Clinical Intervention Study Protocol

Development of Pregnenolone as a Treatment for Depression

R61 Phase

Study Chairman or Principal Investigator:

E. Sherwood Brown, M.D., Ph.D.

Supported by:

The National Center for Complementary and Integrative Health

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Study Intervention Provided by:

MEDISCA

Sponsor of IND:

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Tool Revision History

Version Number: 1.0 (NCCIH protocol approval)
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Summary of Revisions Made: Initial approval issued by NCCIH.

Version Number: 1.1 (Draft version, pending NCCIH and IRB approval)
Version Date: 12/03/2019
Summary of Revisions Made:

- Addressed responses from the monitoring visit to improve clarity and consistency of the protocol.
- Request to extend the maximum visit window allowed between study visits.
- Clarifications to the data management process.
- Added UCLA Loneliness Scale (Russell, 1996).

Version Number: 2.0 (NCCIH protocol approval)
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Summary of Revisions Made: See summary for version 1.1.

Version Number: 3.0
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NCCIH Approval Date: (Approved draft 2.1 on 11/29/2020; converted to 3.0 for main version)
Summary of Revisions Made:

- Request to increase target enrollment to 31 instead of 26 per DSMB recommendation.
- Request to perform Visits 1, 3, 5, and Safety virtually or in-person depending on the status of COVID-19 pandemic and UT Southwestern research operations.
- Request to perform all assessments (exceptions: vital signs, ECG, blood draw, MRI, urine pregnancy and drug screen) virtually.
- Requesting to omit physical exam unless clinically indicated.

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PRÉCIS

Study Title

Development of Pregnenolone as a Treatment for Depression.

Objectives R61 Phase

Primary objective: Determine biosignatures sensitive to pregnenolone

- Determine if an increase in amygdala-PCC functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in dlPFC-insula functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in occipital GABA concentration ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo, in women with MDD.

Secondary objectives:

- Assess safety and tolerability of pregnenolone at the doses tested.
- Demonstrate increases in serum pregnenolone and allopregnanolone levels with pregnenolone administration (bioavailability).
- Identify a dose of pregnenolone that demonstrates bioavailability, tolerability and that is associated with a significant change in a biosignature.

Design and Outcomes

In this phase, 31adult women meeting criteria for Major Depressive Disorder (MDD) as defined in DSM 5, will be randomized to a double-blind, placebo-controlled, crossover phase I clinical trial of pregnenolone (i.e. each participant receives 500 mg/d, 800 mg/d pregnenolone and placebo in random order).

The study will consist of three 7-day treatment exposures to each pregnenolone dose and placebo (in random order) with a 14-day washout between each exposure. Baseline evaluation will include medical and psychiatric history, psychiatric interview, standard laboratory analyses (i.e., blood draw, ECG), and a brief cognitive battery. Neuroimaging will be collected after each study drug or placebo administration. Study drug tolerability and participant safety will be assessed throughout the study (6 in-clinic visits + a safety visit) using structured clinical interviews, self-report questionnaires, and standard laboratory analyses.

NCCIH R61 Study Diagram

Procedure	Baseline	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Safety Visit
Consent	X							
SCID-CV for DSM 5	X							
HRSD	X	X	X	X	X	X	X	X
SAFTEE	X		X		X		X	X
C-SSRS	X		X		X		X	X

UCLA Loneliness Scale	X		X		X		X	
Psychiatric Evaluation	X							
Physical Exam	X						X	
Blood draw	X		X		X		X	
Vital signs	X	X	X	X	X	X	X	X
ECG	X		X		X		X	X
Urine Pregnancy Test & Drug Screen	X							
Physician follow-up		X	X	X	X	X	X	X
MRI scan			X		X		X	
Pregnenolone administration		X		X		X		
Time	**~3 hr**	**~40 min**	**~2.5 hr**	**~40 min**	**~2.5 hr**	**~40 min**	**~2.5 hr**	**~1.5 hr**

Notes: SCID-CV – Structured Clinical Interview Clinician Version. HRSD – Hamilton Rating Scale for Depression. SAFTEE – Systematic Assessment for Treatment Emergent Events. C-SSRS – Columbia-Suicide Severity Rating Scale. ECG – Electrocardiogram. MRI – Magnetic Resonance Imaging.

Interventions and Duration

Pregnenolone, an over-the-counter supplement, is a naturally occurring neurosteroid made in the adrenal glands and brain. In the R61 phase, two different pregnenolone doses will be tested and compared to placebo in a double-blind, crossover design. Participants will be assigned to receive pregnenolone (500 mg/d, 800 mg/d) or placebo for 7 days beginning at Visit 1. The study will consist of three 7-day treatment exposures (in random order) to each pregnenolone dose and placebo with a 14-day washout between each exposure. The first 7-day treatment will begin at Visit 1, the second treatment at Visit 3, and the third treatment at Visit 5. An independent unblinded statistician (see Study Team Roster) not involved in data analysis will perform randomization to determine the order of treatment administration. The 14-day washout period between each treatment will serve to collect neurosteroid serum levels and neuroimaging biosignature.

Pregnenolone and capsules will be purchases from MEDISCA (Irving, TX) and supplied to the Abrams Royal Compounding Pharmacy (Dallas, TX). Abrams Royal Pharmacy is a PCAB accredited pharmacy and will prepare pregnenolone and placebo for encapsulation. The placebo will be identical in appearance to pregnenolone, and will be delivered by the pharmacy staff to the investigator's office.

Sample Size and Population

Twenty-six women (n=31) with MDD, who also meet additional eligibility criteria as outlined in Section 4, will be randomized into the study. A sample size of n= 31 would allow for > 25% attrition (much higher than expected) and still provide > 90% power with an $\alpha = 0.05$ in the R61 phase of the experiment. A sample size of 31 is adequate to detect within-group dose and condition differences with an effect size (Cohen's d) of about 0.82 (medium to large).

1. STUDY OBJECTIVES

1.1 Primary Objectives (biosignature determination)

- Determine if an increase in amygdala-PCC functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in dlPFC-insula functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in occipital GABA concentration ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo, in women with MDD.

1.2 Secondary Objectives

- Assess safety and tolerability of pregnenolone at the doses tested.
- Assess bioavailability of pregnenolone by demonstrating increases in serum pregnenolone and allopregnanolone levels with pregnenolone administration.
- Identify a dose of pregnenolone that demonstrates bioavailability, tolerability and that is associated with a significant change in a biosignature.

2. BACKGROUND AND RATIONALE

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

Pregnenolone, an over-the-counter supplement, is a naturally occurring neurosteroid made in the adrenal glands and brain. Preclinical research suggests pregnenolone has antidepressant, cognitive enhancing, and neuroprotective properties, particularly in women. Given the widespread availability of pregnenolone, as well as promising preclinical and clinical data, this trial proposes to further examine its use for depression. The proposed study will assess the ability of pregnenolone to engage a biosignature in people with major depressive disorder (MDD), as well as assess the safety and tolerability of pregnenolone. Additionally, this trial will provide needed information for further clinical research with this supplement that will be addressed in the R33 phase.

2.2 Study Rationale

Pregnenolone is an endogenous neurosteroid synthesized from cholesterol in the adrenal glands and brain. Pregnenolone serves as a precursor to downstream neurosteroids, including allopregnanolone, pregNANolone (we will use capital letters to distinguish it from pregnenolone), and dehydroepiandrosterone (DHEA). Although

pregnenolone is a readily available, over-the-counter (OTC) supplement, research on its use in humans is quite limited. Studies from the 1950's suggested pregnenolone was associated with improvement in irritability, insomnia, depression and anxiety (for a review, see Sells(1)). Recent studies suggest that pregnenolone may be useful for positive(2) and negative(3) symptoms of schizophrenia. In one of these studies, changes in the Brief Assessment of Cognition in Schizophrenia (BACS) correlated with increases in serum pregnenolone levels.(3)

Preclinical data suggest pregnenolone and other neurosteroids may be useful for MDD. Several lines of preclinical and clinical research suggest pregnenolone may be useful for mood and anxiety disorders. Chronic social isolation in rats is associated with anxiety and depression-like behaviors,(4) as well as reduction in cerebrocortical pregnenolone and allopregnanolone levels.(5) Administration of the pregnenolone metabolite, allopregnanolone, is associated with a reduction in anxiety behaviors in animal models.(6) A synthetic analogue of pregnenolone that specifically binds to microtubule associated protein 2 (MAP-2) receptor had potent antidepressant effects in the forced swim test,(7) as well as more persistent effects than fluoxetine in the isolation-rearing model that induces depression-related behaviors. Pregnenolone and its metabolites also have effects on microtubules,(8, 9) and other mechanisms relevant to depression including effects on N-methyl-D-aspartic acid (NMDA), gamma-amino butyric acid (GABA) and nicotinic receptors, acetylcholine, glycine, and voltage gated calcium and potassium channels.(10) Furthermore, major metabolites of pregnenolone are the GABAergic neurosteroids, allopregnanolone and pregNANolone.(11) Thus, pregnenolone and its metabolites act through mechanisms relevant to depression and anxiety. *Because GABAergic neurosteroids are produced by pregnenolone, and low levels of brain GABA are reported in MDD,(12) brain GABA changes that are consistent with an antidepressant response as biosignatures will be explored in this proposal.*

Human data also suggest pregnenolone may be useful for MDD: George et al. examined pregnenolone levels in the cerebrospinal fluid (CSF) of persons with mood disorders and healthy controls.(13) Patients with mood disorders had mean CSF pregnenolone levels 40% lower than controls. Lower pregnenolone levels appeared to be at least partially state dependent in that levels were 50% lower than controls in those *currently* depressed, but only 30% in those with a past mood disorder. Lower levels of serum pregnenolone were reported in anxiety disorders.(14)·(15) PI on the current application conducted two placebo-controlled pilot studies of pregnenolone in bipolar or unipolar depressive disorders. Both studies had positive findings (section 3.3.).(16) In the first study, 70 outpatients with unipolar (MDD) or bipolar depression were given pregnenolone (100 mg/d) or placebo for eight weeks. The pregnenolone group showed greater improvement in depressive symptoms than placebo group (section 3.3.a.). In the second study, 80 outpatients with bipolar depression were given pregnenolone (500 mg/d) or placebo for 12 weeks. The pregnenolone-treated group showed significant improvement in depressive symptoms as compared to placebo (section 3.3.b.). The

higher dose (500 mg) was associated with a larger effect size for depression improvement. In both studies, women demonstrated a more favorable response to pregnenolone, relative to placebo, than men. In addition, in the second study, women showed improvement in cognition with pregnenolone, and changes in serum pregnenolone and other neurosteroid levels showed very strong correlations with improvement in depressive and anxiety symptoms. Sripada et al. reported a single 400 mg pregnenolone dose by mouth was associated with an increased activity in the dorsal medial prefrontal cortex (dmPFC) relative to placebo, as well as changes in resting state functional connectivity (rsFC) between the amygdala and dmPFC, precuneus and hippocampus, as well as large increases in serum neurosteroid levels.(17, 18) We also collected rsFC data on pregnenolone and observed changes in connectivity consistent with an antidepressant effect in several brain regions (section 3.3.d.). Therefore, rsFC changes as biosignatures will be tested in this proposal.

Pregnenolone and downstream neurosteroids synthesized from it 1) act on neurotransmitters relevant to depression and anxiety, 2) demonstrate antidepressant and anxiolytic properties in animals, 3) have cognitive-enhancing properties that could be particularly useful in MDD, 4) show, based on our pilot data, promise in treating depression, particularly in women. Given the data above, we propose to develop pregnenolone as an MDD treatment in women. As such, pregnenolone would be the first antidepressant to demonstrate sex differences in treatment response. Findings from the study may increase industry interest in development of neurosteroid analogues for brain diseases in the same way that ketamine research has increased research and development in NMDA-receptor antagonists for depression.

Pregnenolone has a favorable safety and tolerability profile. The reported side effects in clinical trials using the 500 mg/day pregnenolone dose were primarily mild restlessness, mild muscle pain or stiffness, and mild coldness in the extremities (3). It is also theoretically possible that pregnenolone (due to its neurosteroid properties) could interact with oral contraceptive agents, thus women taking oral contraceptive medications containing progestin will be excluded from the study (or discontinued from the study if they initiate treatment with oral contraceptives post randomization). It is possible that other unforeseen risks of pregnenolone may exist. All participants will be carefully monitored at each study visit to ensure safety and to minimize any risks that may arise due to pregnenolone. Please refer to Section 7 for a full summary of potential risks.

3. STUDY DESIGN

In the R61 phase, 31 adult women with mild to moderate MDD (based on DSM-5 criteria) meeting inclusion and exclusion criteria will be enrolled in a double-blind, placebo-controlled, crossover phase I clinical trial of pregnenolone (i.e., each participant receives 500 mg/d, 800 mg/d pregnenolone and placebo in random order).

During Visit 1, participants will be started on either 500 mg of pregnenolone, 800mg of pregnenolone, or placebo (order randomized by a study statistician with no participant contact). Participants will continue taking study medication for 7 days before undergoing a 14-day washout period. The second treatment exposure will begin at Visit 3, and the third and final treatment at Visit 5. All visits will have a visit window (see Section 6.2.4) to accommodate participant schedule and MRI scanner schedule. The medication-free washout period will serve to collect neurosteroid serum levels and neuroimaging biosignature. Throughout the study, three total treatment exposures (7 days on/14-day washout) will take place (two pregnenolone doses and placebo).

The following endpoints will be evaluated in the R61 Phase of the trial:

Primary endpoints (biosignature determination):

- Determine if an increase in amygdala-PCC functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in dlPFC-insula functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in occipital GABA concentration ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo, in women with MDD.

Secondary endpoints:

- Assess safety and tolerability of pregnenolone at the doses tested.
- Assess bioavailability of pregnenolone by demonstrating increases in serum pregnenolone and allopregnanolone levels with pregnenolone administration.
- Identify a dose of pregnenolone that demonstrates bioavailability, tolerability and that is associated with a significant change in a biosignature.

All study procedures, with exception of neuroimaging will take place at the offices of the Psychoneuroendocrine (PNE) Research Program (BL8.224) in the Department of Psychiatry at UT Southwestern Medical Center, Dallas, TX over approximately a 2-year period. All neuroimaging procedures will take place at the Advanced Imaging Research Center (AIRC) at UT Southwestern, and will be performed by a licensed MRI technologist.

The approximate duration of study participation for an individual participant will be 9 weeks with additional 2 weeks reserved for unforeseen scheduling events. All participants will be given pregnenolone (2 x 250 mg or 2 x 400 mg capsules) and placebo at some point during the study (depending on the order of randomization). Oral encapsulated pregnenolone and placebo will be prepared by the Abrams Royal Compounding Pharmacy and distributed to the investigator by the pharmacy staff. The laboratory of Dr. Christine Marx at Duke University will perform the neurosteroid assays.

4. SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1 Inclusion Criteria R61

Potential participants must meet **ALL** of the inclusion criteria listed below to participate in this study. Following baseline visit, participants may be discontinued from

the study if they meet any of the criteria outlined in Section 8. Inclusion criteria are as follows:

- Women, ages 18-65 years, with current MDD (mild or moderate severity per DSM-5) based on SCID-CV.
- No psychotropic medications, other than PRN (as needed) hypnotics, within 28 days of randomization (medication free).
- PRN hypnotics allowed up to 3 days prior to study drug administrations but not while receiving study drug.
- Ability to understand study procedures and to comply with them for the entire length of the study.

4.2 Exclusion Criteria R61

Participants who meet **ANY** of the following exclusion criteria at baseline will not be eligible for participation in the study. Following baseline visit, participants may be discontinued from the study if they meet any of the criteria outlined in Section 8. Exclusion criteria are as follows:

- Severe MDD based on DSM-5 severity criteria and/or a baseline HRSD score > 27 (consistent with severe depressive symptom severity).
- High risk for suicide (active SI with plan/intent or > 2 lifetime attempts in lifetime or any in the past 6 months).
- Treatment resistant depression (fail two adequate antidepressant trials or ECT during current episode).
- Vulnerable populations (e.g. pregnant/nursing, severe cognitive or intellectual impairment, incarcerated).
- Coronary artery disease, atrial fibrillation, stroke, deep vein thrombosis, pulmonary embolism or blood clotting disorder, or any severe, life threatening or unstable, medical condition.
- History of allergic reaction or side effects with prior pregnenolone use.
- Current substance use disorder defined as meeting criteria for a use disorder based on the SCID interview and self-reported use within the past 3 months, or a positive baseline urine drug screen.
- Current psychotic features (hallucinations, delusions, disorganized thought processes) or eating disorders.
- Anxiety disorders of sufficient severity to be the primary focus of clinical attention (e.g. severe obsessive compulsive or post-traumatic stress disorders).
- Currently undergoing evidence-based cognitive behavioral therapy (CBT) treatment for depression.
- Hormone-sensitive conditions (i.e. breast cancer; uterine/ovarian cancer, endometriosis, uterine fibroids).
- Clinically significant laboratory, physical examination, or electrocardiogram (ECG) findings, including vital signs of diastolic blood pressure > 100, systolic blood pressure > 150, heart rate > 100 or < 50, temperature > 100.5 degrees F (38 degrees C).

- Currently using oral contraceptives containing progestin (barrier methods allowed)

4.3 Study Enrollment Procedures

Participants will be recruited through various forms of advertising available to the UT Southwestern researchers, specifically 1) paid and free advertising and 2) patients treated at UT Southwestern. IRB-approved flyers will be posted at various clinics affiliated with UT Southwestern, as well at the local mental health clinics (e.g., Dallas Metrocare Clinics), women's health centers, and community clinics. A weekly announcement regarding the study will be posted in "Campus Updates" – a free UT Southwestern weekly news service. Paid advertising may include newspaper and radio ads that the group has used in the past. Other free internet advertising sources may be utilized as needed.

UT Southwestern also has access to a Volunteer Research Participant Registry operated through the Clinical Trial Infrastructure at UT Southwestern. Registry volunteers are UT Southwestern patients who have agreed to be contacted with research opportunities. The registry will be used in the study. Additionally, the study will also be advertised to participants who have previously participated in other research studies and agreed to be contacted for future studies.

Physician referrals will be obtained using medical record review from under the IRB-approved HIPAA waiver. UT Southwestern has a large private practice Psychiatry clinic, as well as numerous other outpatient clinics, from which the physician referrals will be obtained.

Once participants have been identified, a brief phone interview will be held to see if the participant may be eligible for the study. The phone interview will include eligibility questions about the participants (e.g., age, psychiatric and medical history, concomitant medications, etc.). Participants who are determined "ineligible" via phone interview (pre-consent) will be noted on the "Screening Log" as ineligible and no further contact will be made.

If the participant seems eligible for the study, they will be scheduled for a Baseline visit, at which time a written informed consent will be obtained. In order to be eligible for the study, participants will be required to consent for themselves, thus a legal authorized representative will not be needed. Only IRB-approved study personnel will be eligible to consent participants under the direction of the PI.

Participants meeting the required inclusion criteria and none of the exclusion criteria, as determined by the PI or study clinician, will be randomized into the study. Any participants who sign a consent will be noted in the "Enrollment Log". If a participant is determined to be ineligible for the study post-consent, they will be considered a "screen fail" and an appropriate note will be made in the log.

Randomization will be performed by a statistician using a random number sequence (SAS Proc Plan). A randomization list will be maintained in a password protected folder by an unblinded staff member with no participant contact. In the R61 phase, a crossover design will be used so each participant will receive each of three study drug conditions in a random order. All tests will be 2-sided with $p< 0.05$ to assess significance.

5. STUDY INTERVENTIONS

5.1 Interventions, Administration, and Duration

Participants will receive oral encapsulated pregnenolone at two different dosages: 500 mg/day and 800 mg/day, as well as matched placebo that looks identical to pregnenolone. The order of treatment will be randomized for each participant (as described in section 9.2). Three treatment exposures will take place throughout the study, with each treatment lasting 7 days and followed by a 14-day washout period. Both pregnenolone and placebo will be encapsulated by the Abrams Royal Compounding Pharmacy and delivered to the PNE Research Clinic (BL8.224) by the pharmacy staff. An unblinded member of the research staff will dispense the investigational product during the participant's visit to BL8.224. Participants will be supplied 7-days of the product at each visit and additional 3 days of the product should the visit schedule change.

The participants will be asked to discontinue the pregnenolone at completion of the study. Although pregnenolone is available without a prescription, it does not appear ethical, given the current state of the knowledge regarding its effects, to recommend long-term treatment with this agent. The primary risk of discontinuing the study drug (assuming it is effective) is the risk of recurrence of depressive symptoms. The risks of continuing the pregnenolone long-term are not known.

1.2 Handling of Study Interventions

Pregnenolone will be purchased in bulk from MEDISCA (Irving, TX) and will be encapsulated and packaged by the Abrams Royal Compounding Pharmacy. The matching placebo will be prepared at Abrams Pharmacy as well. Both pregnenolone and placebo will be delivered to the investigator's office at BL8.224 by the pharmacy staff. See Appendix II for the Certificate of Analysis (CoA) for pregnenolone and Appendix III for sample pregnenolone and placebo capsules.

The ordering, receiving and tracking inventory will be recorded on an electronic document by the purchasing staff and made available to the research manager and investigator. The accountability log will indicate the date, amounts, batch numbers and conditions at the time of receipt. Both pregnenolone and placebo will be double-locked in the research manager's office (unblinded staff member) and will be dispensed to participants during their in-clinic visits.

The research manager will dispense the study product according to the randomization, and dosage according to the protocol of the study. Each occurrence of product dispensation will be documented on the product accountability log with the date, batch number, expiration date, participant ID number and the amount dispensed to the participant. The balance of the remaining product will be maintained and documented in the study's accountability log. At study completion, the remaining study product will be destroyed by UT Southwestern's Environmental Health and Safety Department according to guidelines for medication disposal. The amount of study product destroyed will be documented in the study's accountability log.

See Appendix I for a letter from Abrams Pharmacy describing the investigational drug (pregnenolone and placebo) preparation, storage, and quality assurance process.

5.3 Concomitant Interventions

5.3.1 Allowed Interventions

Any concomitant interventions are allowed, unless specifically outlined in the Exclusion Criteria and under 5.3.3 Prohibited Interventions.

5.3.2 Required Interventions

There are no required concomitant interventions for this study.

5.3.3 Prohibited Interventions

- Psychotropic medications within 28 days of randomization.
- Hypnotics taken as needed (PRN) are not allowed while receiving study drug, but are allowed up to 3 days prior to study drug administration.
- Chronic hypnotics are prohibited.
- Oral contraceptives containing progestin.

Participants who start psychotropic medications during the trial will be withdrawn from the study. Because the study has a short observation period and a crossover design we suggest that this is both a practical and scientifically justified approach.

5.4 Adherence Assessment

Adherence will be defined as greater than 80% of treatment intervention capsules taken. Study intervention will be provided to participants at Visit 1, Visit 3, and Visit 5. Adherence to a study regimen will be assessed by self-report and using pill counts at each study visit. Participants will be asked to bring their investigational drug bottle back with them at each study visit to assess adherence.

6. STUDY PROCEDURES

6.1 Schedule of Evaluations

Assessment	Baseline	Visit 1 (Randomization, Remote)	Visit 2	Visit 3 (Remote)	Visit 4	Visit 5 (Remote)	Visit 6	Safety Visit (Remote)
Informed Consent Form[a]	X							
Demographics[a]	X							
SCID-CV for DSM 5[a]	X							
Psychiatric Evaluation[a]	X*							
Medical History[a]	X*							
							X	
Concomitant Medications[a]	X	X	X	X	X	X	X	X
Urine Pregnancy Test & Drug Screen	X							
Vital Signs	X		X		X		X	
ECG	X		X		X		X	
Blood Chemistry (CMP, CBC with differential)	X						X	
Blood Chemistry (neurosteroid levels)	X		X		X		X	
Inclusion/Exclusion Criteria[a]	X							
Enrollment/Randomization[a]	X							
HRSD[a]	X	X	X	X	X	X	X	X
SAFTEE[a]	X		X		X		X	X
C-SSRS[a]	X		X		X		X	X
UCLA Loneliness Scale[a]	X		X		X		X	
MRI			X		X		X	
Clinician Follow-Up[a]		X	X	X	X	X	X	X
Treatment Administration (7 days)		X		X		X		
Adverse Events[a]		X	X	X	X	X	X	X

Note. These assessments may be postponed to Visit 1 depending on clinician and participant availability.

[a]*Note.* These assessments may be collected remotely due to COVID-19 safety precautions.

6.2 Description of Evaluations

6.2.1 Screening Evaluation

Consenting Procedure

With PI delegation, the research coordinator assigned to the study (see Study Team Roster for staff education qualifications) will obtain written informed consent at the Baseline visit. All research coordinators have received UT Southwestern-approved training in informed consent process, Human Subjects Protection, HIPAA, and Good Clinical Practices. Participants will be informed of the study procedures that will occur throughout the visit, including the reason the study is being conducted, the duration of the study, assessments and procedures performed at each visit, risks, benefits, payments and alternatives to participation. A PI or Co-I will be available to answer questions. In the event of any protocol changes, participants will be reconsented with the latest consent version. Documentation of signed consent will be kept in the participant's chart, which will be secured in locked offices.

Screening

UT Southwestern IRB has approved a partial waiver of informed consent for screening purposes. The participants will be screened over the phone after they have contacted us expressing an interest in participating in the study. General information about the study will be provided over the telephone. Interested participants, who appear to meet preliminary eligibility criteria, will be scheduled for a Baseline appointment for further evaluation and for obtaining informed consent.

6.2.2 Enrollment, Baseline, and/or Randomization

Enrollment

UT Southwestern IRB considers a participant "enrolled" in the study once they sign an IRB-approved written informed consent. This study will use the same definition of enrollment, and consider participants enrolled in the study once they sign an informed consent at the Baseline visit. Participants are unable to complete any Baseline assessments to determine study eligibility until they sign an informed consent, per UT Southwestern policy.

Baseline Assessments

Baseline assessments to determine eligibility (see Section 4 for criteria) will include. In order to maintain safety precautions during COVID-19 pandemic, all assessments may be done virtually (with exception of vital signs, ECG, urine pregnancy and drug screens, and blood draws):

- Informed Consent Form
- Demographics (self-report)
- Structured Clinical Interview Clinician Version (SCID-CV) for DSM *5*
- Psychiatric Evaluation performed by a study clinician*
- Medical History*

- Physical Examination* (only if clinically indicated in PI judgement; Epic medical record review will be used to collect medical history information).
- Concomitant Medications
- Urine Pregnancy Test & Drug Screen (UPT will be done only for women without a permanent method of birth control, such as hysterectomy or bilateral tubal ligation)
- Vital Signs (temperature, blood pressure, heart rate, respiratory rate, weight)
- Electrocardiogram (ECG)
- Blood Chemistry (Comprehensive Metabolic Panel with lipids, Complete Blood Count with differential)
- Blood Chemistry (neurosteroid levels: pregnenolone, allopregnanolone, pregNANolone, androsterone)
- Hamilton Rating Scale for Depression (HRSD, clinician-rated)
- Systematic Assessment for Treatment Emergent Events (SAFTEE, self-report)
- Columbia-Suicide Severity Rating Scale (C-SSRS, clinician-rated)
- UCLA Loneliness Scale, Version 3 (UCLA, self-report)

*These assessments may be performed at the second part of Baseline or at Visit 1 depending on clinician and participant schedule.

Other procedures performed in the study:

- Neuroimaging (Visit 2, 4, 6)
  - Structrual MRI scan (6 min)
  - Phase contrast (PC) MRI (30 seconds)
  - Resting State Functional MRI (5.5 min)
  - ^{1}HMRS data acquisition (17 min)

The MRI scan should fall on the last day of the study medication dose. In the event that MRI needs to be rescheduled or scheduled for a later date (within the allowable visit window), participants will be instructed on when to take the first medication dose to ensure that the 7th day dose falls on the day of the MRI scan.

Randomization (Visit 1)

If a participant meets all the inclusion criteria and does not meet any of the exclusion criteria at Baseline, as well as indicates willingness to participate in follow-up visits, she will be considered eligible for randomization, and will receive the first treatment course within 14 days of the Baseline visit (at Visit 1). If the Baseline visit needs to be split into two different visits due to scheduling constraints, then the 14-day window will be counted from the second part of the Baseline. A participant will be considered randomized and active in study once they receive the first course of treatment at Visit 1. Depending on the status of COVID-19 pandemic, Visit 1 may be performed remotely using virtual meeting platforms or in person. If Visit 1 occurs remotely, then the investigational drug will be mailed to the participant upon completion of the visit.

If a participant does not come back for Visit 1 within 14 days of Baseline, or is ineligible based on the inclusion/exclusion criteria, then they will be considered a screen fail and not randomized. A participant who does not come back for Visit 1 will be eligible for a rescreening at a future date (at least 30 days from the original Baseline visit). However, a participant who is considered a screen fail based on the inclusion/exclusion criteria will not be eligible for a rescreening.

6.2.3 Blinding

The participants, as well as investigators and research staff who have contact with the participants, will remain blinded to the group assignment. The blind is maintained by using identical procedures during each treatment exposure, as well as using placebo that is identical in appearance to pregnenolone. The randomization will be performed by an unblinded biostatistician (see Study Team Roster) not involved in data analysis. Additionally, the research manager will also be unblinded and will have access to the treatment assignment of participants. To maintain and assure protection of the blind, the research manager will store the randomization information (generated prior to recruitment) in a password-protected folder. The research manager will not be involved in participant interaction, data collection, entry, or quality checking.

The PI and Co-I will be responsible for authorizing any instance when the blind must be broken. The breaking of the blind will only be considered when knowledge of the treatment assignment is deemed essential for the participant's safety. If the blind needs to be broken during the study, one of the unblinded team members will break the blind and provide the information to the physician providing emergency care or the governing institution. The blind may need to be broken in medical emergency cases and/or situations where the IRB, FDA, DSMB or NCCIH are concerned about side effects or the safety of the participant. After the study is complete, the treatment assignments will be unblinded for statistical analyses.

6.2.4 Followup Visits

Baseline visit (~ 3 hours):

- Consent - 30 minutes
- Structured Clinical Interview for DSM 5, Clinician Version (SCID CV) to establish psychiatric diagnosis for inclusion purposes - 1 hour
- Psychiatric evaluation and physical exam performed by a study clinician - 30 minutes
- Safety assessments: HRSD, SAFTEE, C-SSRS (described in detail in the assessments section) - 30 minutes
- Self-report: UCLA – 5 minutes
- Blood work, vital signs, ECG, urine pregnancy test and drug screen - 30 minutes

Psychiatric evaluation and medical history may be postponed until Visit 1 depending on clinician availability and participant schedule. Baseline visit may be split in two visits to accommodate participant schedule.

In order to maintain safety precautions during COVID-19 pandemic, all Baseline assessments may be done virtually (with exception of vital signs, ECG, urine pregnancy and drug screens, and blood draws).

Visit 1 (~ 40 minutes). Depending on the status of COVID-19, Visit 1 may be conducted virtually (to maintain safety precautions) or in person:

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes
- Safety assessments: HRSD - 15 minutes
- At this visit (or within the allowable visit window) (and Visits 3 and 5), participants will begin a 7 day course of pregnenolone 500 mg, pregnenolone 800 mg, or placebo, which will be administered in a double-blind, crossover method. This means that all participants will receive a 7 day exposure to each dose of pregnenolone and placebo throughout the study (each exposure will begin at Visit 1, 3, and 5); however, the order of treatment administration will be random. Each 7-day treatment administration will be followed by a 14 day washout period. (Example order: 800 mg x 7 days; washout x 14 days; placebo x 7 days; washout x 14 days; 500 mg x 7 days). If the visit is conducted remotely, the investigational drug will be mailed to the participant.

Visit 1 must take place within 30 days from the Baseline visit in order for a participant to be randomized into the study. If necessary, Baseline visit may be split in two visits in order to accommodate participant scheduling and decrease participant burden. In the case 30 day window will be counted from the time of the second part of Baseline.

Maximum time allowed between Visit 1 and Visit 2: 14 days (allow +/-3 day window for rescheduling). Participants will be instructed on when to begin their medication, such that the last dose (7^{th} day) of the medication falls on the day of the MRI scan.

Visit 2 (~ 2.5 hours):

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes (done virtually or in person)
- Safety assessments: HRSD, SAFTEE, C-SSRS - 30 minutes (done virtually or in person)
- Self-report: UCLA – 5 minutes (done virtually or in person)
- Blood work for neurosteroid levels, vital signs, ECG - 30 minutes
- MRI scan - 1 hour

Maximum time between Visit 2 and Visit 3: 21 days (allow +/-5 day window for rescheduling)

Visit 3 (~ 40 minutes). Depending on the status of COVID-19, Visit 1 may be conducted virtually (to maintain safety precautions) or in person:

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes
- Safety assessments: HRSD - 15 minutes
- At this visit (or within the allowable visit window) (and Visits 3 and 5), participants will begin a 7 day course of pregnenolone 500 mg, pregnenolone 800 mg, or placebo, which will be administered in a double-blind, crossover method. This

means that all participants will receive a 7 day exposure to each dose of pregnenolone and placebo throughout the study (each exposure will begin at Visit 1, 3, and 5); however, the order of treatment administration will be random. Each 7-day treatment administration will be followed by a 14 day washout period. (Example order: 800 mg x 7 days; washout x 14 days; placebo x 7 days; washout x 14 days; 500 mg x 7 days). If the visit is conducted remotely, the investigational drug will be mailed to the participant.

Maximum time between Visit 3 and Visit 4: 14 days (allow +/-3 day window for rescheduling). Participants will be instructed on when to begin their medication, such that the last dose (7th day) of the medication falls on the day of the MRI scan.

Visit 4 (~ 2.5 hours):

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes (done virtually or in person)
- Safety assessments: HRSD, SAFTEE, C-SSRS - 30 minutes (done virtually or in person)
- Self-report: UCLA – 5 minutes (done virtually or in person)
- Blood work for neurosteroid levels, vital signs, ECG - 30 minutes
- MRI scan - 1 hour

Maximum time between Visit 4 and Visit 5: 21 days (allow +/-5 day window for rescheduling)

Visit 5 (~ 40 minutes). Depending on the status of COVID-19, Visit 1 may be conducted virtually (to maintain safety precautions) or in person:

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes
- Safety assessments: HRSD - 15 minutes
- At this visit (or within the allowable visit window) (and Visits 3 and 5), participants will begin a 7 day course of pregnenolone 500 mg, pregnenolone 800 mg, or placebo, which will be administered in a double-blind, crossover method. This means that all participants will receive a 7-day exposure to each dose of pregnenolone and placebo throughout the study (each exposure will begin at Visit 1, 3, and 5); however, the order of treatment administration will be random. Each 7-day treatment administration will be followed by a 14-day washout period. (Example order: 800 mg x 7 days; washout x 14 days; placebo x 7 days; washout x 14 days; 500 mg x 7 days). If the visit is conducted remotely, the investigational drug will be mailed to the participant.

Maximum time between Visit 5 and Visit 6: 14 days (allow +/-3 day window for rescheduling). Participants will be instructed on when to begin their medication, such that the last dose (7th day) of the medication falls on the day of the MRI scan.

Visit 6 (~ 2.5 hours):

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes (done virtually or in person)
- Safety assessments: HRSD, SAFTEE, C-SSRS - 30 minutes (done virtually or in person)
- Self-report: UCLA – 5 minutes (done virtually or in person)
- Physical exam, blood work for routine labs and neurosteroid levels, vital signs, ECG - 40 minutes
- MRI scan - 1 hour

Time between Visit 6 and Safety Visit: 14 days (allow +/-3 day window for rescheduling)

6.2.5 Completion/Final Evaluation

The final visit while receiving investigational drug is Visit 6. Participants who complete Visit 6 will be considered study "completers". Participants will continue to be monitored for safety purposes until their Safety visit, which will occur 14 days after Visit 6.

Safety visit (~ 1.5 hours). Due to COVID-19, Safety Visit will be conducted virtually to maintain safety precautions:

- Physician follow-up (includes review of concomitant medications and adverse events) – 20 minutes
- Safety assessments: HRSD, SAFTEE, C-SSRS - 30 minutes

ECG and vitals may be requested by PI if clinically indicated.

All procedures at the Safety Visit will be performed for safety purposes, and not data collection purposes. If necessary, participants will continue to be followed after the Safety Visit until safety concerns are adequately resolved and appropriate referrals have been made. Study participants may choose, at any time, to stop participating in the study. In the event that a participant chooses to do so, the study intervention will be stopped, and the participant will be asked to return for a Safety Visit as outlined above.

7. SAFETY ASSESSMENTS

Participants will be evaluated for safety during the course of study intervention using clinician follow-up, safety assessments (HRSD, C-SSRS, and SAFTEE), and clinical laboratory measures (ECG, vital signs, urine pregnancy test, and blood chemistry). Physical examination will be performed at Baseline and completion of the study. Additionally, participants will be given contact information for study staff and instructed to contact study staff in case any problems are experienced during the course of the intervention.

There are no known toxicities of pregnenolone recorded to date. The potential expected risks to the participant are outlined below:

- **Minor side effects.** Limited clinical trials evidence suggests mild restlessness, mild muscle pain/stiffness and mild coldness in the extremities as possible side

effects associated with pregnenolone (3). Side effects will be monitored using SAFTEE.

- **Risks to embryo, fetus or breast-fed infant.** Pregnant or breast-feeding females will be excluded from study participation. In the event of an unexpected pregnancy, the study participation will discontinue and the appropriate referrals will be arranged. All study participants will be required to have a negative urine pregnancy test prior to their participation, and will be asked to use a medically-acceptable birth control (contraceptives) during the study.
- **MRI risks.** There are no known risks from exposure to magnetic fields. However, participants may experience nervousness and/or anxiety due to the loud noise and claustrophobia. If such an event occurs, participants will be able to notify an MRI technologist immediately and stop their participation. Participants with MRI contraindications (e.g., metal implants) will be excluded from participation. An MRI eligibility screening form is completed by a participant and reviewed by an MRI technologist prior to each scan.
- **Pregnenolone interaction with oral contraceptive agents.** It is theoretically possible that pregnenolone, as a neurosteroid, could interact with oral contraceptive agents. Thus, as a precaution, females taking these medications will be excluded from the study participation.
- **Psychological stress.** Mild fatigue, anxiety and discomfort is possible due to the nature of questions included in study assessments. All study researchers have extensive experience in conducting assessments and interviewing participants, and will use breaks to minimize discomfort or fatigue.
- **Risks associated with blood draws** include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting are possible, though unlikely.
- **Risks associated with placebo.** Participants in the placebo group will not receive active medication for their depressive symptoms, and therefore there are risks associated with untreated clinical depression.
- **Risk of untreated depression and worsening of mood symptoms.** If participants are experiencing a worsening of mood symptoms, including active suicidal or homicidal ideations, then study participation will be discontinued and follow-up treatment will be arranged. Depressive symptoms and suicidality will be monitored using HRSD and C-SSRS, as well as through a Mental Status Exam completed by a licensed clinical provider.
- **Loss of confidentiality.** Any time when data are collected, there is a potential for loss of confidentiality. Every effort will be made to keep the information confidential (e.g., double locking participant binders, storing information in password-protected files), and only IRB-approved research personnel will have access to the identified information. Both research sites will use REDCap for the data entry and management purposes. REDCap is a secure and HIPAA

compliant data management system, which is backed up offsite nightly and hosted in a secure environment maintained by Information Resources at UT Southwestern.

It is possible that other study risks and side effects to pregnenolone may exist and are unknown at the time. If there are unforeseen risks that arise, they will be reported to the appropriate entities at per the DSMP. The risks outlined above are considered to be minimal and are addressed in the protocol and consent forms. A licensed clinical provider will monitor the risks at each study visit.

7.1 Specification of Safety Parameters

Potential study participants will be screened at the Baseline visit, prior to randomization, using several laboratory and physiologic measures. These measures will be used to screen out subjects with potentially serious diagnosed or undiagnosed medical or psychiatric conditions.

Laboratory measures:

Study participants must be within normal ranges (or the value must be determined as "not clinically significant" by a study clinician) on several blood tests, including comprehensive metabolic panel (CMP) and complete blood count (CBC).

Abnormal and clinically significant ECGs will be sent to the cardiologist at the Clinical Heart Center in the Department of Cardiology at UT Southwestern for interpretation and recommendations. Based on the information received from the cardiologist, the participant may be excluded or discontinued from the study.

If clinically indicated by the PI, a physical examination will be performed to exclude those with potentially serious undiagnosed clinical findings.

An MRI screening form will be completed by a participant and reviewed by an MRI technologist prior to each scan to exclude participants with any metal objects in their body or any medical conditions precluding a scan.

Psychiatric measures:

To exclude participants with clinically significant severe depressive symptoms potential participants with a baseline HRSD score > 27 will be excluded. Participants who have failed 2 or more adequate antidepressant trials (defined as 4 or more weeks at a therapeutic dose) will also be excluded.

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

Blood draw for CMP and CBC will be performed at Baseline and Visit 6. ECG will be done at Baseline, Visit 2, Visit 4, and Visit 6. Vital signs will be collected at Baseline and at each MRI scan. Urine pregnancy test (UPT) will be done at Baseline. If clinically

indicated, physical examination performed by a study clinician will be done at Baseline and Visit 6. An MRI screening form will be completed prior to each scan. A COVID-19 safety screener mandated by UT Southwestern will be done within 24-48 hours of each in-person visit.

In past two clinical trials (n=70 at 100 mg/day and n=80 at 500 mg/day) of pregnenolone in patients with Bipolar Disorder (BPD) or Major Depressive Disorder (MDD), no difference in side effect burden, as evaluated with a self-report side effect scale that assesses a variety of somatic symptoms, was observed with pregnenolone as compared to placebo. No adverse events classified as likely related to pregnenolone were observed in either study.

A clinical trial by Marx et al. in patients with schizophrenia (n=21) administered pregnenolone at the same dose as the current study (500 mg) and also reported excellent safety and tolerability in combination with psychotropic medications.(3) The only reported side effects with pregnenolone were mild restlessness, mild muscle pain or stiffness, and mild coldness in the extremities. No changes in TSH or blood glucose levels were observed with pregnenolone. Total cholesterol and LDL levels significantly decreased with pregnenolone, but the levels were not obtained in the fasting state. No ECG changes were observed. **Lipids will be collected as part of the CMP panel to see if a reduction in LDL and total cholesterol can be replicated. ECG will be obtained as a precautionary measure, although changes are not anticipated.** The GABAergic pregnenolone metabolite allopregnanolone has a benzodiazepine-like effect on sleep.(11) However, pregnenolone administration at the dosages that have been studied in humans do not appear to be associated with sedation or somnolence. Nonetheless, these symptoms will be monitored through both side effect and depressive symptom scales. There are no known drug-to-drug interactions with pregnenolone or its metabolites.

Further evidence of the safety and tolerability of pregnenolone comes from a study of 120 patients (n=38 women) with schizophrenia receiving 500 mg of pregnenolone or placebo for eight weeks.(3) The number of participants experiencing treatment-emergent adverse events was comparable between the placebo and pregnenolone groups (58% vs. 55%, respectively). Moderate-intensity adverse events were reported by 8.3% of participants in the placebo group and 13.3% of participants in the pregnenolone group. No participants in either group reported adverse events of severe intensity. Three participants in the pregnenolone group and none in the placebo group had serious adverse events (SAEs); no SAEs were related to pregnenolone. Study medication was discontinued in only one participant (secondary to reasons unrelated to study drug).

Studies of pregnenolone for arthritis from the 1950s provide longer-term and higher-dose safety data. McGavack gave pregnenolone with doses ranging from 400-1000 mg/day for a duration, in some cases, longer than one year with good safety and

tolerability.(19) Freeman et. al. also gave pregnenolone doses of 700-800 mg/day for up to 30 weeks with good tolerability.(20)

As mentioned above, pregnenolone appears to increase levels of progesterone. Marx et al. reported a mean increase in progesterone from 0.38±0.15 ng/ml to 2.17±0.64 ng/ml in patients with schizophrenia given pregnenolone for eight weeks at the dose proposed in the current application.(3) Eight of these nine participants were men. Thus, the impact of pregnenolone on progesterone in women is not well studied. The mean progesterone level achieved during pregnenolone administration in this study was higher than is typical for men (0.3-1.2 ng/ml), postmenopausal women (<0.7 ng/ml), or premenopausal women during the follicular phase (0.2-1.2 ng/ml), but was lower than during the luteal phase (3.3-25.0 mg/ml). Oral contraceptives containing the combination of progestin (a synthetic progesterone) AND estradiol were shown in the Women's Health Initiative, following several years of treatment, to increase the risk of deep vein thrombosis, pulmonary embolism, myocardial infarction, breast cancer, and dementia in postmenopausal women.(21) Therefore, this study excludes persons at an elevated risk, based on personal, and also excludes women over 65 years of age.

7.3 Adverse Events and Serious Adverse Events

An **adverse event (AE)** is any untoward medical occurrence in a subject during participation in the clinical study or with use of the experimental agent being studied. An adverse finding can include a sign, symptom, abnormal assessment (laboratory test value, vital signs, electrocardiogram finding, etc.), or any combination of these regardless of relationship to participation in the study.

A **serious adverse event (SAE)** is one that meets one or more of the following criteria:

- Results in death
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred)
- Results in inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect

An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

During each study visit, participants will be asked to report any potential side effects and the SAFTEE score will be reviewed by a clinician. Abnormal blood panels and ECGs will be monitored by a clinician and repeated as needed. In addition, participants will be asked the general question "Has anything else felt different since

you started the study medication?" in order to capture unknown side effects or AEs. A clinician will meet with a study participant at each visit and document the occurrence of any potential side effects. The side effects will be classified using the Adverse Event grading scale as outlined in the DSMP. All AEs will be documented in both the participant chart and the REDCap data capture system.

An SAE can also meet criteria for an **Unanticipated Problem (UP)**. The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.4 Reporting Procedures

All AEs will be initially reviewed by the PI and a determination will be made on the nature of the event. A DSMB will be established to monitor the safety of the trial and independently review the safety findings, as well as provide guidance to the PI regarding participant safety. Additional safety reports will be made to the IRB, NCCIH, and FDA per individual guidelines and as outlined in the DSMP.

The DSMB for this study is comprised of Richard Auchus, MD, PhD (endocrinologist), Robin Jarrett Ph.D. (clinical psychologist), and Thomas Carmody, Ph.D (biostatistician). Dr. Jarrett will chair the DSMB. Drs. Auchus, Jarrett, and Carmody are not associated with this research project and work independently of the PI, E. Sherwood Brown, MD, PhD. They are not part of the key personnel involved in this grant. No member of the Committee has collaborated or co-published with the PI within the past three years. They are qualified to review the patient safety data generated by this study because of their unique expertise.

Unanticipated problems reporting

- In accordance with UT Southwestern IRB reporting policy, unanticipated problems will be reported to the IRB within 5 working days of the investigator becoming aware of the event.

- Unanticipated problems that are serious adverse events will be reported to the DSMB, and NCCIH within 7 days of the investigator becoming aware of the event.
- Any other unanticipated problem will be reported to the DSMB and NCCIH within 14 days of the investigator becoming aware of the problem.
- All unanticipated problems should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and OHRP within one month of the IRB's receipt of the report of the problem from the investigator.

Serious Adverse Events (SAE) UT Southwestern IRB Guidance

Any SAE, including death to any cause, will be reported to the UT Southwestern IRB only if the event is considered an Unanticipated Problem Involving Risks to Subjects or Others (UPIRSO). UT Southwestern IRB reporting policy specifies that only events that are unexpected, and possible/definitely related to the study, and place a subject or other participants at a greater risk (thus meeting criteria for UPIRSO) need to be reported to the IRB. For example, an SAE (including, but not limited to, a death unrelated to the study or the study medication) will not need to be reported to the UT southwestern IRB. It will, however, be reported to the DSMB and NCCIH according to the specified guidelines, and will be reported to the IRB (along with any other AEs) during the annual continuing review. UT Southwestern will reject any reportable events that do not meet criteria for UPIRSO or warrant a substantial protocol change.

Adverse Events (AE) reporting for IND Studies

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to the DSMB, IRB, FDA, and NCCIH in accordance with requirements. For the IND:

- 7-day IND Safety Report (unexpected fatal or life-threatening AEs related to the intervention); a copy of the report sent to the FDA will be submitted to the NCCIH Program Officer and DSMB within 24 hours of FDA notification.
- 15-day IND Safety Report (any other serious and unexpected AE related to the intervention); a copy of the report submitted to the FDA will be submitted to the NCCIH Program Officer and DSMB within 24 hours of FDA notification.
- All other AEs documented during the course of the trial will be reported to NCCIH on an annual basis by way of inclusion in the annual report and in the annual AE summary which will be provided to NCCIH and to the DSMB. The DSMB Report will state that all AEs have been reviewed.

Reporting of Pregnancy

All participants will be required to have a negative pregnancy test prior to the study enrollment. In the event of an unexpected pregnancy, the study participation will be discontinued and the appropriate referrals will be provided to the participant. . Participants will be provided with contact information to the UT Southwestern and Parkland Hospital OBGYN clinics, as well as additional local resources as needed. If a participant elects to continue pregnancy, and with participant permission, she will be contacted at 3 month intervals (months 3, 6, and 9) to check the status of pregnancy, as well as at 3 months post-partum to collect information on pregnancy outcome. The pregnancy and the follow-up plan for the participant will be reported to the DSMB and NCCIH within 48 hours of PI awareness. The pregnancy will be reported to the UT Southwestern IRB if it also meets criteria for UPIRSO and requires a modification to the protocol.

7.5 Followup for Adverse Events

The PI will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. The study clinician, with PI delegation, will make the final determination on whether an AE/SAE is considered resolved.

7.6 Safety Monitoring

The NCCIH requires that all Human Subjects research studies undergo independent monitoring, and NCCIH Program Officials will provide specific guidelines to the PI for the study.

8. INTERVENTION DISCONTINUATION

Study intervention may be discontinued for participants for any of the following reasons:

- Development of any exclusion criteria for the study while participating in the study (e.g., development of severe MDD).
- Development of active suicidal or homicidal ideation with plan and intent, clear and progressive worsening of psychiatric symptoms that in the opinion of the investigator makes continued care within the research study unsafe.
- Development of psychotic features (hallucinations, delusions, disorganized thoughts)
- Development of severe or life-threatening medical condition, pregnancy or psychiatric hospitalization.
- Withdrawal of informed consent by the participant.
- Opinion of the investigator that continued participation is unsafe or unwise.
- Participants who start psychotropic medications during the trial will be withdrawn from the study. Because the study has a short observation period and a crossover design we suggest that this is both a practical and scientifically justified

approach.

Participants will continue to be followed with their permission if study intervention is discontinued. Participants discontinued prior to Visit 6 will be asked to return for a Safety Visit. Participants who meet discontinuation criteria at Visit 6 will return for the regularly scheduled Safety Visit within 14 days of study discontinuation.

At the Safety Visit, assessments will be given based on their clinical relevance to the discontinuation criteria met by the study participant, in addition to a doctor visit, routine vital signs, AE review, and side effects scales. Clinically significant abnormal labs will be repeated at the Safety Visit, if necessary, and participants will continue to be followed until safety concerns are adequately resolved and appropriate referrals have been made.

Study participants may choose, at any time, to stop participating in the study. In the event that a participant chooses to do so, the study intervention will be stopped and the participant will be asked to return for a Safety Visit as outlined above.

Due to the short length of each treatment exposure (7 days), no temporary discontinuations will be allowed. In the event of participant discontinuation, no further evaluations will be performed for outcomes, only the safety measures as indicated above.

9. STATISTICAL CONSIDERATIONS

9.1 General Design Issues

The R61 phase involves a randomized, double-blind, cross-over design of 500 mg/d pregnenolone, 800 mg/d pregnenolone, and placebo for seven days with a 14-day washout between each exposure in 31 women with MDD.

Hypothesis: Pregnenolone is associated with improvement in depressive symptom severity in women that is associated with changes in rsFC and GABA.

Primary Study Aims R61 Phase (biosignature determination):

- Determine if an increase in amygdala-PCC functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in dlPFC-insula functional connectivity ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo in women with MDD.
- Determine if an increase in occipital GABA concentration ($d \geq 0.5$) is observed with pregnenolone, as compared to placebo, in women with MDD.

Secondary Study Aims R61 Phase:

- Assess safety and tolerability of pregnenolone at the doses tested.
- Assess bioavailability of pregnenolone by demonstrating increases in serum pregnenolone and allopregnanolone levels with pregnenolone administration.

- Identify a dose of pregnenolone that demonstrates bioavailability, tolerability and that is associated with a significant change in a biosignature.

9.2 Sample Size and Randomization

Data from pooled randomized, controlled trials of how standard depression therapies influence proposed neuroimaging biosignatures (GABA) in a depressed population, and our rsFC connectivity data with pregnenolone, suggest that a sample size of n=31 would allow for > 25% attrition (higher than expected), and provide > *90% power* with an $\alpha = 0.05$ in the R61 phase of the experiment.(22) A sample size of 31 is adequate to detect within-group dose condition differences (500 mg vs. 800 mg vs. placebo) with an effect size (Cohen's *d*) of about 0.82 (medium to large) in the first phase. Thus, each pregnenolone dose will be compared to placebo separately. The reported *d* for changes in occipital GABA with depression treatment ranges from 1.28 to 1.59. The rsFC increase with pregnenolone, as compared to placebo, between the amygdala (seed region) and PCC in our pilot had an effect size of $d = 3.28$.

Randomization will be performed by an independent unblinded statistician (see Study Team Roster) not involved in data analysis prior to recruitment using a random number sequence (Statistical Analysis System [SAS] Proc Plan). A randomization list will be maintained in a password protected folder by an unblinded staff member with no participant contact. See section 6.2.3 Blinding for more information regarding the unblinding procedures.

9.3 Definition of Populations

The intent-to-treat (ITT) sample will be defined as follows: if a participant completes visits with at least one MRI scan, then useable data will be obtained. There is no baseline MRI, but only an MRI after each condition. Thus, having one scan will define ITT. In the case of early dropouts, the last data point collected will be entered into analyses for that participant (e.g. data on one dose condition but not all conditions).

9.4 Interim Analyses and Stopping Rules

No interim analysis is planned for the R61 phase. The analysis will be conducted after the R61 phase is completed to determine whether the trial progresses to the R33 phase.

The DSMB in consultation with NCCIH and the PI may recommend a temporary suspension of enrollment and/or study intervention if pregnenolone is associated with adverse effects that occur with greater than anticipated frequency and severity, and call into question the safety of pregnenolone. If a temporary suspension is recommended, then a complete safety review will be conducted in order to determine whether the study should proceed per protocol, proceed with enhanced monitoring, be further investigated, be discontinued, or be modified and then proceed.

9.5 Outcomes

9.5.1 Primary Outcomes (biosignature determination)

1. Amygdala-PCC functional connectivity
2. dlPFC-insula functional connectivity

Changes in rsFC using a region of interest (ROI) approach for the following regions 1) amygdala (seed region) and PCC, and 2) dorsolateral PFC (dlPFC, seed region) and insula. ROIs will be anatomically defined using MarsBaR.

3. Occipital GABA concentration

LCModel software(23) will be used to analyze spectra using calculated (model) spectra of 20 metabolites as basis sets, and similar analysis will be conducted for preliminary ^{1}HMRS studies. Spectral fitting range will be set to 0.5 – 4.2 ppm. We will estimate GABA, and other metabolite, concentrations with respect to brain tissue water concentration (GM+WM) (24, 25), excluding CSF water. GABA and Glu will be measured using MEGA-PRESS difference spectra. NAA, Cr and Cho will be estimated using edit-OFF subscan spectra. GM, WM, and CSF fractions within ^{1}HMRS voxel will be estimated from T1-weighted images using the Statistical Parametric Mapping software.

9.5.2 Secondary Outcomes

4. **Tolerability.** Tolerability will be defined as no SAEs related to the study medication and no more than 10% of participants have a moderate or severe AE that is related to the product.
5. **Bioavailability.** Serum pregnenolone and allopregnanolone levels.
6. **Dose of pregnenolone.** Determine pregnenolone dose that demonstrates bioavailability, tolerability and that is associated with a significant change in a biosignature.

9.6 Data Analyses

Primary Aims:

To **determine biosignature(s)** sensitive to pregnenolone, scores on biosignatures will be compared between conditions using a random regression model with time and dose (500mg, 800mg, placebo) as within-subject factors using the PROC MIXED function. In determining statistical significance, Bonferroni corrections will be made to control for Type I error rates. Additionally, to explore the role neurosteroid levels have in the relationship between dose condition and biosignatures, separate mediational path models will be run. Specifically, following Preacher and Hayes' (2008)(26) bootstrap procedure, 5,000 bootstrap resamples will be examined using the Process macro (Hayes, 2013)(27) for SAS 9.4. In the path model, each biosignature will be regressed separately on to dose condition with neurosteroid levels included as a mediator (indirect effect). The following covariates will be included in this exploratory model: baseline scores on HRSD, age, depression severity, and depression duration.

95% confidence intervals will be examined and if the confidence interval does not contain zero, the effect will be considered statistically significant.

Secondary Aims: Tolerability

Tolerability will be defined as no SAEs related to the study medication and no more than 10% of participants have a moderate or severe AE that is related to the product. For dichotomous outcomes (e.g. drop out vs. completer) PROC NLMIXED will be used where dose and time will be added as within-subject factors and status as a dichotomous outcome variable.

Secondary Aims: Bioavailability

To assess **bioavailability** of pregnenolone and allopregnanolone, mean pregnenolone and allopregnanolone levels in each condition will be compared using a one-way repeated measures ANOVA. PROC ANOVA will be used to examine overall differences for each outcome, Bonferroni corrections will be made to control for Type I error rates, and Tukey's post hoc procedure will be used to examine pairwise comparisons for each condition.

Secondary Aims: Pregnenolone Dose

To identify a **dose of pregnenolone that demonstrates bioavailability, tolerability and that is associated with a significant change in a biosignature,** a one-way repeated measure multivariate analysis of variance (MANOVA) with dose as a within subject variable and exit biosignature engagement and overall tolerability (as assessed by total SAFTEE scores) as outcome measures will be performed using the MANOVA statement in the PROC GLM function in SAS 9.4.

Additionally, the results from the R61 phase will be used to determine whether the trial progress to the R33 phase. Using the results of these analyses, go/no-go rules will then be applied to determine if the study progresses to the R33 phase. The "go" criteria will be defined as meeting 2 of 3 of the biological signatures, with a medium effect size (defined as Cohen's $d \geq 0.5$) in the proposed direction.

Go:

- At least 2 of 3 biosignatures (amygdala-PCC, dlPFC-insula, occipital GABA concentration) achieve at least a medium effect size (defined as Cohen's $d \geq 0.5$) in the proposed direction (increased rsFC with fMRI, increased GABA with MRS). Biomarkers were selected based on their relevance to depression, antidepressant response and postulated pregnenolone (or active metabolite) mechanism(s) of action. A detailed justification of the biosignatures with literature references is provided in the Research Strategy (section 3.3.e.)

- If more than one biosignature achieves the proposed effect size, then the project proceeds to the R33 phase and includes any biosignatures reaching the proposed effect size. Two of three biosignatures need to be in the proposed direction. If one or two of the biosignatures is on the opposite direction of prediction, then this will result in a "no-go" signal.
- The dose (500 mg/d or 800 mg/d) with a go on biosignature must show adequate tolerability. Tolerability will be defined as no SAEs related to the study medication and no more than 10% of participants have a moderate or severe AE that is related to the product.
- If both doses of pregnenolone achieve the go threshold, including having adequate tolerability, (on either the same or different biosignature) then the dose with the largest effect size on a biosignature (defined using Cohen's *d*) on a biosignature will be selected for the R33 phase.

No-go:

- None of the go rules are achieved.
- A biomarker(s) achieves proposed effect size, but in the opposite direction of prediction.
- A biomarker(s) achieves proposed effect size, but tolerability is not adequate as judged by PI and DSMB. Tolerability will be defined as no SAEs related to the study medication and no more than 10% of participants have a moderate or severe AE that is related to the product.

10. DATA COLLECTION AND QUALITY ASSURANCE

10.1 Data Collection Forms

Data will be collected from each participant by the lead research coordinator. If the lead research coordinator is unavailable, the back-up research coordinator will complete the participant's study visit. All data collection and study visits will be held in BL8.224 (Psychoneuroendocrine Research Program Office at UT Southwestern). All the neuroimaging data collection will take place at AIRC.

When available, NCCIH templates (part of Clinical Research Toolbox) will be used to aid in data collection. These include the adverse events form, demographics form, documentation of informed consent, inclusion/exclusion criteria, medical history, physical exam, prior and concomitant medications, randomization and enrollment, serious adverse events form, study completion, and vital signs. The psychiatric evaluation and clinician follow-up will be performed by a PI or a study clinician and completed on forms created by the office staff to include specific data requirements for this study. Blood panel results will be received from Quest Diagnostics, Inc. via fax, signed and dated by a study clinician, and kept in the participant's binder. ECG results will be printed immediately, reviewed and signed by a study clinician, and added to the participant's binder as well.

The majority of study assessments will be given on standardized and validated paper forms or, where possible, entered directly into REDCap data collection system.

This includes the SCID-CV, HRSD, C-SSRS, SAFTEE, and UCLA loneliness scale. SCID-CV, HRSD, and C-SSRS are observer-rated and SAFTEE and UCLA is a self-report document.

Blood samples for routine labs (CMP and CBC) will be sent to Quest Diagnostics, Inc. for analysis. The blood for neurosteroid assays will be aliquoted into a 2mL cryovial and stored at McDermott Center for Human Growth and Development until the samples are ready to be shipped to Dr. Marx at Duke University. All blood samples will be labeled with a 4-digit unique identifier assigned to each participant. Quest Diagnostics also requires specific information to be provided in order to aid in sample processing. This information will include year of birth (but not date and month) and participant sex in addition to the 4-digit identifier.

Neuroimaging data will be stored on UT Southwestern servers as part of the standard AIRC protocol until the data are ready for analysis by the labs of Dr. Choi and Dr. Filbey.

All study documents will be kept in secure locations. Participant binders are double-locked will be kept in locked file cabinets behind locked office doors at all times. Electronic forms will be kept on password encrypted files that are backed-up to secure, encrypted, off-site servers maintained by UT Southwestern Medical Center.

10.2 Data Management

REDCap – a self-managed, secure, web-based solution that is designed to support data collection strategies for research studies – will be used in this study. This tool provides functionality and features enabling researchers to rapidly develop databases for collecting and managing research data. All data entered in REDCap will have an associated case report form kept in a participant's binder. The REDCap system will be set up by the study personnel at UT Southwestern, and will be validated by the UT Southwestern biostatistician (Jayme Palka, B.S.).

Data will be transcribed from source documentation into REDCap, and will be limited to questionnaire data (i.e., no protected health information). The transcribed data will be consistent with the source documents or the discrepancies will be explained in a note-to-file. All entries, corrections, and alterations will be made by the investigator or other authorized study personnel. The REDCap system maintains a full audit trail of data entry, data corrections, and data queries. Participant binders will be double-locked, and any electronic study logs will be kept in password-protected files that are backed-up to secure, encrypted, off-site servers maintained by UT Southwestern Medical Center.

The PNE Office at UT Southwestern Medical Center will be in charge of the majority of data collection and management. All study procedures, with the exception of the MRI scan, will occur at the PNE Office in private offices and exam rooms. ECGs for these visits will be performed at the P-CRI office of UT Southwestern Medical Center (BL6). The research coordinator will collect data and perform data entry for these visits.

Once the study visit has been completed, all assessments will be scored. The lead research coordinator will input each participant's data into their specific chart. Per PNE Program guidelines, the research coordinator must have scored and entered data for a study visit within, ideally, 2 business days of completion of said study visit.

Once data have been entered, the back-up research coordinator will validate the lead research coordinator's data. Chart validation consists of the back-up research

coordinator scoring the data again to ensure that the lead research coordinator's calculations and entry are accurate. Per PNE Program guidelines, chart validation must be completed within, ideally, 7 days of initial data entry (9 days after study visit), including resolution of any errors or discrepancies.

Participants will be identified in REDCap via a unique 4-digit participant ID assigned to all randomized participants at Baseline visit. No personal identifiers will be used in any publication or communication used to support this research study. The subject number will be used if it becomes necessary to identify data specific to a single subject. Personal identifiers will be removed from photocopied or electronic medical and research records.

10.3 Quality Assurance

10.3.1 Training

Staff members are already familiar with the assessments from prior or ongoing research projects. However, additional refresher training will be set up if needed on SCID-CV, HRSD, and C-SSRS and conducted by Dr. David Morris (Departmental Trainer).

Furthermore, a mock patient interview will be conducted using the HRSD to establish inter-rater reliability, and the intraclass correlation coefficients (ICC) will be calculated. Additional training will be provided, when needed, for individual raters with scores outside the acceptable range. The goal will be an ICC of at least 0.8. Inter-rater reliability training will be completed every 6 months.

In addition, staff members have completed training requirements for Human Subjects Protection, HIPAA and Good Clinical Practice. These courses have to be completed every 3 years as per UT Southwestern IRB policy.

The research coordinators associated with this study have been phlebotomy certified as a Certified Venous Access Specialist (CVAS) through Becksford Health Services, LLC. Renewals of phlebotomy certification will be obtained through Becksford Health annually.

10.3.2 Quality Control Committee

The PNE Quality Control Committee oversees all training and data maintenance to ensure the highest quality data is obtained across all study procedures. To do this, the Committee will perform regular reviews of study participant charts and electronic databases, as well as perform regular chart audits. Chart validation will be monitored via checklists that show the progress of chart completion. The committee will also ensure that all study staff remain updated on their trainings and certifications (e.g. CVAS phlebotomy, HIPAA, Conflict of Interest) in conjunction with the training committee.

10.3.3 Metrics

Inter-rater reliability on mood assessments will be measured using a mock patient interview that will be administered by the research coordinators. Scores will be compared using an ICC. The PNE Program standard is to achieve an ICC ≥ 0.8 on all mood assessments. Any mood assessment that results in an ICC below the goal will require an additional training to resolve any administration discrepancies.

10.3.4 Protocol Deviations

Protocol deviations will be avoided if possible. However, should deviations occur, they will be captured as they occur. Major protocol deviations will be documented using the online IRB Protocol Deviation Form and reviewed by the research coordinator and the PI. Major deviations will be reported to both NCCIH and the UT Southwestern IRB within 5 business days of the PI becoming aware of the deviations. Minor deviations will be summarized and reported to the UT Southwestern IRB at the annual continuing review.

10.3.5 Monitoring

Internal monitoring will occur on an ongoing basis and at least quarterly via chart audits, and will be supervised by the PI. NCCIH will also perform site monitoring visits. All materials and resources will be made available to NCCIH site monitors to provide a fully encompassing review of site performance and protocol compliance.

11. PARTICIPANT RIGHTS AND CONFIDENTIALITY

11.1 Institutional Review Board (IRB) Review

This protocol and the informed consent document (to be attached once final and approved) and any subsequent modifications will be reviewed and approved by the UT Southwestern IRB in accordance with the Federal Wide Assurance (FWA) on file with the Department of Health and Human Services.

11.2 Informed Consent Forms

A UT Southwestern IRB-approved written signed informed consent form will be obtained from each participant after full disclosure to the participant. Full disclosure means that the participant has been given sufficient information and time to understand the nature of the research project and risks that may be associated with the treatment. 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, and this fact will be documented in the participant's record. The research coordinator, with PI delegation, will obtain the informed consent and answer any questions about the study. The PI will always be available to answer any additional questions and provide more information about the study.

This research study will exclude vulnerable populations including pregnant or nursing women, the incarcerated, and people with severe cognitive disorders. Because many of the assessment tools have not been validated in other languages, non-English speaking individuals will be excluded. Participants with language limitations will be accommodated if the adaptations allow for clear understanding and completion of the assessments. The locations of the research study are all handicapped accessible. Children under the age of 18 and adults over the age of 65 are excluded from the study.

The consent forms are reviewed annually by the UT Southwestern IRB.

11.3 Participant Confidentiality

The research site follows the policies and procedures for maintaining participant confidentiality according to HIPAA. The participant records from the research study and the executed consent forms will be maintained in locked file cabinets in one of the PI's offices on campus. All data will either be maintained in locked file cabinets or in computers with password-protected access. Information will not be released without written permission of the participant, except as necessary for monitoring by IRB, DSMB, the FDA, the NCCIH, and the OHRP. Any data, specimens, forms, reports, and other records that leave the site will be de-identified by a participant identification number (PID) to maintain confidentiality.

Any email communication using identifiable participant information will utilize the UT Southwestern encryption system. During the consent process, all participants will be given the Notice of Privacy Practices (NPP) brochure that outlines the rights and responsibilities of UT Southwestern (hospitals, healthcare providers, research laboratories, and pharmacies) and the participant when dealing with the provision of protected health information related to care received and participation in research. After presenting the participant with the brochure, the staff members will collect a signature from the participant acknowledging receipt of the NPP brochure.

11.4 Study Discontinuation

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

12. COMMITTEES

Quality Control Committee: Alexandra Kulikova (chair).

The goal of the committee is to ensure that the highest quality of data is being obtained, collected, entered, and reported for all clinical studies. In addition, the chair ensures staff members understand and follow current protocol and updated consent forms. She also maintains and oversees data entry and validation. The quality control committee will audit reliability of assessment administration, chart validation, back-up training, protocol and consent form continuity.

Training Committee: Stephanie Reyes (chair)

The committee chair will assist with overseeing the training process for new PNE employees, interns and medical students. The chair will ensure that all training requirements are met by newly hired PNE employees, as well as current staff members. The chair will also schedule inter-rater reliability testing for the group every 6 months.

13. PUBLICATION OF RESEARCH FINDINGS

The findings of this research project may be presented at a national meeting and/or published in a peer-reviewed journal. Any presentation, abstract, or manuscript will be made available for review by NCCIH prior to submission

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15. SUPPLEMENTS/APPENDICES

Appendix I Abrams Pharmacy Letter of Support

ABRAMS ROYAL
compounding pharmacy

Lark Swofford RPh, FIfHI, FAARM
8220 Abrams Rd, Dallas, TX 75231
214-349-8000 (P) 214-341-7966 (F)
lark@arp-rx.com www.arp-rx.com

To all parties,

May 23, 2018

Thank you for allowing us to participate in your study. Abrams Royal Pharmacy is a PCAB accredited pharmacy, and as such, will be happy to provide you with any information you need on the formulation of your pregnenolone study medication and matching placebo.

All of the ingredients and capsules used for your study will come from FDA approved facilities within the United States and we will be happy to provide certificates of analysis on all ingredients and capsules. Our "regular" suppliers are PCCA, Medisca and Letco Medical.

Pregnenolone capsules (active medication) will contain the prescribed amount of pregnenolone and enough filler to fill the capsule to capacity. No added colors, additional fillers, glidants, etc will be added. We usually use either lactose or microcrystalline cellulose as fillers since they are both used as inert fillers in OTC and Rx medications. If you require a different filler, just let me know.

Placebo capsules (no active medication) will match the active capsules in color and size and will contain a filler only. Again, we usually use either lactose or microcrystalline cellulose, but if you need something different, we will accommodate.

We have several color choices available for the capsules, so you can pretty much pick your favorite. The size of the capsule will be determined by the strength of the active medication – higher strengths will most likely need to be encapsulated into larger capsules. If you have several strengths in your study and want all the capsules to look alike, regardless of strength, we can definitely work that out.

For quality assurance, we follow our standard operating procedures (SOP) for compounding which include electronic weighing/logs and calculation of theoretical vs. actual weight of the final product. USP guidelines allow for a (+/-)10% weight error in the final product, but our computer setup allows for only a (+/-)5% error and our lab average for final product weight is about (+/-)3%.

We assign a beyond use date (BUD) of 6 months on our capsules according to USP and this date cannot be extended. As such, we would rather make several batches for you along your study timeline than have you order too much medication and have to destroy it.

Please let me know what other information you need and I will be happy to supply it for you. On a personal note, I recommend pregnenolone quite a bit for my clients to help with memory, anxiety, PMS, hormone regulation, etc with nice results, so I'd be excited to see what you learn in your study in regards to depression.

Yours in health,

Lark Swofford RPh

PCAB ACCREDITED Compounding Pharmacy

Appendix II Certificate of Analysis

MEDISCA®
YOUR TRUSTED PARTNER IN COMPOUNDING

CERTIFICATE OF ANALYSIS

PREGNENOLONE (Micronized)

Batch/Lot Number : 140625

Manufacturing Date : 05/22/2016

Expiration Date : 04/30/2019

Retest Date : 04/30/2019

† All dates in this document are in format mm/dd/yyyy unless otherwise specified

TESTS	SPECIFICATIONS	RESULTS
ASSAY	>= 99.0 %	99.6 %
DESCRIPTION	White or almost white crystalline powder.	CONFORMS
SOLUBILITY	Very slightly soluble in water.	CONFORMS
IDENTIFICATION A	Melting Point: 185° - 194°C	189° - 192 °C ; POSITIVE
IDENTIFICATION B*	IR: Reference to standard spectrum	POSITIVE
SPECIFIC ROTATION	+27° to +30°	+29 °
LOSS ON DRYING	<= 0.50 %	0.15 %
HEAVY METALS	<= 10 ppm	<= 10 ppm
RELATED SUBSTANCES	<= 0.5 % (Individual impurities)	0.24 %
	<= 1.0 % (Total impurities)	0.41 %
TOTAL BACTERIAL COUNT	<= 1000 cfu/g	<= 1000 cfu/g
MOLD & YEAST	<= 100 cfu/g	< 10 cfu/g
SALMONELLA	Negative	CONFORMS
STAPHYLOCOCCUS	Negative	CONFORMS
E.COLI	Negative	CONFORMS
PSEUDOMONAS	Negative	CONFORMS
COLIFORM	<= 100 cfu/g	< 10 cfu/g
RESIDUAL SOLVENTS	Meets the requirements	CONFORMS
PARTICLE SIZE	To be reported	100 % < 74 µm 95 % < 48 µm
SOURCE	Yam	
PACKAGING AND STORAGE	Preserve in tight containers. at controlled room temperature. Protect from light.	

*TESTED ON 03/03/2017

The above mentioned product conforms to the manufacturer's specifications.

The above test results are a direct transcription of information provided to Medisca Inc. from the Certificate of Analysis provided by the manufacturer / supplier. Additional testing conducted by Medisca Inc. is represented by an asterisk.

Dated: 3/6/2017

Appendix III Sample Pregnenolone and Matched Placebo Capsules