STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Official Title: A Neurosteroid Intervention for Menopausal and Perimenopausal Depression

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The University of Texas Southwestern Medical Center at Dallas
Institutional Review Board

Protocol

NIH – National Institute of Aging
A Neurosteroid Intervention for Menopausal and Perimenopausal Depression

1. Introduction and Purpose:

Major depressive disorder (MDD) during menopausal transition affects a larger number of women; however, few clinical studies have examined treatments for depression and associated menopausal symptoms beyond prescription antidepressants and estrogen. The proposed clinical trial seeks to demonstrate the efficacy of pregnenolone as an antidepressant among women in the menopausal transition and to examine the effect of pregnenolone on somatic symptoms, cognition, and quality of life. The following specific aims are proposed:

Primary Aim:

1. Determine if pregnenolone is associated with greater reduction in depressive symptom severity than placebo in women with current menopausal MDD (mMDD).

Secondary Aims:

1. Determine if pregnenolone is associated with greater reduction in anxiety symptom severity than placebo in women with mMDD.
2. Determine if pregnenolone is associated with greater improvement in cognition than placebo in women with mMDD.
3. Determine if pregnenolone is associated with greater improvement in quality of life than placebo in women with mMDD.
4. Determine if pregnenolone is associated with greater improvement in vasomotor symptoms of menopause than placebo.

Mechanistic Aims:

1. Determine whether changes in neurosteroid levels with pregnenolone mediate clinical response.
2. Determine if baseline neurosteroid levels predict pregnenolone response.
3. Determine whether depressive symptoms, anxiety, sleep or vasomotor symptoms improve first.

Other Analyses:

- The safety and tolerability data (side effects scales, laboratory values, ECG data) will also be assessed.

2. Background:

Women are twice as likely as men to have major depressive disorder (MDD) (>20% lifetime prevalence). Across both genders, MDD in women may be more severe and lead to greater functional impairment compared to men. Women are particularly at risk for depressive symptoms during the menopausal transition (comprised of perimenopause and early menopause), as demonstrated consistently by epidemiological data and longitudinal studies supporting a higher rate of depression during menopause (15-18% prevalence) than pre-menopause (8-12%). Perimenopause, which typically starts after age 40, can last several years and into the period of early menopause, and is characterized by marked hormonal fluctuations, mood symptoms, and various somatic symptoms. There is, therefore, a several year span when women are at increased risk of MDD onset associated with this transition (menopausal depression, mMDD). Due to this particular window of vulnerability marked by hormonal variability, mMDD requires specific treatments to meet the needs of this particular population.

The primary treatments for mMDD are antidepressants with serotonergic activity and estrogen replacement therapy, either with or without concomitant antidepressant medication. Although women are often treated for MDD during the menopausal transition, there is a paucity of data to guide treatment tailored to this population. The limited available data suggest that estrogen may be effective for mMDD, but safety concerns greatly limit its use, and standard antidepressant treatments demonstrate relatively modest efficacy in this population. For this reason, studies of additional treatments for mMDD are needed.

Pregnenolone is an endogenous neurosteroid that is synthesized from cholesterol both in the adrenal glands and in the brain. Pregnenolone serves as a precursor to other downstream steroids, including cortisol, allopregnanolone, pregNANolone (capital letters used throughout to distinguish the spelling from pregnenolone), dehydroepiandrosterone (DHEA) and progesterone. Although pregnenolone is a readily available, over-the-counter (OTC) supplement, research on its use in humans is quite limited. We suggest that pregnenolone may be especially effective in women with mMDD based on the evidence that pregnenolone and downstream neurosteroids synthesized from it: 1) act on neurotransmitters relevant to depression and anxiety, 2) show antidepressant and anxiolytic properties in animal models, 3) may have cognitive-enhancing properties that could be particularly useful in mMDD, 4) based on our pilot data, show promise in treating depression, particularly in older women, 5) show a particularly robust response in women with baseline anxiety, fatigue or physical symptoms, all common in the menopause transition and 6) act on brain regions pertinent to menopause and perimenopause. In summary, this study seeks to demonstrate the efficacy of pregnenolone as an antidepressant among women in the menopausal transition, with expected improvement of secondary variables (e.g., cognition, quality of life, anxiety and vasomotor symptoms.) as well.

3. Concise Summary of Project:

A total of 144 (72 at each site: UT Southwestern and Massachusetts General Hospital, Center for Women's Mental Health (CWMH)), outpatient women with menopausal or perimenopausal depression and meeting inclusion and exclusion criteria will be enrolled in a double-blind, placebo-controlled, Sequential Parallel Comparison Design (SPCD) trial of pregnenolone. SPCD is a clinical trial design that may reduce the placebo response, improve signal detection, and maximize the ability to assess efficacy by dividing the trial into two phases (8 weeks each). At phase one, participants will be randomized 2:1 placebo to pregnenolone, and then at phase two, placebo non-responders will be re-randomized to receive either active or placebo treatment.
Figure 1. SPCD Layout

Drug treatment pregnenolone or placebo will be initiated on a fixed titration schedule starting at 50 mg twice daily (100 mg/day) for one week, 150 mg in the morning and 150 mg in the evening (300 mg/day) for one week, and then 250 mg in the morning and 250 mg in the evening (500 mg/day). If clinically significant side effects are reported with either active medication or placebo, based on their clinical judgment the protocol psychiatrist may stop or delay titration or decrease dose, in a double-blinded fashion.

The 16-week, double-blind treatment will be divided into two stages of 8 weeks each. Stage 1 will be 8 weeks (baseline to Week 8) and Stage 2 will be 8 weeks (Week 8 to Week 16), for a total of 16 weeks. During the first stage of SPCD, the focus is on comparing pregnenolone and placebo treatment across all participants. The second stage re-randomizes placebo non-responders to pregnenolone and placebo, and as such focuses on comparing pregnenolone and placebo in placebo non-responders only.

During Stage 1, participants meeting all eligibility criteria will be randomized to receive placebo or pregnenolone in a 2:1 ratio. More participants receive placebo in Stage 1 so that there is a sufficient number of participants who receive placebo to be randomized again in the second phase.

For the second 8-week stage, placebo non-responders in phase one will be re-randomized to receive pregnenolone or placebo with a 1:1 ratio. Placebo non-responders are defined as having < 50% decrease in their MADRS score between Stage 1 baseline and Week 8. All other participants

(Stage 1 participants on pregnenolone and placebo responders) will remain in the same group as assigned in the original randomization. A study psychiatrist and a research coordinator will reassess participants for 16 weeks (Baseline, Weeks 1, 2, 4, 8, 9, 10, 12, 16) on mood, neurocognitive, medication adherence, and safety measures. Weeks 6 and 14 will be done over the phone and will include MADRS only. Baseline, Week 8, and Week 16 visits will be done in-person. Virtual appointment options will be available for Week 1,2,4,9,10 visits. Vital signs and urine tests will not be collected for virtual appointments.

4. Study Procedures:

Individuals who contact the research clinic (BL8.224 or the Simches Research Building at MGH) will first be screened over the phone (short 15-minute interview) to determine initial eligibility and will be asked questions about their demographic information, medical and psychiatric history, symptoms, medications, allergies, substance use and smoking history. Individuals who appear to meet initial eligibility after completing the phone screen will be invited to come to the clinic for an in-person eligibility assessment visit (Baseline, Visit 1). Participants who sign the informed consent, meet the inclusion criteria and do not meet any of the exclusion criteria at Baseline will be randomized into the study within 2 days of the Baseline visit using the randomization protocol described in section 3 (Concise Summary of Project). While eligibility as determined by mood assessments, cognitive tasks, and some safety assessments (i.e., Section 7, “Clinically significant laboratory, physical examination) will take between 24-48 hours to be assessed. Once this is complete, the subject will be randomized by the unblinded statistician at UTSW.

If the subject initially seems eligible we will distribute her study medication to her in the Baseline V1, but will instruct her not to use the medication until their eligibility has been officially determined and she receives notification from the study team. If the study medication cannot be distributed to the study subject at V1, it will be sent to her via overnight mail.

The study participation will last 16 weeks, with reassessments during 48-96 hours post baseline (via phone), Weeks 1, 2, 4, 6 (via phone), 8, 48-96 hours post baseline (via phone), 9, 10, 12, 14 (via phone), and 16 (See Assessment Schedule Table below). All visits can be scheduled $\pm$ 2 business days of the timeline set on the assessment schedule. Participants will be paid $100 at Baseline, Week 8, Week 16, at UTSW ($50 at MGH), $50 at Weeks 1, 2, 4, 9, 10, 12, and $25 at phone calls on Weeks 6 and 14 (at UTSW only) with maximum compensation for the study being $650 at UTSW and $390 at MGH. DART transportation passes will also be provided to UTSW participants and parking vouchers for MGH participants.

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11	Visit12	Visit 13
Instrument	**Wk 0 BL & R**	**48-96 (+/-24) hr**	**Wk 1**	**Wk 2**	**Wk 4**	**Wk 6 (Phone)**	**Wk 8**	**48-96 (+/- 24) hrs Post Wk 8 visit (phone)**	**Wk 9**	**Wk 10**	**Wk 12**	**Wk 14 (Phone)**	**Wk 16**

		sPost baseline (phone)											
SCID, HRSD, TII, RLHQ (Module 1)	X												
MADRS	X	X	X	X	X	X	X	X	X	X	X	X	X
HRSA, PSQI, RLHQ (Module 3)	X		X	X	X		X		X	X	X		X
GCS	X		X	X	X		X		X	X	X		X
Cognitive tasks: RAVLT, TMT, CPFQ	X						X				X		X
MEN-QOL	X						X						X
Safety Assessments: SAFTEE, C-SSRS, Vital signs	X		X	X	X		X		X	X	X		X
UPT, UDS	X						X						X
Safety Assessments: Physical Exam	X						X						X
Safety Assessments: Routine labs, weight	X						X						X
Blood draw (Neurosteroid levels)	X						X						X
Psychiatric evaluation/check-in	X		X	X	X		X		X	X	X		X
Exit survey													X
Approximate visit duration	*3hr*	*20 mins*	*1.5hr*	*1.5hr*	*1.5hr*	*20 min*	*2hr*	*20 mins*	*1.5 hr*	*1.5hr*	*2hr*	*20min*	*2hr*
	SCID= Structured Clinical Interview for DSM-5 Clinician Version; HRSD= Hamilton Rating Scale for Depression; MADRS= Montgomery Asberg Depression Scale; HRSA= Hamilton Rating Scale for Anxiety; PSQI= Pittsburgh Sleep Quality Index; GCS= Greene Climacteric Scale; RAVLT= Rey Auditory Verbal Learning Test; TMT= Trail Making Test; CPFQ= Cognitive and Physical Functioning Questionnaire; MEN-QOL= Menopause Specific Quality of Life; SAFTEE= Systematic Assessment for Treatment Emergent												

	Events; C-SSRS= Columbia-Suicide Severity Rating Scale; UDS= Urinary Drug Screen; UPT= Urinary Pregnancy Test.

Table 1. Assessments Schedule

All assessments in this study have been validated for research and/or clinical use, with descriptions provided below:

Measures – Interviews/Questionnaires/Surveys:

Structured Clinical Interview for DSM-5 Clinician Version (SCID-CV) - brief structured interview for major Axis I disorders including mood, psychotic disorders, anxiety disorders, eating disorders and alcohol and substance use disorders. Administered at Baseline.

Montgomery Asberg Depression Rating Scale (MADRS) – 10-item observer-rated measure of depressive symptomatology designed for use in clinical trials, and will also serve as the primary outcome measure. Administered at every visit, **including** phone calls at 48-96 (+/- 24) hours post baseline and post week 8 visit and at weeks 6 and 14.

Hamilton Rating Scale for Depression (HRSD) – 17-item observer-rated measure of depressive symptom severity, which will be used at Baseline for inclusion purposes. Administered at Baseline.

Hamilton Rating Scale for Anxiety (HRSA) – 14-item observer-rated scale that assesses the degree and pathology associated with anxiety such as anxious mood, tension, fear, and insomnia. Administered at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

Pittsburgh Sleep Quality Index (PSQI) – 9-item self-report scale used to assess sleep quality and disturbances during the past week. Administered at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

Greene Climacteric Scale (GCS) – 21-item checklist providing an objective measure of mood disturbance, hot flushes, night sweats, and vaginal dryness. Administered at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

Menopause Specific Quality of Life (MEN-QOL) – 29-item measure used to assess the presence and bother associated with 29-different menopausal symptoms. Administered at Baseline, Week 8, Week 16.

Female Reproductive Lifecycle and Hormones Questionnaire (RLQH) Module 1 – a 6-item clinician-rated questionnaire designed to evaluate childbearing potential, menopausal status, and menstrual cycle. RLHQ Module 3 is a self-report menstrual cycle tracking form design to count menstrual cycle dates and track premenstrual/menstrual symptoms on each day. Module 1 will be administered at Baseline only. Module 3 will be administered at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

Treatment Impressions Inventory (TII) – 50-item self-report measure used to assess participant's feelings and impressions regarding their medical treatment. Administered at Baseline only.

Systematic Assessment for Treatment Emergent Events (SAFTEE) – 56-item self-report checklist used to assess study drug side effects and tolerability. Administered at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

Columbia-Suicide Severity Rating Scale (C-SSRS) – observer-rated measure for assessing suicidal ideation and risk in clinical trials. C-SSRS consists of subscales assessing severity of ideation, intensity of ideation, suicidal behavioral, and lethality. Administered at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

Measures – Cognitive/Behavioral:

The following cognitive assessments will be administered to participants during Baseline, Week 8, and Week 16:

Rey Auditory Verbal Learning Test (RAVLT) – an auditory assessment of verbal learning and memory, in which the participant is asked to recall a list of words first immediately following presentation (immediate recall) and later following a set period of time (delayed recall)

Trail Making Test (TMT) – a diagnostic tool measuring executive functioning and information processing in which the participant is asked to link a set of randomly distributed numbered and lettered points

Cognitive and Physical Functioning Questionnaire (CPFQ): The CPFQ is a 7-item self-report questionnaire that assesses possible cognitive and physical side effects.

At week 16 (or, in case of early discontinuation, the last study visit done by participant), an exit survey will be administered to evaluate participant's perception of in-person vs. virtual study visits.

Measures – Physiological/Other:

Blood draw – approximately 2.5 tablespoons of blood will be drawn at Baseline, Week 8, and Week 16 to assess routine laboratory values, including lipid levels (Comprehensive Metabolic Panel – CMP and Complete Blood Count – CBC), as well as neurosteroid levels (pregnenolone, pregnenolone sulfate, allopregnanolone, pregNANolone, progesterone, DHEA, dehydroepiandrosterone sulfate (DHEAS)). Additional 1.5 mL may be drawn if participant consents to DNA research participation. An additional laboratory value may be also conducted (per clinician judgment) to assess Follicle Stimulating Hormone (FSH) levels, to determine menopause eligibility.

Physical Examination – a study physician will perform a physical examination of the oral cavity, head, eyes, ears, nose, and throat, cardiovascular system, lungs, abdomen, extremities, skin, neuropsychiatric mental status and sensory/motor status, musculoskeletal system and general appearance during Baseline, Week 8, and Week 16.

Vital signs (blood pressure, temperature, heart rate, respiratory rate, weight) will be assessed at each in-person study visit.

Urine sample will be obtained to conduct a Urine Drug Screen (UDS) and a Urine Pregnancy Test (UPT), as per inclusion/exclusion criteria. UDS, and UPT will be done at Baseline, Week 8, , Week 16.

Study Adherence: Study adherence to the study medication will be assessed by pill count and asking the patient about the number of missed doses at the following visits: Baseline, Week 1,2,4,8,9,10,12,16.

5. Sub-Study Procedures:

With participant consent at Baseline visit, DNA will be obtained for future analysis. Plasma samples for DNA will be coded and sent to UT Southwestern McDermott Center for Human Growth and Development. Samples will have their DNA banked in a secure freezer with coded identification that will not include any personal identifiers such as the subject's name or date of birth.

6. Criteria for Inclusion of Subjects:

The participants must meet the following criteria:

- Women aged 40-67 years who are perimenopausal or early postmenopausal (within 5 years of the last menstrual period if not surgically postmenopausal), including:
    - Women who have experienced changes in menstrual cycle frequency or duration, and/or physical symptoms indicative of menopausal transition, as determined by clinician
    - Women who are using hormonal IUDs (i.e. brands Mirena and Skyla), with FSH level > 20 mIU/m (as menstrual periods are irregular with IUDs that utilize hormones, making irregular/absent periods difficult to assess as related to the menopausal transition).

- Women with significant menopause-related physical symptoms, indicated by ***any*** of the following criteria:
    - Greene Climacteric Scale total scores > 20
    - Greene Climacteric Scale sub-score for vasomotor symptoms >3
    - 5 or more bothersome hot flashes per week (self-reported)
- Women meeting DSM-*5* criteria for current major depressive disorder (assessed by the SCID)
- Baseline HRSD score of ≥ 18
- Participant agrees to abstain from disallowed medications (specified in the Excluded Concomitant Medication in Section 7) for the duration of the trial

7. Criteria for Exclusion of Subjects:

The participants must not meet any of the following criteria:

- Vulnerable populations (e.g. pregnant/nursing, severe cognitive or intellectual impairment, incarcerated)
- Pregnancy (determined by urine pregnancy test), intending pregnancy or breast feeding
- Psychiatric disorder other than MDD that is acute and the primary focus of symptom burden or treatment.
- History of bipolar disorder or psychotic disorder
- Current substance use disorder
- Positive baseline urine drug screen of an illicit substance (in this study: opioids and cocaine,) with the exception of a medication used with a prescription (use of a detected substance that is used with a prescription, such as an opioid pain medication, is not necessarily exclusionary and will be based upon judgment of the PI, particularly in the cases of chronic opioid use).. Participants are allowed to use THC up to one time per week as long as there is no evidence of cannabis abuse
- Current eating disorder
- Treatment resistant depression (failure of 2 adequate antidepressant trials or electroconvulsive therapy (ECT) during current episode; adequate antidepressant trials are defined as within the US FDA approved dosage for the medication and used for at least 6 weeks, with failure described by the patient as <50% improvement based on her subjective experience).
- High risk for suicidal acts including active suicidal ideation with plan and intent or > 2 suicide attempts in lifetime or any attempt in the past 6 months
- Use of selective estrogen-receptor modulators (SERMs), hormone replacement therapy, hormonal contraceptives (hormonal IUDs allowed), episodic sleep medications (chronic, regular, stable-dose benzodiazepines and hypnotics such as zolpidem, Sonata (Zaleplon), and Lunesta (Eszopiclone) OR sleep-seating antihistamines such as Unisom (Doxylamine succinate) or diphenhydramine allowed) within 2 weeks of the baseline visit and randomization. Antidepressants will be allowed for those participants who have been taking the antidepressant for 6 weeks with a stable dose for at least 4 weeks.
- Use of natural menopause and depression supplements, phytoestrogens, soy-based medications, steroids within 2 weeks of baseline visit and randomization.
- Use of any disallowed medications (specified in the Excluded Concomitant Medication section below).
- Women who have received a gonadal hormonal intervention within 1 month prior to study entry (stable thyroid medications are allowed).
- Not using a medically approved method of birth control, if sexually active and not 12 or more months since last menstrual period
    - IUDs, condoms, abstinence are acceptable forms of contraception in this study; due to the possible interactions with the study medication, oral contraceptive pills will be prohibited.
- Uncontrolled hypertension (>160/95mmHg)
- Active coronary artery disease, atrial fibrillation, stroke, deep vein thrombosis, pulmonary embolism or blood clotting disorder
- Any severe, life threatening or unstable medical condition that, based on clinician-judgment, would make participation in the study unsafe or inappropriate

- Personal or first degree family history of known hormone sensitive tumors
- History of allergic reaction or side effects with prior pregnenolone use
- Clinically significant laboratory or physical examination findings
- Concurrent enrollment in another clinical trial

Excluded Concomitant Medications:

- Selective estrogen-receptor modulators (SERMs)
- Hormone replacement therapy
- Hormonal contraceptives, excluding Mirena IUD or other IUD with localized progesterone
- Natural menopause or antidepressant supplements
- Episodic sleep medications (chronic, regular, stable-dose benzodiazepines and hypnotics such as zolpidem, Sonata (Zaleplon), and Lunesta (Eszopiclone) OR sleep-sedating antihistamines such as Unisom (Doxylamine succinate) or diphenhydramine allowed)
- Phytoestrogens
- Soy-based medications or supplements
- Oral systemic corticosteroids (prednisone, dexamethasone, methylprednisone, etc) with the exception of low-dose nasal steroids and inhaled corticosteroids for asthma

8. Sources of Research Material:

All research materials will be obtained during the time of study participation and will only be used only for research purposes. The data that will be used to answer the primary and secondary aims of the study will be depressive and anxiety symptoms, cognitive rating scales, sleep and hot flash inventories, and quality of life assessments. When the results are published, none of the data will be identifiable with specific participants.

9. Recruitment Methods and Consenting Process:

The Dallas-Fort Worth (DFW) area has about 6.5 million people with 1.2 million living within the City of Dallas. To access these patients, the UTSW site will primarily use two recruitment approaches 1) patients treated at UT Southwestern and 2) with paid advertising. The UT Southwestern Department of Psychiatry has a large private practice clinic (BL7.749) in the same building as the PI's offices. They have about 11,200 patient visits annually. In addition, they receive approximately nine new referrals each day as well as numerous calls from the community. The majority of these referrals are for women with depression. Thus, we will utilize these clinic referrals for the study and have budgeted in a modest amount of salary support for a staff member at the clinic who will screen the calls and refer participants. In addition, Dr. Matan White from Psychiatry, leads a UT Southwestern Women's Mental Health initiative and sees many depressed patients. Other sources of advertising will involve posting flyers in the community and community clinics (e.g., Metrocare), using paid advertising using media outlets that we successfully utilized in other studies (e.g., radio, newspaper, TV advertising, print ads, social media ads), and utilizing i2b2 Volunteer Research Registry available through UT Southwestern.

With respect to the Boston site at MGH, subjects will be recruited from psychiatry, primary care, gynecology clinics, and from mental-health treatment centers at MGH, BWH and Partners-affiliated health centers. The PI, Dr. Marlene Freeman, is the Associate Director of the Center of Women's Mental Health (CWMH) at MGH, a group of perinatal psychiatrists that have a large outreach to the surrounding geographical areas in New England and see approximately 1,200 new subject consults per year. Every woman who comes to either the Research Program to participate in a clinical trial or for a consult with one of the psychiatrists in our program has the opportunity to fill out a form allowing us to enter her information into our tracking system. This is also complemented by our internet resource center (www.womensmentalhealth.org) as well as a weekly e-newsletter with nearly 4,000 subscribers. These critical recruitment tools generate a constant flow of patients that may be eligible and interested in participating in the current study. Subjects will also be recruited using the

Partners Clinical Trials page and Partners-associated recruitment portals, Craigslist, flyers posted in healthcare settings in Boston, mailings sent to potential research participants, and paid advertising. Past studies with similar eligibility criteria have used these advertising techniques and have yielded a diverse patient population.

All participants will undergo the informed consent process during their Baseline visit (before any study procedures can take place). A research coordinator, with PI delegation, will consent the participants and go over all study-related information. The PI or a study-approved clinician will always be available to answer questions about the informed consent process and provide additional information about the study, including risks, benefits and alternatives to participation. All investigators, research coordinators and other staff (including administrative staff) have received training in the protection of human research participants. All investigators and staff involved with patient care will remain updated on issues related to patient rights and safety through educational programs and updates.

10. Potential Risks:

The likely risks from the study are primarily medication side effects and risks related to inadequately treated depression.

Pregnenolone Risks:

Animal studies have not found pregnenolone to be have a lethal dose or be associated with chronic toxicity (Henderson et al., 1950), while pregNANolone had the LD50 IV of 144 mg/kg in male mice and 175 mg/kg in females. With intraperitoneal administration the LD50 for pregNANolone was 148 mg/kg for males but could not be determined in females due to no deaths at 136 mg/kg. Oral doses of 460 mg/kg for females and 328 mg/kg in males resulted in 100% mortality (Brewster et al., 1995).

Pregnenolone administration is associated with large increases in levels of the neuroactive steroids pregNANolone and allopregnanolone, serum pregnenolone sulfate and progesterone, and with little or no change in other neuroactive steroids including DHEA, DHEAS, androsterone and androstenedione, as well as in serum testosterone, free testosterone, cortisol and estradiol (Marx et al., 2009). Nonetheless, ***we will assess for changes in all of these steroids in the proposed study to confirm this finding.***

Based on a study in three people (Roberts, 1995) and a study in one person (Marx, unpublished data used with permission), the half-life of pregnenolone appears to be about 5-25 hours. PregNANolone has an elimination half-life of about 1.2-3.5 hours when administered intravenously (Carl et al., 1994), while the half-life of intravenous allopregnanolone is about 4.4 hours (Timby et al., 2006).

The UTSW PI has conducted 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 assessed with a 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 (Marx et al., 2009). ***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. The GABAergic pregnenolone metabolite allopregnanolone has a benzodiazepine-like effect on sleep (Lancel et al., 1997). 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 recent study by Dr. Christine Marx (consultant) in 120 patients (n=38 women) with schizophrenia given 500 mg of pregnenolone or placebo for eight weeks (data reported with permission from Dr. Marx). The number of participants experiencing treatment-emergent adverse events was comparable in 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).

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. 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 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 (Rossouw et al., 2002). ***Therefore, we have excluded persons at an elevated risk, based on personal and family history for any of these disorders, and also exclude women over 62 years of age.***

Because it is theoretically possible that pregnenolone may have interactions with oral contraceptive agents, ***we will exclude patients taking these medications but include participants using other forms of birth control***. This is a precautionary procedure for a research study.

Although pregnenolone is available without a prescription, we do not feel that it is ethical, given the current state of our knowledge, 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 (tapering schedule is described in section 11 below). The risks of continuing the pregnenolone long-term are not known yet.

Pregnenolone will be purchased from a supplier (MEDISCA) the UTSW researchers have used in prior research studies. MEDISCA is a leading FDA-registered supplier of quality pharmacy compounding products. A certificate of analysis will be provided documenting the content of the sample. In addition, pregnenolone and other neurosteroids serum levels will be assessed at baseline and after pregnenolone or placebo exposure. Although pregnenolone is made by the human body, commercially available products are synthesized like other drugs and not extracted from natural sources. Diosgenin (3β,25R)-spirost-5-en-3-ol) is typically used in the commercial synthesis of pregnenolone, progesterone, cortisone and other compounds containing a steroid nucleus. Study medication and identical placebos will be prepared at the UTSW site by Abrams Royal Pharmacy, a Dallas compounding pharmacy we have used in numerous studies, and at the MGH site by Johnson Compounding and Wellness, a local compounding pharmacy with experience in supplying medication for research studies. The filler will be lactose or sodium bicarbonate. Abrams Royal Pharmacy is a member of the International Academy of Compounding Pharmacies and the Professional Compounding Centers of America.

Risks to an Embryo, Fetus, or Breast-fed Infant: Pregnant or breastfeeding women, as well as women planning pregnancy will be excluded from the study. A negative urine pregnancy test will be mandated before a woman of child-bearing potential can participate in this study, as well as recurrently throughout the study in case a pregnancy is suspected. Women who do not agree to use an acceptable form of birth control (as outlined in the inclusion/exclusion criteria) will not be enrolled in the study. The risks to embryo/fetus/breast-fed infant will be clearly outlined in the consent form for the participant.

Physiological Measures: Risks associated with drawing blood from your arm include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although it is unlikely.

Psychological Stress: Mild fatigue, anxiety and discomfort are potential, but are unlikely (20% chance), adverse effects associated with the study. Investigators and staff with extensive experience and expertise, who are sensitive to the needs of participating individuals, conduct clinical and behavioral evaluations. Breaks will be taken in order to minimize fatigue and discomfort, and participants may choose to refrain from answering any questions that induce undue discomfort and may stop study participation at any time.

Loss of Confidentiality: Any time that data is collected; there is the potential for loss of confidentiality. This risk is deemed unlikely (<2% chance) due to the multiple efforts that are in place to ensure that all study information is protected and remains confidential.

Other Risks: It is possible that other unforeseen risks may exist. If at any point, the investigators become aware of another risk, it will be reported immediately to the IRB and appropriate measures will be taken to minimize the new risk.

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11. Subject Safety and Data Monitoring:

The likely risks from the study are primarily medication side effects and the risk of untreated depressive symptoms. As with the UTSW research group's prior pregnenolone studies, an IND application will be filed with the FDA. Pregnenolone is considered by the Food and Drug Administration (FDA) as an over-the-counter (OTC) supplement, and extensive literature suggests that its side effects are generally mild and comparable to those of placebo. Pregnenolone appears to be well tolerated; however, all participants will receive a safety and tolerability assessment (mental status exam, mood symptoms, suicidality, and side effects) by a study psychiatrist and a research coordinator at each study visit. Although the available data suggest that pregnenolone administration does not alter estrogen levels, women with a history of estrogen sensitive tumors or a first degree relative (e.g. mother, sister) with estrogen sensitive tumors will be excluded.

At study completion, participants will be instructed on how to taper and discontinue the study drug (pregnenolone or placebo) over one week (150 mg twice a day (BID) x 4 days, 50 mg twice a day (BID) x 4 days, discontinue), and will also be given referral information for the local treatment facilities. If needed, we will provide a longer period of aftercare until treatment can be transitioned to another provider. A final telephone safety phone call will also be scheduled after the end of study participation. If a participant informs the research team that she would like to discontinue the study, every effort will be made to schedule a final in-clinic appointment, during which all assessments for Weeks 8 or 16 (depending on the time of discontinuation) will be conducted.

Another potential risk is worsening of mood symptoms. If participants have a worsening of mood symptoms, such as active suicidal or homicidal ideation with clear plan and the intent to act on the plan, study participation will be discontinued and follow-up treatment will be arranged. Some level of suicidal ideation is common in participants with depressive symptomatology, thus recurrent suicidal thoughts accompanied by a clear understanding that the participant does not plan to act on these thoughts (as assessed and documented by a study clinician) would not require discontinuation. In general, in the event of a medical or psychiatric emergency (e.g., active suicidal ideation), appropriate medical advice will be provided, including recommendations for emergency room evaluation if necessary. Dr. Brown and other UTSW physicians involved with the study have staff privileges at the Parkland and William P. Clements University Hospital Emergency Rooms, which are likely to be the most commonly used hospitals by the participants. In addition, Dr. Freeman and the MGH study staff will be conducting study procedures on the same hospital campus as the MGH Emergency Rooms and Acute Psychiatric Service if appropriate medical attention is warranted. In the event of an acute medical emergency, the researchers will also be able to summon medical assistance either by calling the Rapid Response Team for the clinic of calling 911.

An investigator will be available 24 hours a day. The consent form will have both daytime and nighttime numbers for reaching study personnel. Business cards for the research coordinator and psychiatrists will also be provided. In the event an investigator or research coordinator is on vacation or out-of-town, coverage will be provided, and both the research coordinator and UT Southwestern answering service are informed of the name and pager number of the physician on-call. The MGH Triage line will page the study physician on-call.

Potential pharmacokinetic or pharmacodynamic drug-drug interactions will be managed using inclusion, exclusion, and discontinuation criteria and dose adjustments. Clinical assessments are collected by trained and experienced staff members who will be able to reassure participants if they are distressed during study visits. If participants become fatigued or stressed during the testing, rest periods will be provided. Standard, appropriate measures will be used to minimize risk of infection during venipuncture.

All women of childbearing potential (as outlined in the inclusion/exclusion criteria) must have a urine pregnancy test with negative results prior to receiving study medication. These participants will also be asked to use effective forms of contraception and dates of last menstrual period during the study. Pregnancy or failure to use contraceptives will result in discontinuation from the study.

Potential safety concerns will result in discontinuation from the study protocol. However, standard care will be provided until referral to another physician for further care can be arranged. Examples of these safety concerns are given below:

- 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, or development of psychotic features
- Development of severe or life-threatening medical condition, pregnancy or psychiatric hospitalization
- Withdrawal of informed consent by the participant

These are general guidelines. Additionally, clinician judgment, as well as specific discontinuation criteria, will be used to determine whether or not a participant can safely remain in the study.

12. Data Management and Monitoring

At the beginning of the study, a REDCap database will be set up by the Dallas site in collaboration with the Boston site. Original data (including PHI) will be maintained at the site of collection (Dallas or Boston). However, de-identified data used in the data analysis (e.g., depressive symptoms) will be stored centrally using the secure electronic REDCap system. These data will be reviewed recurrently by the Dallas data monitor and any errors or inconsistencies will be discussed. A data monitor from both the Dallas and Boston sites will review data charts and corresponding digital database content weekly and provide feedback to the PIs and research staff on data quality and recommend remediation, if necessary.

An independent Data and Safety Monitoring Board (DSMB) will be formed to oversee the progress of this clinical trial. The board will provide an independent review and oversight mechanism for the study and will meet immediately prior to the initiation of enrollment in the study and again at least twice per year, as per National Institute of Aging (NIA) guidelines. The researchers will present safety data to the board for review, including changes in psychiatric symptom severity and side effects. In addition, materials provided to the IRBs, including serious adverse events leading to death or hospitalization will be forwarded to board members. The board will review preliminary data on adverse events and outcomes. If the board feels that protocol modifications are needed, it will have the authority to recommend modifications of safety procedures and recommend temporary or permanent discontinuation of enrollment. However, the final decision on these safety matters will rest with the institutional IRBs and NIH. The suggested DSMB plan will be approved by NIH prior to study enrollment.

13. Procedures to Maintain Confidentiality:

The patient 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 on computers with password-protected access. We will not disclose information about individual patients in the study without the patient's consent. A certificate of confidentiality will be obtained.

14. Potential Benefits:

The primary benefit of the study will be to determine whether pregnenolone therapy will improve depressive symptoms and anxiety. Based on our pilot data, some participants may benefit from the study. However, the primary goal is to obtain information that will benefit future patients. All patients in the study will receive a thorough assessment and careful longitudinal monitoring of depressive symptoms. The medication selected (pregnenolone) appears to be generally safe and well-tolerated. We feel it is essential for this study that a placebo group be included as the efficacy is not well established. Thus, the benefits of the study are great and will include a better understanding of the treatment of depressed patients. We believe that the risks, though clearly present, are greatly outweighed by the benefits of this study.

15. Power Analysis & Sample Size Determination

The sample size calculation was performed using a customized program in SAS (v9.2), and was based on the SPCD sample size formula that takes into account the mean treatment effect

combined with equal weighting of each stage-wise estimate and its variance, and subject retention rates for the trial. We assume 67% placebo non-responders in Stage 1, 10% dropout rate for Stage 1 and 5% dropout in Stage 2. The sample size calculation was performed for a continuous response assuming a Stage 1 mean change from baseline to Week 8 in total MADRS score between pregnenolone and placebo of 3.5 (standard deviation (SD) of 8) and a Stage 2 difference of 5 (SD=8). Given these assumptions, a sample size of 144 subjects (randomized in 2:1 ratio at Stage 1 for placebo and pregnenolone) will have 90% power to detect a change from baseline in total MADRS score between pregnenolone and placebo. This assumes a two-sided hypothesis test with 0.05 significance is used for the comparison between pregnenolone and placebo.