 Metrics

Data submission and quality control. Data forms will be delivered from the originating location to the central data unit within one week of being generated. When errors, omissions, or unclear information is detected on the paper forms, a copy will be returned for corrections. The data manager will generate monthly reports summarizing missing and incorrect data, and accrual report will be sent to the PI. Data entry will be facilitated by 1) having visual screen formats similar to the actual forms; 2) having range boundaries on each field, where appropriate, to be checked automatically as data is entered; 3) having default values incorporated into the data entry system to minimize typing.

10.3.4 Protocol Deviations

Protocol deviations will be captured, documented, and reviewed using the Concomitant Medications / Interventions Form, and UKU Side-effect scale, as well as any deviation from the protocol

10.3.5 Monitoring

Data submission and quality control. Data forms will be delivered from the originating location to the central data unit within one week of being generated. All consent forms and the assessment forms will be reviewed weekly during staff meeting for any missing data or errors. When errors, omissions, or unclear information is detected on the paper forms, a copy will be returned for corrections. The data manager will generate monthly reports summarizing missing and incorrect or incomplete data, range check and accrual report will be sent to the PI. Data entry will be facilitated by 1) having visual screen formats similar to the actual forms; 2) having range boundaries on each field, where appropriate, to be checked automatically as data is entered; 3) having default values incorporated into the data entry system to minimize typing. To ensure the accuracy of data entry, we will apply double-entry of each data point into the database. Dr. Siddarth will provide additional assistance with monthly monitoring of the out of range data entry.

In order to minimize missing data during the study, research assistants will be present to answer subjects' questions and to double check all self-reported questionnaires, making sure that all

items were completed. At the analysis stage, our primary analysis will consist of generalized linear mixed models (GLMMs) that are valid under the assumption of missing-at-random. To study missingness patterns, we will compare mean response trajectories of the primary outcome measure HAMD for subjects who dropped out and those who did not. We will further compare treatment groups for drop-out rates, time to drop-out and drop-out reasons. Since it is theoretically impossible to rule out data missing-not-at-random, we will also perform sensitivity analysis taking a pattern mixture approach. Our pattern mixture approach will make assumptions on how the outcome trajectory for subjects who dropped out will differ from those for subjects who did not. These differences will be allowed to be vary across treatment arms and the amount of difference will be varied in a plausible range to obtain a full sensitivity analysis.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document (Appendix A) and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study.

11.2 Informed Consent Forms

A signed consent form will be obtained from each participant (Appendix A). For participants who cannot consent for themselves, such as those with a legal guardian (e.g. person with power of attorney), this individual must sign the consent form. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy will be given to each participant or legal guardian and this fact will be documented in the participant's record.

11.3 Participant Confidentiality

All files will be kept in locked cabinets, as will copies of the signed informed consent forms to maintain the anonymity of participants and to bar any unauthorized access. The computerized database will be protected through the use of entry codes available only to authorized personnel.

In conjunction with the UCLA-NPI Human Subject Protection Committees (HSPC), as well as with the HSPC in all participating institutions, all legal and ethical safeguards for participants will be implemented. All participants will receive a copy of the Subject's Bill of Rights prior to giving consent to participate and will sign the Informed Consent form approved by the HSPC of UCLA-NPIH and affiliated institutions. They will receive a copy of the Patient Consent Form.

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be done using PIDs only. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, the FDA, the NCCAM, and the OHRP.

11.4 Study Discontinuation

The study may be discontinued at any time by the IRB, the NCCAM, the OHRP, the FDA, or other government agencies as part of their duties to ensure that research participants are protected.

12. COMMITTEES

Data Safety Monitoring Committee (DSMC): Regulatory safety monitoring

UCLA Institutional Review Board (IRB): UCLA-NPI Human Subject Protection Committees (HSPC), as well as with the HSPC in all participating institutions, all legal and ethical safeguards for participants will be implemented. All participants will receive a copy of the Subject's Bill of Rights prior to giving consent to participate and will sign the Informed Consent form approved by the HSPC of UCLA-NPIH and affiliated institutions.

13. PUBLICATION OF RESEARCH FINDINGS

A publication committee will be arranged and include the PI, and Drs Narr, Siddarth and Senturk who will consider interest from other investigators and trainees in publishing. Any presentation, abstract, or manuscript will be reviewed by the Committee prior to submission.

14. REFERENCES

1. Surgeon General Report on Mental Health in older adults. http://www.surgeongeneral.gov/library/mentalhealth/toc.html2004.
2. Report of the 2005 White House Conference on Aging. *The Booming Dynamics of Aging: From Awareness to Action*. http://www.whcoa.gov/2005.
3. Retooling for an Aging America: Building the Health Care Workforce In: IOM, ed. http://www.iom.edu/Reports/2008/Retooling-for-an-Aging-America-Building-the-Health-Care-Workforce.aspx2008.
4. Charney DS, Nemeroff CB, Lewis L, et al. National Depressive and Manic-Depressive Association consensus statement on the use of placebo in clinical trials of mood disorders. *Arch Gen Psychiatry.* 2002;59(3):262-270.
5. Thase ME. Achieving remission and managing relapse in depression. *J Clin Psychiatry.* 2003;64 Suppl 18:3-7.
6. Alexopoulos GS. Depression in the elderly. *Lancet.* 2005;365(9475):1961-1970.
7. Cho KL. Effect of Tai Chi on depressive symptoms amongst Chinese older patients with major depression: the role of social support. *Med Sport Sci.* 2008;52:146-154.
8. Lavretsky H, Irwin, M. R.,. Resilience and aging. *Aging health.* Perspective 2007;3(3):309-323.
9. Institute of Medicine of National Academics. *Retooling for an Aging America. Reducing suicide: A national imperative.* Washington, D.C.: National Academic Press; 2008.
10. Zajecka JM. Treating depression to remission. *J Clin Psychiatry.* 2003;64 Suppl 15:7-12.
11. Chi I, Jordan-Marsh M, Guo M, Xie B, Bai Z. Tai chi and reduction of depressive symptoms for older adults: a meta-analysis of randomized trials. *Geriatrics & gerontology international.* 2013;13(1):3-12.
12. Redwine LS, Tsuang M, Rusiewicz A, et al. A pilot study exploring the effects of a 12-week t'ai chi intervention on somatic symptoms of depression in patients with heart failure. *J Altern Complement Med.* 2012;18(8):744-748.
13. Lavretsky H, Alstein LL, Olmstead RE, et al. Complementary Use of Tai Chi Chih Augments Escitalopram Treatment of Geriatric Depression: A Randomized Controlled Trial. *American Journal of Geriatric Psychiatry.* 2011.
14. Eisenberger NI, Inagaki TK, Mashal NM, Irwin MR. Inflammation and social experience: An inflammatory challenge induces feelings of social disconnection in addition to depressed mood. *Brain Behav Immun.* 2010.
15. Eisenberger NI, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. An fMRI study of cytokine-induced depressed mood and social pain: the role of sex differences. *Neuroimage.* 2009;47(3):881-890.
16. Irwin M, Pike J, Oxman M. Shingles Immunity and Health Functioning in the Elderly: Tai Chi Chih as a Behavioral Treatment. *Evid Based Complement Alternat Med.* 2004;1(3):223-232.
17. Irwin MR, Pike JL, Cole JC, Oxman MN. Effects of a behavioral intervention, Tai Chi Chih, on varicella-zoster virus specific immunity and health functioning in older adults. *Psychosom Med.* 2003;65(5):824-830.

18. Irwin MR, Olmstead R, Motivala SJ. Improving sleep quality in older adults with moderate sleep complaints: A randomized controlled trial of Tai Chi Chih. *Sleep.* 2008;31(7):1001-1008.
19. Irwin MR, Olmstead R, Oxman MN. Augmenting immune responses to varicella zoster virus in older adults: a randomized, controlled trial of Tai Chi. *Journal of the American Geriatrics Society.* 2007;55(4):511-517.
20. Harmer CJ, Cowen PJ, Goodwin GM. Efficacy markers in depression. *Journal of Psychopharmacology.* 2010.
21. Clark L, Chamberlain SR, Sahakian BJ. Neurocognitive Mechanisms in Depression: Implications for Treatment. *Annual Review of Neuroscience.* 2009;32(1):57-74.
22. Robinson OJ, Sahakian BJ. A Double Dissociation in the Roles of Serotonin and Mood in Healthy Subjects. *Biological psychiatry.* 2009;65(1):89-92.
23. Aizenstein HJ, Butters MA, Wu M, et al. Altered functioning of the executive control circuit in late-life depression: episodic and persistent phenomena. *Am J Geriatr Psychiatry.* 2009;17(1):30-42.
24. Okada G, Okamoto Y, Morinobu S, Yamawaki S, Yokota N. Attenuated left prefrontal activation during a verbal fluency task in patients with depression. *Neuropsychobiology.* 2003;47(1):21-26.
25. Siegle GJ, Thompson W, Carter CS, Steinhauer SR, Thase ME. Increased amygdala and decreased dorsolateral prefrontal BOLD responses in unipolar depression: related and independent features. *Biol Psychiatry.* 2007;61(2):198-209.
26. Rogers MA, Kasai K, Koji M, et al. Executive and prefrontal dysfunction in unipolar depression: a review of neuropsychological and imaging evidence. *Neuroscience Research.* 2004;50(1):1-11.
27. Harvey P-O, Fossati P, Pochon J-B, et al. Cognitive control and brain resources in major depression: An fMRI study using the n-back task. *Neuroimage.* 2005;26(3):860-869.
28. Fitzgerald PB, Srithiran A, Benitez J, et al. An fMRI study of prefrontal brain activation during multiple tasks in patients with major depressive disorder. *Human Brain Mapping.* 2008;29(4):490-501.
29. Langenecker SA, Kennedy SE, Guidotti LM, et al. Frontal and limbic activation during inhibitory control predicts treatment response in major depressive disorder. *Biol Psychiatry.* 2007;62(11):1272-1280.
30. Matthews S, Simmons A, Strigo I, Gianaros P, Yang T, Paulus M. Inhibition-related activity in subgenual cingulate is associated with symptom severity in major depression. *Psychiatry Res.* 2009;172(1):1-6.
31. Wagner G, Sinsel E, Sobanski T, et al. Cortical inefficiency in patients with unipolar depression: an event-related FMRI study with the Stroop task. *Biol Psychiatry.* 2006;59(10):958-965.
32. Clark L, Chamberlain SR, Sahakian BJ. Neurocognitive mechanisms in depression: implications for treatment. *Annu Rev Neurosci.* 2009;32:57-74.
33. George MS, Ketter TA, Parekh PI, et al. Blunted left cingulate activation in mood disorder subjects during a response interference task (the Stroop). *J Neuropsychiatry Clin Neurosci.* 1997;9(1):55-63.

34. Thomas EJ, Elliott R. Brain imaging correlates of cognitive impairment in depression. *Front Hum Neurosci.* 2009;3:30.
35. Aizenstein HJ, Butters MA, Clark KA, et al. Prefrontal and striatal activation in elderly subjects during concurrent implicit and explicit sequence learning. *Neurobiology of Aging.* 2006;27(5):741-751.
36. Aizenstein HJ, Butters MA, Figurski JL, Stenger VA, Reynolds Iii CF, Carter CS. Prefrontal and Striatal Activation During Sequence Learning in Geriatric Depression. *Biological Psychiatry.* 2005;58(4):290-296.
37. Elliott R, Zahn R, Deakin JF, Anderson IM. Affective cognition and its disruption in mood disorders. *Neuropsychopharmacology.* 2011;36(1):153-182.
38. Sahakian B, Morein-Zamir, S. . Depression and resilience: Insights from cognitive, neuroimaging and psychopharmacological studies. In: Delgado M, Phelps, E., Robbins, T., ed. *Decision making, affect and learning Attention and Performance XXIII* Oxford: Oxford University Press; 2011.
39. Fales CL, Barch DM, Rundle MM, et al. Altered emotional interference processing in affective and cognitive-control brain circuitry in major depression. *Biol Psychiatry.* 2008;63(4):377-384.
40. Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol Psychiatry.* 2001;50(9):651-658.
41. Surguladze SA, Young AW, Senior C, Brebion G, Travis MJ, Phillips ML. Recognition accuracy and response bias to happy and sad facial expressions in patients with major depression. *Neuropsychology.* 2004;18(2):212-218.
42. Taylor Tavares JV, Clark L, Furey ML, Williams GB, Sahakian BJ, Drevets WC. Neural basis of abnormal response to negative feedback in unmedicated mood disorders. *NeuroImage.* 2008;42(3):1118-1126.
43. Hamilton JP, Gotlib IH. Neural Substrates of Increased Memory Sensitivity for Negative Stimuli in Major Depression. *Biological Psychiatry.* 2008;63(12):1155-1162.
44. Roberson-Nay R, McClure EB, Monk CS, et al. Increased Amygdala Activity During Successful Memory Encoding in Adolescent Major Depressive Disorder: An fMRI Study. *Biological Psychiatry.* 2006;60(9):966-973.
45. Lisiecka D, Meisenzahl E, Scheuerecker J, et al. Neural correlates of treatment outcome in major depression. *Int J Neuropsychopharmacol.* 2011;14(4):521-534.
46. Buckner RL, Andrews-Hanna JR, Schacter DL. The brain's default network: anatomy, function, and relevance to disease. *Ann N Y Acad Sci.* 2008;1124:1-38.
47. Mason MF, Norton MI, Van Horn JD, Wegner DM, Grafton ST, Macrae CN. Wandering minds: the default network and stimulus-independent thought. *Science.* 2007;315(5810):393-395.
48. Anand A, Li Y, Wang Y, et al. Antidepressant effect on connectivity of the mood-regulating circuit: an FMRI study. *Neuropsychopharmacology.* 2005;30(7):1334-1344.
49. Bluhm R, Williamson P, Lanius R, et al. Resting state default-mode network connectivity in early depression using a seed region-of-interest analysis:

decreased connectivity with caudate nucleus. *Psychiatry Clin Neurosci.* 2009;63(6):754-761.
50. Cullen KR, Gee DG, Klimes-Dougan B, et al. A preliminary study of functional connectivity in comorbid adolescent depression. *Neurosci Lett.* 2009;460(3):227-231.
51. Greicius MD, Flores BH, Menon V, et al. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. *Biol Psychiatry.* 2007;62(5):429-437.
52. Sheline YI, Pieper CF, Barch DM, et al. Support for the vascular depression hypothesis in late-life depression: results of a 2-site, prospective, antidepressant treatment trial. *Arch Gen Psychiatry.* 2010;67(3):277-285.
53. Li M, Chen K, Mo Z. Use of qigong therapy in the detoxification of heroin addicts. *Altern Ther Health Med.* 2002;8(1):50-54, 56-59.
54. Li JY, Zhang YF, Smith GS, et al. Quality of reporting of randomized clinical trials in tai chi interventions-a systematic review. *Evid Based Complement Alternat Med.* 2011;2011:383245.
55. Hamilton JP, Furman DJ, Chang C, Thomason ME, Dennis E, Gotlib IH. Default-mode and task-positive network activity in major depressive disorder: implications for adaptive and maladaptive rumination. *Biol Psychiatry.* 2011;70(4):327-333.
56. Anand A, Li Y, Wang Y, Gardner K, Lowe MJ. Reciprocal effects of antidepressant treatment on activity and connectivity of the mood regulating circuit: an FMRI study. *J Neuropsychiatry Clin Neurosci.* 2007;19(3):274-282.
57. Davidson RJ, Irwin W, Anderle MJ, Kalin NH. The neural substrates of affective processing in depressed patients treated with venlafaxine. *Am J Psychiatry.* 2003;160(1):64-75.
58. Frodl T, Moller HJ, Meisenzahl E. Neuroimaging genetics: new perspectives in research on major depression? *Acta Psychiatr Scand.* 2008;118(5):363-372.
59. Robertson B, Wang L, Diaz MT, et al. Effect of bupropion extended release on negative emotion processing in major depressive disorder: a pilot functional magnetic resonance imaging study. *J Clin Psychiatry.* 2007;68(2):261-267.
60. Kemp AH, Gordon E, Rush AJ, Williams LM. Improving the prediction of treatment response in depression: integration of clinical, cognitive, psychophysiological, neuroimaging, and genetic measures. *CNS Spectr.* 2008;13(12):1066-1086; quiz 1087-1068.
61. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct.* 2008;213(1-2):93-118.
62. Lorenzetti V, Allen NB, Fornito A, Yucel M. Structural brain abnormalities in major depressive disorder: a selective review of recent MRI studies. *J Affect Disord.* 2009;117(1-2):1-17.
63. MacQueen GM. Magnetic resonance imaging and prediction of outcome in patients with major depressive disorder. *J Psychiatry Neurosci.* 2009;34(5):343-349.
64. Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry.* 2004;161(11):1957-1966.

65. Kumar A, Bilker, W., Zhisong, J., Udupa, J. Atrophy and high intensity lesions: complimentary mechanisms in late-life depression. Presented at the Society for Neuroscience; 1998.
66. Lavretsky H, Kurbanyan K, Ballmaier M, Mintz J, Toga A, Kumar A. Sex differences in brain structure in geriatric depression. *Am J Geriatr Psychiatry.* 2004;12(6):653-657.
67. Lavretsky H, Lesser IM, Wohl M, Miller BL. Relationship of age, age at onset, and sex to depression in older adults. *Am J Geriatr Psychiatry.* 1998;6(3):248-256.
68. Lesser IM, Boone KB, Mehringer CM, Wohl MA, Miller BL, Berman NG. Cognition and white matter hyperintensities in older depressed patients. *Am J Psychiatry.* 1996;153(10):1280-1287.
69. Hamilton JP, Gotlib IH. Neural substrates of increased memory sensitivity for negative stimuli in major depression. *Biol Psychiatry.* 2008;63(12):1155-1162.
70. Qiu A, Taylor WD, Zhao Z, et al. APOE related hippocampal shape alteration in geriatric depression. *Neuroimage.* 2009;44(3):620-626.
71. Zhao Z, Taylor WD, Styner M, Steffens DC, Krishnan KR, MacFall JR. Hippocampus shape analysis and late-life depression. *PLoS One.* 2008;3(3):e1837.
72. Frodl T, Scheuerecker J, Albrecht J, et al. Neuronal correlates of emotional processing in patients with major depression. *World J Biol Psychiatry.* 2009;10(3):202-208.
73. Diaconescu AO, Kramer E, Hermann C, et al. Distinct functional networks associated with improvement of affective symptoms and cognitive function during citalopram treatment in geriatric depression. *Hum Brain Mapp.*32(10):1677-1691.
74. Tang YY, Lu Q, Geng X, Stein EA, Yang Y, Posner MI. Short-term meditation induces white matter changes in the anterior cingulate. *Proc Natl Acad Sci U S A.*107(35):15649-15652.
75. Xue S, Tang YY, Posner MI. Short-term meditation increases network efficiency of the anterior cingulate cortex. *Neuroreport.*22(12):570-574.
76. Kilpatrick LA, Suyenobu BY, Smith SR, et al. Impact of Mindfulness-Based Stress Reduction training on intrinsic brain connectivity. *Neuroimage.*56(1):290-298.
77. Abbott R, Lavretsky H. Tai Chi and Qigong for the treatment and prevention of mental disorders. *The Psychiatric clinics of North America.* 2013;36(1):109-119.
78. Kabat-Zinn J, Wheeler E, Light T, et al. Influence of a mindfulness meditation-based stress reduction intervention on rates of skin clearing in patients with moderate to severe psoriasis undergoing phototherapy (UVB) and photochemotherapy (PUVA). *Psychosom Med.* 1998;60(5):625-632.
79. Alexander CN, Schneider RH, Staggers F, et al. Trial of stress reduction for hypertension in older African Americans. II. Sex and risk subgroup analysis. *Hypertension.* 1996;28(2):228-237.
80. Hyman RB, Feldman HR, Harris RB, Levin RF, Malloy GB. The effects of relaxation training on clinical symptoms: a meta-analysis. *Nurs Res.* 1989;38(4):216-220.

81. Lavretsky H, Alstein LL, Olmstead RE, et al. Complementary use of tai chi chih augments escitalopram treatment of geriatric depression: a randomized controlled trial. *Am J Geriatr Psychiatry.* 2011;19(10):839-850.
82. Salzman C, Wong E, Wright BC. Drug and ECT treatment of depression in the elderly, 1996-2001: a literature review. *Biol Psychiatry.* 2002;52(3):265-284.
83. Flint AJ. Choosing appropriate antidepressant therapy in the elderly. A risk-benefit assessment of available agents. *Drugs Aging.* 1998;13(4):269-280.
84. Roose SP, Schatzberg AF. The efficacy of antidepressants in the treatment of late-life depression. *J Clin Psychopharmacol.* 2005;25(4 Suppl 1):S1-7.
85. Mulsant BH, Pollock BG. Treatment-resistant depression in late life. *J Geriatr Psychiatry Neurol.* 1998;11(4):186-193.
86. Salzman C. Psychiatry in the elderly, 2nd ed. . *Am J Psychiatry.* 2000;157(2):296-297.
87. Stone JF. *Tai-Chi-Chih! Joy through movement.* Boston, MA: Good Karma Publishing; 1996.
88. Lawlor DA, Hopker SW. The effectiveness of exercise as an intervention in the management of depression: systematic review and meta-regression analysis of randomised controlled trials. *Bmj.* 2001;322(7289):763-767.
89. Spitzer RL, Williams JB, Gibbon M, First MB. The Structured Clinical Interview for DSM-III-R (SCID). I: History, rationale, and description. *Arch Gen Psychiatry.* 1992;49(8):624-629.
90. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry.* 1960;23:56-62.
91. Guy W. Clinical Global Impressions (CGI). In: Services UDoHaH, ed. *ECDEU Assessment Manual for Psychopharmacology*. Rockville, Alcohol Drug Abuse and Mental Health Administration, NIMPH Psychopharmacology Research Branch1976:218-222.
92. Yesavage JA. Depression in the elderly. How to recognize masked symptoms and choose appropriate therapy. *Postgrad Med.* 1992;91(1):255-258, 261.
93. Hamilton M. The assessment of anxiety states by rating. *The British journal of medical psychology.* 1959;32(1):50-55.
94. Marin RS, Biedrzycki RC, Firinciogullari S. Reliability and validity of the Apathy Evaluation Scale. *Psychiatry Res.* 1991;38(2):143-162.
95. Montgomery GK, Reynolds NC, Jr., Warren RM. Qualitative assessment of Parkinson's disease: study of reliability and data reduction with an abbreviated Columbia Scale. *Clin Neuropharmacol.* 1985;8(1):83-92.
96. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 1989;28(2):193-213.
97. Kannel WB, Wolf PA, Garrison RJ, (eds). . The Framingham Study: AN Epidemiological Investigation of Cardiovascular Disease, Section 34. Paper presented at: National Heart, Lung, and Blood Institute1987; Bethesda, MD.
98. Truelsen T, Lindenstrom E, Boysen G. Comparison of probability of stroke between the Copenhagen City Heart Study and the Framingham Study. *Stroke.* 1994;25(4):802-807.

99. American Heart Association. Stroke Risk Factor Prediction Chart. *Dallas, TX.* 1990.
100. Ware JEJ, Kosinski M, Keller SD. *SF-36 physical and mental health summary scores: a user's manual.* Boston, MA: The Health Institute, New England Medical Center; 1994.
101. Connor KM, Davidson JR. Development of a new resilience scale: the Connor-Davidson Resilience Scale (CD-RISC). *Depress Anxiety.* 2003;18(2):76-82.
102. Endicott J, Nee J, Harrison W, Blumenthal R. Quality of Life Enjoyment and Satisfaction Questionnaire: a new measure. *Psychopharmacol Bull.* 1993;29(2):321-326.
103. Gleeson-Kreig JM. Self-monitoring of physical activity: effects on self-efficacy and behavior in people with type 2 diabetes. *Diabetes Educ.* 2006;32(1):69-77.
104. Lockwood KA, Alexopoulos GS, van Gorp WG. Executive dysfunction in geriatric depression. *Am J Psychiatry.* 2002;159(7):1119-1126.
105. Bhalla RK, Butters MA, Becker JT, et al. Patterns of mild cognitive impairment after treatment of depression in the elderly. *Am J Geriatr Psychiatry.* 2009;17(4):308-316.
106. Butters MA, Bhalla RK, Mulsant BH, et al. Executive functioning, illness course, and relapse/recurrence in continuation and maintenance treatment of late-life depression: is there a relationship? *Am J Geriatr Psychiatry.* 2004;12(4):387-394.
107. Alexopoulos GS, Kiosses DN, Klimstra S, Kalayam B, Bruce ML. Clinical presentation of the "depression-executive dysfunction syndrome" of late life. *Am J Geriatr Psychiatry.* 2002;10(1):98-106.
108. Wechsler D. *Wechsler test of adult reading.* San Antonio, TX: Psychological Corporation; 2001.
109. Lingjaerde O, Ahlfors UG, Bech P, Dencker SJ, Elgen K. The UKU side effect rating scale. A new comprehensive rating scale for psychotropic drugs and a cross-sectional study of side effects in neuroleptic-treated patients. *Acta Psychiatr Scand Suppl.* 1987;334:1-100.
110. Knopman DS, DeKosky ST, Cummings JL, et al. Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology.* 2001;56(9):1143-1153.
111. Lyketsos CG, Lopez O, Jones B, Fitzpatrick AL, Breitner J, DeKosky S. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA : the journal of the American Medical Association.* 2002;288(12):1475-1483.
112. Kressig RW, Beauchet O, Tharicharu J. [T'ai chi in the elderly: practical aspects]. *Rev Med Suisse Romande.* 2003;123(11):671-675.
113. van der Kouwe AJ, Benner T, Salat DH, Fischl B. Brain morphometry with multiecho MPRAGE. *Neuroimage.* 2008;40(2):559-569.
114. Hariri AR, Drabant EM, Weinberger DR. Imaging genetics: perspectives from studies of genetically driven variation in serotonin function and corticolimbic affective processing. *Biol Psychiatry.* 2006;59(10):888-897.

115. Hariri AR, Drabant EM, Munoz KE, et al. A susceptibility gene for affective disorders and the response of the human amygdala. *Arch Gen Psychiatry.* 2005;62(2):146-152.
116. Manuck SB, Brown SM, Forbes EE, Hariri AR. Temporal stability of individual differences in amygdala reactivity. *Am J Psychiatry.* 2007;164(10):1613-1614.
117. Foland LC, Altshuler LL, Sugar CA, et al. Increased volume of the amygdala and hippocampus in bipolar patients treated with lithium. *Neuroreport.* 2008;19(2):221-224.
118. Townsend J, Bookheimer SY, Foland-Ross LC, Sugar CA, Altshuler LL. fMRI abnormalities in dorsolateral prefrontal cortex during a working memory task in manic, euthymic and depressed bipolar subjects. *Psychiatry Res.* 2010;182(1):22-29.
119. Damoiseaux JS, Rombouts SA, Barkhof F, et al. Consistent resting-state networks across healthy subjects. *Proc Natl Acad Sci U S A.* 2006;103(37):13848-13853.
120. Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M. Learning sculpts the spontaneous activity of the resting human brain. *Proc Natl Acad Sci U S A.* 2009;106(41):17558-17563.
121. Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and modulatory interactions in neuroimaging. *Neuroimage.* 1997;6(3):218-229.
122. De Luca M, Beckmann CF, De Stefano N, Matthews PM, Smith SM. fMRI resting state networks define distinct modes of long-distance interactions in the human brain. *Neuroimage.* 2006;29(4):1359-1367.
123. Di Martino A, Scheres A, Margulies DS, et al. Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex.* 2008;18(12):2735-2747.
124. Ballmaier M, Narr KL, Toga AW, et al. Hippocampal morphology and distinguishing late-onset from early-onset elderly depression. *Am J Psychiatry.* 2008;165(2):229-237.
125. Narr KL, Thompson PM, Szeszko P, et al. Regional specificity of hippocampal volume reductions in first-episode schizophrenia. *Neuroimage.* 2004;21(4):1563-1575.
126. Yang Y, Raine A, Narr KL, Colletti P, Toga AW. Localization of deformations within the amygdala in individuals with psychopathy. *Arch Gen Psychiatry.* 2009;66(9):986-994.
127. Chiang MC, Dutton RA, Hayashi KM, et al. 3D pattern of brain atrophy in HIV/AIDS visualized using tensor-based morphometry. *Neuroimage.* 2007;34(1):44-60.
128. Gogtay N, Lu A, Leow AD, et al. Three-dimensional brain growth abnormalities in childhood-onset schizophrenia visualized by using tensor-based morphometry. *Proc Natl Acad Sci U S A.* 2008;105(41):15979-15984.
129. Hua X, Leow AD, Parikshak N, et al. Alzheimer's Disease Neuroimaging Initiative. Tensor-based morphometry as a neuroimaging biomarker for Alzheimer's disease: an MRI study of 676 AD, MCI, and normal subjects. *Neuroimage.* 2008 Nov 15;43(3):458-469.

130. Lee JS, Potter GG, Wagner HR, Welsh-Bohmer KA, Steffens DC. Persistent mild cognitive impairment in geriatric depression. *Int Psychogeriatr.* 2007;19(1):125-135.
131. Leow AD, Klunder AD, Jack CR, Jr., , et al. Longitudinal stability of MRI for mapping brain change using tensor-based morphometry. *Neuroimage.* 2006;31(2):627-640.
132. Schwarz C, Fletcher E, DeCarli C, Carmichael O. Fully-automated white matter hyperintensity detection with anatomical prior knowledge and without FLAIR. *Inf Process Med Imaging.* 2009;21:239-251.

15. SUPPLEMENTS/APPENDICES: Appendix A. Schedule of Evaluations

Assessment schedule	Screen/Base 1	Baseline2	Wk 2	Wk 4	Wk 6	Wk 8	Wk 10	Wk 12	Month 4	Month 5	Month 6	Month 12 (or Month 24)	COVID-19 One-time Assessment
Visit Number	**1-3**	**4**	**5**	**6**	**7**	**8**	**9**	**10-11**	**12**	**13**	**14-15**	**16-17**	**18**
Demographics / Medical History	X												
HAMD	X	X	X	X	X	X	X	X	X	X	X	X	X
MMSE	X							X			X	X	
SCID	X												
MADRS	X	X	X	X	X	X	X	X	X	X	X	X	X
GDS	X	X	X	X	X	X	X	X	X	X	X	X	

CGI (Severity & Improvement)			X	X	X	X	X	X	X	X	X	X	
HAM-A	X							X			X	X	X
Clinical Dementia Rating Scale	X							X			X	X	
Apathy Evaluation Scale	X							X			X	X	
UPDRS	X							X			X	X	
CIRS	X							X			X	X	
CVRF	X							X			X	X	
SF-36	X							X			X	X	
Q-LES-Q	X							X			X	X	
Brief Pain Scale	X							X			X	X	
The Pittsburgh Sleep Quality Index	X							X			X	X	
The Connor-Davidson Resilience Scale	X							X			X	X	
Neuropsychological Assessment	X							X			X	X	
Vital Signs: blood pressure	X							X			X	X	
Vital Signs: Weight	X							X				X	
The UKU Side Effect Rating Scale			X	X	X	X	X	X	X	X	X	X	

Clinical Procedure (fMRI, blood draw)	X							X				X	
EKG	X												
Monitoring of therapeutic expectations	x	x						X			X	X	
Homework Diaries/Notes			x	x	x	x	x	x					
COVID-19 Questionnaire													X