Complementary Neurosteroid Intervention in Gulf War Veterans' Illnesses (GWVI)

NCT01956279

Study Protocol and Statistical Analysis Plan

Version 4 – 2/20/2015

PROTOCOL OVERVIEW
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PURPOSE

Gulf War Veterans' Illnesses (GWVI) profoundly influence quality of life and functional outcome in many Veterans with a history of deployment to the Persian Gulf theater (Gulf War and Health, Vol 8; Update of Health Effects of Serving in the Gulf War; 2009; Institute of Medicine [IOM]). Deployed Gulf War Veterans consistently report increased rates of the majority of symptoms examined compared to non-deployed Gulf War Veterans, and more Gulf War Veterans who were deployed meet criteria for "multi-symptom illnesses" compared to non-deployed Gulf War Veterans (IOM Update 2009, Kang et al 2009). Further, these higher rates of reported symptoms in deployed Gulf War Veterans compared to non-deployed Gulf War Veterans also occur among those who served from other countries, including Canada, Australia, the United Kingdom, and Denmark (IOM Update 2009). The increased prevalence of multiple symptoms in deployed Gulf War Veterans compared to non-deployed Gulf War Veterans is currently considered to be "beyond dispute," and it is thought that "the excess of unexplained medical symptoms reported by deployed Gulf War Veterans cannot be reliably ascribed to any known psychiatric disorder" (IOM Update 2009); these assessments are supported by reports from several research groups (Iversen et al 2007, Fukuda et al, 1998, Gray et al 1996, Unwin et al 1999). Although elucidation of the precise physiological underpinnings of these complex symptom constellations remain a focus of ongoing scientific inquiry, it is clear that multisystem involvement is a hallmark GWVI.

The Institute of Medicine Committee on Gulf War and Health also notes that *"inadequate numbers of clinical trials have been undertaken to develop more effective and evidence-based treatments for multi-system illness,"* and calls for "a renewed research effort to identify and treat multi-system illness in Gulf War Veterans." The IOM Committee also states that it is *"optimistic that a rigorous, adequately powered study could identify useful biomarkers that are helpful for symptomatic Veterans of the Gulf War and for non-deployed Veterans and civilians who have a variety of medically unexplained symptoms, including chronic fatigue, muscle and joint pain, sleep disturbance, difficulty with concentration, and depression"* (IOM Update 2009). The current proposal investigating a novel complementary neurosteroid intervention in Gulf War Veterans with GWVI and candidate biomarkers of therapeutic response is thus clearly aligned with this Institute of Medicine mandate. The rationale for utilizing a neurosteroid intervention in Gulf War Veterans with GWVI is outlined below.

RESEARCH DESIGN AND METHODS

Specific Aim 1: To conduct an RCT with the neurosteroid pregnenolone in 140 Gulf War Veterans with GWVI and a history of deployment (70 Veterans randomized to adjunctive pregnenolone, 70 Veterans randomized to placebo), targeting functional outcome as the primary endpoint (as assessed by the SF-36). Secondary endpoints will be pain symptoms, cognitive symptoms, fatigue, and global psychological functioning. ***Based on our preliminary data from multiple investigations, we hypothesize that adjunctive pregnenolone will significantly improve functional outcomes, pain symptoms, cognitive symptoms, fatigue, and global psychological symptoms in Gulf War Veterans with GWVI.***

Recent investigations support the possibility that pregnenolone may be a promising therapeutic intervention in Gulf War Veterans with GWVI. Trial duration will be 10 weeks, consisting of a 2-week placebo lead-in period, followed by 8 weeks of randomly assigned adjunctive treatment with either pregnenolone or placebo (add-on to treatment-as-usual). The primary endpoint will be functional outcome (as assessed by the Physical Component of the SF-36) in the group randomized to pregnenolone compared to the group randomized to placebo. Secondary outcome measures will be pain symptoms (as assessed by the Brief Pain Inventory), cognitive symptoms (specifically executive functioning, as assessed by the Tower of London component of the BACS), fatigue (as assessed by the Multidimensional Fatigue Inventory), and overall psychological functioning (as assessed by the Global Severity Index [GSI] of the SCL-90R).

Specific Aim 2: To conduct candidate biomarker investigations quantifying pregnenolone and pregnenolone metabolite levels (allopregnanolone, pregnanolone, androsterone, others) in serum samples at baseline, during treatment, and post-treatment with pregnenolone using mass spectrometry-based technologies, and to investigate potential genetic predictors of therapeutic response. These investigations will thus: a.) characterize the pharmacokinetics of pregnenolone and its metabolic profile - which could yield valuable dosing information for pregnenolone, characterize pharmacological windows of optimal therapeutic efficacy, and identify potential neurosteroid predictors of therapeutic response, and b.) examine single nucleotide polymorphisms (SNPs) of genes coding for enzymes involved in neurosteroid synthesis and metabolism. *Together these studies could contribute to the development of new neurosteroid interventional strategies that build on the current investigation and exhibit promise as pharmacological candidates in GWVI*. Based on our preliminary candidate biomarker data that neurosteroids are altered in CNS disorders (Marx et al 2006a, Marx et al 2006b, Marx et al 2006c, Naylor et al 2008, Naylor et al 2010, Kilts et al 2010), and that neurosteroid alterations following treatment with pregnenolone may be associated with therapeutic response (Marx et al 2009), we hypothesize that changes in pregnenolone levels (and pregnenolone metabolite levels) post-treatment will predict therapeutic response to this intervention. We also hypothesize that neurosteroids are dysregulated in GWVI, and that specific SNPs of genes coding for neurosteroidogenic enzymes will be associated with therapeutic response. Additionally, we hypothesize that changes in one or more pregnenolone metabolites may also predict therapeutic response, and potentially lead to the identification of additional neurosteroid targets that demonstrate promise for treating diverse symptoms impacting functional outcome and quality of life in Gulf War Veterans with GWVI.

SUBJECT RECRUITMENT

We propose to conduct a randomized controlled trial (RCT) investigating a novel neurosteroid intervention in Gulf War Veterans with GWVI. Research participants will be recruited via Durham VA IRB-approved local advertising (flyers), Durham VA IRB-approved letters (and follow-up phone calls utilizing a Durham VA IRB-approved phone script), Vet Centers, and the VISN 6 MIRECC Post-Deployment Mental Health Data Repository (over 95% of these Registry participants have provided permission to be re-contacted for future research studies; over a quarter of Veterans in this OEF/OIF/OND era Registry are also Gulf War era Veterans), and by referral from the Durham VA, Raleigh CBOC, Greensboro CBOC, and Morehead City CBOC medical and behavioral health care providers. The target number of participants for this study is 140 subjects who complete at least 4 weeks of the study post-randomization (70 subjects per group, randomized to either adjunctive pregnenolone or placebo). Based on our prior investigations with pregnenolone, we conservatively estimate a drop-out rate of 30%. The power analysis for this study in GWVI was conducted under the assumption that 140 participants reach Visit 4 of the study (i.e. 4 weeks post-randomization). With an estimated 30% drop-out rate, we thus anticipate that we will have to randomize approximately 200 participants to achieve this goal.

Enrolled subjects will be 1). Veterans deployed to the Gulf War theater of operations between 1990 and 1991, 2). Gulf War Veterans who report at least 2 of the following 3 symptoms that began in 1990 or thereafter, that have lasted for more than 6 months, and that are present at the time of screening: a) fatigue that limited usual activity, b) musculoskeletal pain involving 2 or more regions of the body, c) cognitive symptoms (memory, concentration, or attentional difficulties by self-report, 3) on a stable medication regimen (no change in past 4 weeks) and no anticipated change in medications during study, 4) Able to provide informed consent for study participation. Only Gulf War era Veterans will be enrolled into this study. There will be no non-Veteran participants enrolled in this study.

RECRUITMENT PROCEDURES

Potential participants may be contacted initially via U.S. mail with a signed and IRB-approved recruitment letter. For individuals who have been sent a letter, a follow-up telephone call will take place to confirm receipt of letter, to inquire if a participant may be interested in study participation, and to pre-screen for study qualification (via IRB-approved telephone script). If potential participants initiate contact with the study team by telephone (i.e. if participants are responding to an IRB-approved advertisement or have been referred to the study by another healthcare professional and thus did not receive an IRB-approved recruitment letter), individuals will be pre-screened for study qualification

using the IRB-approved telephone script entitled "call from patient." Participants who meet pre-qualification criteria will be invited to schedule an appointment at the Durham VAMC to complete a screening visit, in which they will learn details about study involvement and will be asked to provide informed consent. Participants who attend this visit will be compensated for their time, whether or not they decide to participate further in the study.

SELECTION OF SUBJECTS

Inclusion Criteria:

1. Veterans deployed to the Gulf War theatre of operations between 1990 and 1991.
2. Veterans who report at least 2 of the following 3 symptoms that began in 1990 or thereafter, that lasted for more than 6 months, and that are present at the time of screening: 1) fatigue that limited usual activity, 2) musculoskeletal pain involving 2 or more regions of the body, 3) cognitive symptoms (memory, concentration, or attentional difficulties by self-report)
3. Stable medication regimen (no change in past 4 weeks) and no anticipated change in medications during study.
4. Able to provide informed consent for study participation.

Exclusion Criteria:

1. Subjects with a history of clinically significant neurological, metabolic, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, and/or urological disorders (e.g. unstable angina, seizures, cerebrovascular accident, decompensated congestive heart failure, CNS infection, cancer [other than non-melanoma skin cancer], or history of HIV seropositivity), which would pose a risk to the patient if s/he were to participate in the study or that might confound the results of the study.
2. Concurrent enrollment in another clinical trial.
3. Pregnant women or women of child-bearing potential who are not surgically-sterile or not using appropriate methods of birth control.
4. Use of oral contraceptives or other hormonal supplementation such as estrogen [although early studies suggested no effects on menstrual cycle, alterations in downstream metabolites of pregnenolone (such as estradiol) could theoretically impact the efficacy of oral contraceptives and/or estrogen replacement]. Similarly, it is theoretically possible that pregnenolone could be metabolized to other steroids such as DHEA, resulting in hair, skin, or other steroid-related changes. Since we have determined in our prior study that pregnenolone administration does not result in downstream elevations in DHEA, DHEAS, estradiol, or testosterone, these possibilities may be unlikely.
5. Women who are breast-feeding.
6. Use of narcotic interventions.
7. Use of benzodiazepines.
8. Known allergy to study medication.
9. History of moderate or severe TBI (with loss of consciousness greater than 30 minutes). (Mild TBI is permissible.)
10. A clearly defined disease entity that accounts for the Veteran's symptoms.
11. Current DSM-IV/DSM-IVTR/DSMV diagnosis of bipolar I disorder, schizophrenia or other psychotic disorder, or dementia. (PTSD and/or depression are permissible.)
12. Subjects with a DSM-IV/DSM-IVTR/DSMV diagnosis of alcohol or substance dependence (other than nicotine or caffeine) within the last month.
13. Subjects with current suicidal or homicidal ideation necessitating clinical intervention or representing an imminent concern
14. If in the judgement of the PI it is not in the subject's best interest to participate.
15. Final eligibility decisions will be determined by the PI.

CONSENT PROCESS

A subject who is judged likely to meet all of the inclusion criteria and none of the exclusion criteria will meet with a member of the research team to discuss the research protocol, and to determine if the patient is capable of providing informed consent. This study utilizes an informed consent form. The subject will be provided with a description (verbal and written) of the informed consent form, which includes the risks along with procedures to minimize these risks, and the subjects' rights and responsibilities. Subjects will be provided the opportunity to read the consent form and ask questions. Subjects will be assured that participation in this research study is voluntary and that they may withdraw from the study at any time without adversely affecting their medical care or any benefits they might be receiving. Veterans will be informed that participation in this research study is completely voluntary and distinct from usual clinical care. They may also refuse to answer any research questions during interviews. Subjects who are eligible for the study and choose to participate will sign the consent form in the presence of a member of the research team.

STUDY INTERVENTIONS

Primary Outcome: Functional Outcome (as assessed by the Physical Component of the SF-36).

Hypothesis: Gulf War Veterans with GWVI who are randomized to pregnenolone will demonstrate significantly greater improvements in the Physical Component Score of the SF-36 compared to Veterans randomized to placebo.

We will assess this hypothesis in the context of a two factor repeated measures mixed model ANOVA, with one between subjects factor with two levels (pregnenolone vs. placebo) and one within subjects factor with three levels (Weeks 0, 4, 8 of treatment post-randomization, which correspond to Visits 2, 4, and 6). This hypothesis will be tested using the treatment-by-time interaction, followed by contrasts in least squares means. These contrasts will examine the difference between the treatments at Weeks 4 and 8 on the changes between least squares means between Week 0 to Week 4, and Week 0 to Week 8 of treatment post-randomization. We will perform several such analyses, one for functional outcome as assessed by the Physical Component of the SF-36 (primary endpoint), and others for secondary endpoints, including: pain symptoms (as assessed by the Brief Pain Inventory), executive functioning (as assessed by the Tower of London test of the BACS), fatigue (as assessed by the Multidimensional Fatigue Inventory), and overall psychological functioning (as assessed by the Global Severity Index of the SCL-90R). The response variable will be change from baseline, and we will use baseline as a covariate. Exploratory outcomes will include the BACS composite score (cognitive symptoms), resilience (as assessed by the Connor-Davidson Resilience Scale), anxiety and depression symptoms (as assessed by the Hospital Anxiety and Depression Scale), PTSD symptoms (as assessed by the PTSD Symptom Checklist), and depression symptoms (as assessed by the Beck Depression Inventory-II scale).

There are likely many characteristics of patients with GWVI that could influence observed treatment effects. Patients with GWVI often have other conditions that may modify study outcomes. The randomization process is intended to make the treatment group and control group equivalent, on average, with respect to these known and unknown confounding factors. However, by random chance the experimental groups may differ sizably with respect to some confounding factors. We thus intend to measure several potential confounding factors, including history of mild TBI, among other possibilities (Veterans with a history of moderate or severe TBI will be excluded). Initial bivariate analyses to characterize and compare experimental groups will evaluate whether these groups differ sizably with respect to these known confounders. Analyses will be adjusted for potential confounders by adding them or their interactions with treatment to the models and assessing these effects. These mediator/moderator type analyses are exploratory and will suggest hypotheses for further study.

It is also possible that patients with or without specific conditions (e.g. history of mild TBI) may find the treatment especially beneficial. However, so little is known regarding how GWVI may interact with these conditions, that we have no specific grounds by which to exclude or enrich the study population to examine effects in mild TBI subpopulations (or other subpopulations) in this study. We thus expect that our study population will reflect the heterogeneity of the general patient population with this condition. We intend to explore interactions between the treatment effect and a number of co-occurring conditions and other characteristics as part of our analyses. However,

the investigation of these interactions is not the primary aim to this study (and any negative findings with respect to potential interactions may thus reflect insufficient statistical power to detect them).

Pregnenolone:
Pregnenolone doses were chosen following careful review of prior dosing strategies in the existing literature that were tolerated without significant side effects. Subjects randomized to pregnenolone following a two-week placebo lead-in will receive this neurosteroid according to the following titration schedule:

Pregnenolone 100 mg in divided doses (50 mg BID) for 2 weeks, then
Pregnenolone 300 mg in divided doses (150 mg BID) for 2 weeks, then
Pregnenolone 500 mg in divided doses (250 mg BID) for 4 weeks.

Compliance with study drug treatment will be assessed by counting the returned study drug supply. Subjects that fall below 80% compliance at 2 consecutive visits or miss 6 consecutive doses in a row (3 days of study drug) for two consecutive visits should be withdrawn from the study.

Study Timeline:
1. Referred patients and patients responding to IRB-approved advertisements and/or IRB-approved letters with follow-up phone calls (using an IRB-approved phone script) will meet with a member of the research team to discuss the study and the risks and benefits of participation. In addition, patients will be screened for exclusion and inclusion criteria. If subjects are interested in potentially participating in the study, the informed consent document will be discussed with them. Patients will have the option of taking the informed consent with them and discussing the matter with family, friends and/or clinicians. If applicable, the participant's physician or other VA provider will be consulted regarding the patient's appropriateness of the patient's enrollment in the study.

2. Once the informed consent is signed, the subject will proceed to the initial screening procedures. Each participant will receive the MINI diagnostic assessment tool (Mini-International Neuropsychiatric Interview).

3. A licensed physician, nurse practitioner (NP), or physician's assistant (PA) who is clinically privileged at the Durham VA will examine patients prior to randomization to study drug (pregnenolone) or placebo. Patients will have a venous puncture for laboratory/genetic testing (please also the Schedule of Events). Patients will be asked to provide approximately 3.5 tablespoons of blood on the first study visit, and approximately 2.5 tablespoons of blood on Visits #2, 3, 4, 5, 6 and 7. This is a total of approximately16 tablespoons of blood over the duration of the study. Subjects will receive an ECG at Visits #1 and 6. Patients with clinically significant abnormal physical exam, blood tests, or ECG that preclude safe study participation will be excluded from the study and referred to their primary care provider or appropriate specialist.

4. Following the screening visit (Visit 1), all patients will begin a two-week placebo-only lead-in period (prior to randomization at Visit 2). On the day that patients are given the study medication and randomized (and at each visit during the study), a set of vital signs will be done including pulse, blood pressure, and weight. With regard to the study medication, participants will be told that they could be getting placebo (a sugar pill) or active medication (pregnenolone). The subjects will be told that they may be receiving a different dose every two weeks. They will be asked to take the medication twice a day; once in the morning and once in the evening.

5. After a week of the placebo lead-in period, a member of the research team will contact the participant to ask about study medication compliance and to answer any questions. A member of the research team will continue to contact patients by phone every two weeks to ask about compliance to study medication, possible suicidality, and potential adverse events (these telephone check-in contacts will be staggered with in-person patient visits every two weeks; please see also Schedule of Events for details).

6. Subjects will return for a study visit every two weeks. Vital signs will be repeated at each visit. A member of the research team will ask subjects about possible side effects and adverse events (utilizing a structured Adverse Event

Form) and will administer the Columbia Suicide Severity Scale to assess possible suicidality. Patients will be asked to return tablets of pregnenolone or placebo to determine compliance with study medication by pill counting. The subject will be given a new supply of tablets (either pregnenolone or placebo, depending upon random assignment) every two weeks. Patients will receive a laboratory testing (Chem 7, GI panel, CBC) at all study visits. Female patients will also receive a pregnancy test at Visits #1, 2, 4 and 6. TSH, free T4, B12/folate, and a lipid panel will be assessed at Visits #1 and 6. A urinalysis and urine tox screen will be conducted at Visits 1, 2, 4, and 6.

7. At Visits #1 (screening visit), 2 (randomization visit), 3, 4, 5 and 6 (each visit is 2 weeks apart), three extra red-top tubes will be collected for pregnenolone levels and determination of pregnenolone metabolite levels, proteins, and other small molecules. A PaxGene tube and two purple top tubes will be collected at Visit #1 (for genetic analyses).

8. At Visit 6 (=8 weeks post-randomization), the study medication will be tapered (150 mg BID x 2 days, followed by 50 mg BID x 2 days, followed by study medication discontinuation). Subjects will return 1-2 weeks after Visit 6 for a follow-up visit that includes vital signs, laboratory tests, administration of the CSSRS to assess for suicidality, and adverse event assessment.

9. The WRAT, HADS, and TBI screen will be administered at Visit #1. The SF-36, BAC-A, BPI, MFI, SCL-90, HADS, BDI, PCL, WHODAS, SDS, and CDRS will be administered at Visits #2, 4 and 6. The Adverse Event Scale and CSSR (to assess for suicidality) will be administered at each in-person visit and at each telephone visit.

Randomization and Maintenance of the Blind: Randomization will occur in blocks of 10, and will be conducted by the research pharmacist at the Durham VAMC. Only the research pharmacist will have access to the randomization code during conduction of the study, and the PI and all raters, investigators and other study staff will be blinded during the study. The pharmacist does not participate in assessing any of the primary symptom or side effect dependent variables, and conveys no information about treatment assignment to subjects or staff. Study medication will be dispensed every two weeks. Subjects will be given six extra days of medication in case of a delayed or rescheduled appointment. The blind will be broken only if a medical emergency requires this information. If this occurs, the subject will be withdrawn from the study.
After the study is completed and the last participant leaves the study, the aggregate blinded data will be cleaned (i.e. reviewed for possible outliers that may reflect data entry errors, etc.) and the database will be locked. A de-identified copy of the locked data will be given to our PhD-level statistician for analysis. A de-identified copy of the locked data will also be give to a second statistician for independent replication of the study analyses. The PI will not be involved in study data analyses.

Neurosteroid Quantification:

Gas Chromatography/Mass Spectrometry (GC/MS) Preceded by High Performance Liquid Chromatography (HPLC)

Neurosteroid quantifications in serum samples will be were performed by a highly sensitive and specific gas chromatography-mass spectrometry method, as described previously (Marx *et al*, 2006), preceded by high performance liquid chromatography (HPLC) purification, with modifications (the electron impact ionization mode will be utilized for this investigation, rather than negative ion chemical ionization). One mL of serum will be extracted three times in ethyl acetate prior to HPLC purification using tetrahydrofuran, ethanol, and hexane in the mobile phase.

For neurosteroid quantification, the standard curve for the steroid of interest is prepared by combining varying known quantities of steroids (Steraloids) ranging from 2 to 3000 pg/2 μL with a constant amount of the respective deuterated internal standard and tritiated neurosteroid of interest. Identical to the experimental samples, the standard curve is extracted three times in ethyl acetate prior to HPLC purification and GC/MS injection; standard curve $r^2 = 0.99$ for each neurosteroid. The area under the peak of each known quantity of steroid is divided by the area under the peak of the internal standard. This ratio is then plotted on the y-axis against known quantities of each steroid to generate the

standard curve. Only peaks with a signal to noise ratio greater or equal to 5:1 are integrated. The limit of neurosteroid detection with this method is 1 pg for pregnenolone, allopregnanolone, pregnanolone, and androsterone. Intra-assay coefficients of variation range between 1.5-8.0% for each neurosteroid.

ADVERSE EVENTS AND SAFETY EVALUATIONS

Adverse Events Assessment: Adverse events will be assessed by administration of the Hillside Adverse Events Form and reviewed by a licensed physician, NP, or PA with clinical privileges at the Durham VA. Adverse events will be assessed at all study visits after the screening visit (i.e. Visits 2 through 6 and at the follow-up visit) as well as at each phone call (phone calls staggered with study visits – i.e. participants will receive an adverse events assessment weekly, either during a telephone check-in call every two weeks or in person at a study visit every two weeks). In addition, adverse events will be assessed during a follow-up visit one-two weeks after study medication taper.

Withdrawal from Study:

As stated on the Informed Consent Form, all subjects reserve the right to withdraw from the clinical investigation at any time.

Subjects may be discontinued from the study by the Principal Investigator for the following reasons:

- Subject withdraws consent to participate in the study;
- Subject is noncompliant with procedures set forth in the protocol;
- Subject experiences an AE that warrants withdrawal from the study;
- It is in PI's opinion that it is not in the subject's best interest to continue;
- Other laboratory, medical, or clinical finding for which clinical care should take precedence over study participation.

Adequacy of Protection from Risks

a) *Physical Risk and Subject Safety*: Possible side effects from drawing blood will be attended to as usual in the phlebotomy laboratory. If a subject experiences serious adverse effects from the study medication, it will be discontinued and s/he will be withdrawn from the study. The subject will immediately be referred for appropriate evaluation and treatment. Adverse events (AEs) and serious adverse events (SAEs) will be documented and reported as required by the Durham VA IRB committee, the FDA, and the national VA Data Safety and Monitoring Board. Progress reports summarizing AEs and SAEs will be submitted to the Durham VA IRB and FDA annually, and to the national VA DSMB every 6 months as required. Any SAEs will be rapidly reported as required within the timeframe and guidelines of the Durham VA IRB, the FDA, and the national VA DSMB.

b) *Emotional Distress and Subject Safety*: All subjects will be carefully assessed before the study and will be made aware of emergency services (available 24/7 at the Durham VA). In addition, they will be assessed at screening and at each study visit. During the informed consent process, they will be advised that the study procedures could potentially lead to distress (some questionnaires could be upsetting, for example), and that they may withdraw from the study at any time without adversely affecting their medical care or any benefits they may be receiving. If a subject is significantly distressed during a study visit (and if safety is a concern, i.e. expressing thoughts of self-harm or harm to others requiring clinical intervention), or reporting significant medical problems requiring clinical attention, s/he will be walked to the emergency room onsite for rapid evaluation and treatment (including potential hospitalization if clinically indicated). Dr. Marx will be available by cell phone to study participants for any concerns 24/7 (her cell phone number is 919-[redacted], as listed in the consent form of this study; all consented participants will receive a hard copy of this consent form). When Dr. Marx is traveling or on annual leave, a licensed and clinically privileged psychiatrist or other physician at the Durham VA will assume coverage - such as Dr. Daniel Bradford (who has extensive experience in clinical trials in Veterans with psychotic disorders), Dr. Michael Hertzberg (who has extensive

experience in clinical trials in Veterans with PTSD), or Dr. Richard Weiner (who has extensive experience in clinical trials in depression and who is the Durham VA Mental Health Service Line Chief). In terms of risks to confidentiality, pertinent information regarding potential harm, including suicidal and homicidal intent, will be shared as necessary and as required by law with clinicians and/or the appropriate authorities. In such circumstances, records may be made available to authorities, even without the subject's consent, in the setting of potential risk of harm to self or others.

Safety Reporting:

The National VA Data Monitoring Committee (DMC) will receive a report of adverse events every six months. Serious adverse event reporting will adhere to requirements of the Durham VA IRB, the FDA, and the national VA DSMB (also referred to as the national VA DMC). Adverse events will be reported to appropriate regulatory agencies within mandated timeframes.

Suicidality Safety Procedures for Phone Calls and In-Person Suicidality Assessments:

If a participant reports suicidality (suicidal ideation, gesture, or attempt) as per the Columbia Suicide Severity Rating (CSSRS, which is an FDA-recommended assessment for suicidality in clinical trials – and which will be administered at every check-in phone call and in person at every visit – hence weekly, either in person on by phone) during any phone call or during an in-person study visit, a study physician will be notified immediately to determine whether the situation requires immediate clinical intervention. If a participant is thought be at imminent risk to self, he/she will be referred immediately to emergency psychiatric care at the Durham VA Emergency Department, to Psychiatric Emergency Services (PEC) at the Durham VA, or to the participant's local Emergency Department for further evaluation (if the assessment was conducted by phone). If the participant is unwilling or unable to get to emergency psychiatric care and appears to be in imminent danger of self-harm, the study team will contact local law enforcement who will be asked to check on the participant in person. If the study assessment was conducted at an in-person study visit, a study physician will be contacted immediately to assess the patient and to develop a clinical plan; if the participant is judged to require acute intervention, s/he will be walked to the Psychiatry Emergency Care at the Durham VA or to the Durham VA Emergency Room.

Risks for Study Personnel: There is minimal risk involved to study personnel. A trained laboratory phlebotomist will draw the participant's blood for laboratory analysis and serum/blood collection. There is a slight risk of potential needle stick, however the Durham VA Chemical Laboratory provides extensive risk prevention training for all phlebotomists, and thus the likelihood of an accidental needle stick is low. The blood samples will be transported to a -80º Celsius freezer for storage by study personnel. In order to reduce the risk of direct exposure, personnel will wear personal protective equipment while transporting patient serum/blood samples.

ADDITIONAL SAFETY PLAN DETAILS:

Overview of Facilities:

Durham VA Medical Center (DVAMC) Psychiatric and Medical Services:

i. The DVAMC has acute psychiatric care available onsite 24 hours per day/7 days per week provided by the Psychiatric Emergency Care (PEC) team and staffed by a Duke psychiatry resident, psychiatric social worker, and VA psychiatry attending.

ii. The DVAMC has an inpatient psychiatric unit onsite with 28 inpatient psychiatric beds. It is staffed by three VA Mental Health Service Line psychiatry attendings, three Duke psychiatry residents, and other mental health care professionals.

iii. The DVAMC has an Emergency Room onsite providing care 24 hours per day/7 days per week, which is staffed by Duke/VA internal medicine residents, fellows, and attendings.

iv. Extensive specialty and subspecialty consultation services are also available at the DVAMC, including renal, GI, dermatology, cardiology, orthopedic surgery, otolaryngology, endocrinology, pain management, dental health, vocational rehabilitation, and occupational health, among others.

Vital Signs and Side Effect Monitoring:

Procedures for Addressing Abnormal Vital Signs and Potential Side Effects:

i. Vital signs will be assessed at each study visit. Blood pressure >160/95 and other clinically significant vital sign abnormalities will be followed-up with the patient's primary care physician (PCP), the internal medicine physician in the Emergency Room, or an appropriate consult physician, as clinically indicated.

ii. If vital sign abnormalities require same-day medical follow-up, the patient will be assessed in the DVAMC Acute Care Clinic/Emergency Room.

iii. If vital sign abnormalities do not require same-day medical follow-up, an appointment will be scheduled with the patient's PCP.

iv. Patients will receive an extensive side effect scale at each study visit (i.e. every two weeks, as outlined above; specifically with the Hillside Adverse Events Scale). Patients will also be queried regarding potential side effects utilizing this scale at staggered phone check-in contact during the weeks when patients do not receive a study visit. Potential side effects will be monitored closely by the PI and other MD and/or NP and/or PA members of the research team. A participant may be asked to return for an unscheduled appointment to assess a potential side effect if clinically indicated. The PI will consult as necessary with the patient's PCP, internal medicine physician in the ER, psychiatrist, or relevant consult physician.

v. Clinical worsening of psychiatric assessments (>25% increase in PTSD or depression symptoms over two consecutive visits, as assessed by the PCL and BDI-II) will be discussed with the patient's treating psychiatrist, other mental health care provider, or PCP, and the option of study withdrawal will be discussed with the patient and his/her provider. As always, clinical care will take precedence over study participation.

Laboratory Results:

Procedures for Addressing Abnormal Laboratory Results:

i. Patients will receive a CBC, clinical chemistry panel, and liver function tests at each of the six study visits in this clinical trial. At screening, mid-point, and final study visit, patients will also receive a urinalysis and urine toxicology test (and serum pregnancy test if female). At the screening study visit, patients will receive a TSH, B12/folate, and lipid levels. Lipid levels will also be checked at Visit 6. Laboratory results out of the normal range (range determined by the Durham VA Medical Center Clinical Chemistry Laboratory that services both the outpatient and inpatient services at our hospital, and that will conduct the above lab assessments for this study) will receive appropriate review and follow-up as described below.

ii. The PI (or MD/NP/PA member of the research team supervised by the PI) will review laboratory results within 24 hours. Clinically relevant laboratory abnormalities will receive prompt follow-up attention with the patient's PCP or other health care provider, the internist physician in the ER, and/or a follow-up visit for additional lab work as clinically indicated.

iii. The DVAMC Clinical Chemistry Laboratory that services the hospital will phone the PI with any "alert" laboratory values that require immediate attention.

iv. Any laboratory abnormalities that may potentially preclude study participation will be discussed with the patient's PCP, the internal medicine ER physician, the patient's treating psychiatrist, or other relevant health care provider.

EKG Monitoring:

Procedures for Addressing Abnormal EKG Findings:

i. Final EKG readings issued by a cardiologist are available from the DVAMC Heart Station within 48 hours.

ii. Each patient will have an EKG at baseline and completion of the study. If the patient does not complete the study and withdraws prior to the final study visit, every effort will be made to obtain an EKG at the follow-up visit post-withdrawal from the study (please see withdrawal plan below).

iii. Any abnormal EKG findings will be discussed with cardiologist Dr. Joseph Greenfield (former Chair of the Department of Medicine at Duke), who generously consults with our clinical trial team. If Dr. Greenfield suggests

additional medical follow-up, we will proceed according to his recommendations (see below). If Dr. Greenfield is unavailable, the EKG will be discussed with another cardiologist, the ER physician, or the patient's PCP.

iv. Additional potential follow-up actions may include, but are not limited to: Primary care provider notification by pager or VA email, the placement of a cardiology consult, a repeat EKG (if lead placement is suspected to have been suboptimal, for example), or referral to the onsite Acute Care Clinic/Emergency Room for assessment.

Psychiatric Worsening:

Procedure for Addressing Psychiatric Worsening:

i. Patients who demonstrate psychiatric worsening as assessed by a 25% increase over baseline of the PCL (PTSD symptoms) or BDI-II total score (depression symptoms) on two consecutive visits (which are two weeks apart) may be withdrawn from the study (even if this change is thought to be unrelated to the study), and they will be referred to their treating psychiatrist, other mental health care provider, PCP, or the onsite Psychiatric Emergency Care team. Please see also above description.

ii. Patients who exhibit suicidal or homicidal ideation requiring urgent evaluation during a study visit will be referred to the onsite DVAMC Psychiatric Emergency Care team for prompt assessment and possible inpatient psychiatric admission.

Addressing Hormonal Changes in Response to Test Drug or Drug Metabolites:

Procedures for Quantifying Neurosteroid and Neurosteroid Metabolite Levels:

Neurosteroids and neurosteroid metabolites will be quantified by gas chromatography/mass spectrometry (preceded by high performance liquid chromatography), radioimmunoassay, or ELISA, as previously described. Our prior data suggest that pregnenolone, pregnenolone sulfate, progesterone, and allopregnanolone increase following treatment with pregnenolone (please see below for additional detail).

In our pilot randomized controlled trial with pregnenolone in schizophrenia (PDF attached), pregnenolone administration at the identical doses to those proposed in the current study did not significantly increase cortisol, testosterone, free testosterone, estradiol, androstenedione, or DHEA, however (Marx et al 2009, attached; please see table on p.10). A modest 20% increase was observed in DHEAS (Marx et al 2009). In this pilot study of pregnenolone in schizophrenia, as well as in subsequent investigations [specifically, a) a pilot investigation in OEF/OIF Veterans with mild TBI, b) a randomized controlled trial in schizophrenia enrolling 120 participants that was recently completed in Singapore, c) a pilot investigation in Veterans with PTSD, and d) a randomized controlled trial of 80 patients with bipolar disorder], pregnenolone was well-tolerated.

For the last several clinical studies with pregnenolone, we have quantified pregnenolone and pregnenolone metabolite levels after the trial was completed so that samples could be run in one batch with the same standard curve in order to minimize inter-assay variation. The FDA did not require monitoring of neurosteroid levels or neurosteroid metabolite levels during these studies. Neurosteroid and neurosteroid metabolite levels will thus be quantified at the completion of this study, as we have done with prior clinical investigations utilizing adjunctive pregnenolone as a pharmacological intervention.

Follow-up Plans:

Follow-up Procedures:

The follow-up plan after unanticipated withdrawal from the study (defined as withdrawal from the study at any point prior to completion and subsequent to randomization to study drug) will be to schedule a follow-up visit within 7-21 days of cessation of study participation. At this follow-up visit, patients will receive vital signs, laboratory testing (CBC, chemistry panel, liver function tests) and the Columbia Suicide Severity Rating Scale. We will also make certain that the patient has a follow-up appointment with his/her PCP or mental health care provider, and that the patient has adequate refills of psychiatric medications until the next scheduled appointment (if taking psychiatric

medications). If there are any medical issues to be addressed, these will be discussed with the patient's PCP, psychiatrist, the internal medicine physician in the ER, or other relevant health care provider. For any urgent medical issues, patients will be escorted to the onsite Acute Care Clinic/Emergency Room at the Durham VA Medical Center, which provides 24/7 services. For non-urgent medical issues, patients will be scheduled for an outpatient appointment with their PCP or other health care provider.

The follow-up plan for patients who complete the entire study (6 visits total over the course of 10 weeks, consisting of a 2-week single-blind placebo lead-in period followed by randomization to 8 weeks of adjunctive pregnenolone or placebo) will be an additional follow-up visit to be conducted within 7-21 days after the final visit. At this follow-up visit, patients will receive vital signs, laboratory testing (CBC, chemistry panel, liver function tests), and the Columbia Suicide Severity Rating Scale. Similarly to the above follow-up plan for unanticipated withdrawal, we will also make certain that the patient has a follow-up appointment with his/her PCP or mental health care provider, and that the patient has adequate refills of psychiatric medications until the next scheduled appointment (if taking psychiatric medications). If there are any medical issues to be addressed, these will be discussed with the patient's PCP, psychiatrist, the internal medicine physician in the ER, or other relevant health care provider. For any urgent medical issues, patients will be escorted to the onsite Acute Care Clinic/Emergency Room at the Durham VA Medical Center, which provides 24/7 services. For non-urgent medical issues, patients will be scheduled for an outpatient appointment with the patient's PCP or other health care provider.

PAYMENTS TO SUBJECTS

Subjects will be paid $75.00 for each study visit (six study visits total, plus a follow-up visit or $525.00 total if a subject completes all seven study visits). If a subject attends two study visits, the subject will be paid $150; if s/he attends 3 study visits, s/he will be paid $225, etc.). There are no research-related costs to subjects participating in this study. Participants will be provided with a paper voucher, which is redeemable for cash at the Durham VAMC Cashier's Office (payment for funds will be provided by the VA).

There will be an additional allowance for travel to the Durham VAMC based on the distance traveled by the subject. The travel allowance will be approximated by the distance traveled. Travel allowance for several cities and major towns in VISN6 are included in the table below. There will be no further compensation for meals, parking, childcare, lost wages, etc.

Distance (roundtrip miles)	Allowance	Typical towns and cities
0-50	$10	Raleigh, Durham, Cary, Chapel Hill
50-100	$20	Henderson, Wake Forest, Burlington
100-200	$30	Greensboro, Fayetteville, Goldsboro
200-300	$40	Salisbury, Greenville, Rocky Mount
300-400	$50	Wilmington, Charlotte, Hickory
> 400	$60	

DATA AND SAFETY MONITORING

Sources of Materials: All of the data for this study will be collected specifically for research purposes. All study data will be kept in separate locked file cabinets, in locked offices within locked (card accessible only) building and will be accessible only to qualified research personnel (in VAMC Building [redacted], Room [redacted]; Building [redacted], [redacted] floor; or Building [redacted], [redacted] floor). Access to research data will be removed for staff that are no longer part of the study team. Each subject will be assigned a study number and all data and specimens will be identified by that study number. The list linking this number to subject identity will be kept in a file on a secure VA network drive ([redacted]), accessed through a password-protected computer to which only the Principal Investigator and study staff have access. Seven sources of data will be maintained for this study: a) the initial telephone screening, b) diagnostic interviews, c) physical examinations, d) psychiatric rating scales and cognitive assessments (both self-report and clinician-administered, e) genetic analyses, and f) neurosteroid profiles, other small molecule and protein results, and g) laboratory assessments and ECG results.

Data Monitoring and Confidentiality: Reports from subjects' clinical records concerning research observations will not be made available to outside medical facilities without the written consent of the patient. All clinical and biological data obtained from research interviews and the laboratory will be de-identified. The data will be kept in locked file cabinets, in locked offices within locked (card accessible only) buildings and will be accessible only to qualified research personnel (in VAMC Building [redacted], Room [redacted]; Building [redacted], [redacted] floor; or Building [redacted], [redacted] floor). and kept in accordance with VA Records Control Schedule. Only study numbers will appear on data, documents and biological specimens used for evaluation or statistical analysis (i.e., de-identified). The key-code linking subjects to study data identified by study number will be kept on a secure VA network drive ([redacted]). In addition, any publications resulting from this research will not identify individual subjects.

PRIVACY AND CONFIDENTIALITY

Patient confidentiality will be maintained through the assignment of patient identification numbers. These numbers will be used in keeping of all research records. All hard copy research materials will be kept in locked file cabinets with keys available only to the principal investigator and research personnel participating directly in this protocol. Access to research data will be removed for staff that are no longer part of the study team. Patients will be informed during the consent process about the limits of confidentiality.

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

-What protected health information (PHI) will be collected from subjects in this study
-Who will have access to that information and why
-Who will use or disclose that information
-The rights of a research subject to revoke their authorization for use of their PHI.

PHI collected as part of this study will include: name, address, telephone number, date of birth, dates of service, dates of attendance, social security number, gender, close relative's name, close relative phone number, marital status, race, education level, work status, employer, length of employment, occupation, service connection (reason, percentage), disability status, medical and mental health diagnoses and treatments, medication, substance use, history of suicide attempts, family history of suicide attempts, smoking status, incarceration history, twin status, military service (time period, branch, number of tours, whether or not subjects served in a war zone, fired a weapon in a combat situation, were under enemy fire, were wounded in a war zone, awarded medals or were prisoners of war). Genetic data will also be collected.

As part of the study (and as indicated in the HIPAA Authorization), patient information and medical and/or research records may be disclosed to the Office for Human Research Protections (OHRP), the VA Office of the Inspector General (OIG), the Office of Research Oversight (ORO), other government agencies, the Durham VAMC Institutional Review Board (IRB), and/or local Research Compliance Officers, and Food and Drug Administration (FDA).
In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization.

INFORMATION SECURITY

Collection, Use and Storage of Research Information: Participant data will be completely de-identified according to 18 identifiers listed in Appendix B of the VHA Handbook 1605.1, and participant information will be identifiable only by a unique, linked numerical code. The master key code will be maintained on the secure VA network drive ([redacted]) and which is accessible only through a VA, password protected computer (in Building [redacted], Room [redacted]; Building [redacted], [redacted] floor; or Building [redacted], [redacted] floor). All electronically recorded PHI (i.e., any patient identifying information) will be maintained in a separate file on a secure VA password protected network drive ([redacted]) on a VA computer. Any PHI collected on paper which needs to be maintained (i.e., Informed Consent Form, HIPAA Authorization, Subject

Payment Forms, etc.) will be stored in a folder separate from deidentified paper forms (i.e., study assessments). These two folders will be filed in two different locked file cabinets inside a locked room within the Durham VAMC (Building [redacted], Room [redacted]; Building [redacted], [redacted] floor; or Building [redacted], [redacted] floor). Only approved VA researchers and study personnel will have access to this information. No PHI will be removed from the Durham VAMC. De-identified information collected using paper forms (i.e., study assessments) will be stored in a locked filing cabinet inside Room [redacted] in VAMC Building [redacted]; Building [redacted], [redacted] floor; or Building [redacted], [redacted] floor. These paper forms will be entered into a database stored on a separate, secure, password protected database ([redacted]) within the Durham VAMC (so that information cannot be linked to participant PHI) by study personnel and will be stored indefinitely according to VA guidelines. Paper form and electronic data will be destroyed in accordance with the Records Control Schedule 10-1, which currently does not allow for the destruction of research data.

Patient blood and serum samples will be collected during the study visit(s) and coded with a corresponding unique, linked numerical code (i.e. the study ID number). Specimens will be completely de-identified. Blood and serum samples will be stored at the Durham VAMC in a locked freezer room in Building [redacted] or [redacted] located in a secure research wing (requires special permission and a security activated badge to gain access) within the Durham VAMC.

Subjects will be asked to provide permission to store their contact and demographic information and study interview results in a secure database (an excel spreadsheet maintained by the PI and her study staff on a secure VA server) to be used to select potentially eligible participants for other ongoing and future studies. If patients provide permission to do so, they may then be re-contacted in the future and invited to participate in other studies for which they may qualify, and of which Dr. Marx is an investigator.

Any incident regarding theft/loss of data, authorized access of sensitive data or storage devices or non-compliance with security controls will be addressed according to VA policy (VHA Handbook 1200.05, 10j; 1058.01 11.a; 6500, Appendix D and 6500.2) and will be reported to Durham VAMC PO and ISO immediately.

Study members who are initially part of the study team, but leave the study team for whatever reason will no longer have access to participant study data.

Risks/Benefit Assessment:
Subjects will NOT be tapered from their current stable medication regimen; adjunctive pregnenolone treatment will be "add-on" only to treatment-as-usual. Pregnenolone is available over-the-counter as a nutritional supplement. Pregnenolone has been well-tolerated at the doses proposed in this study. Previously reported uncommon adverse reactions include headache, rash, insomnia, stomach upset, a report of palpitations in the existing literature. We will perform ECGs at Visits 1 and 6 to closely monitor patients, and perform a Chem 7, GI panel, and CBC at each study visit. Additional laboratory tests will be conducted at Visit 1 and 6 (TSH, etc.). Blood draws at each visit are minimal risk. Possible blood draw side effects include bruising, bleeding, or pain at the injection site, and (rarely) fainting and infection. It is possible that pharmacological intervention with pregnenolone will improve functional outcomes, cognitive symptoms, pain symptoms, fatigue, and global psychological functioning in Gulf War Veterans with GWVI.

Potential Benefit of the Proposed Research to the Subject and Others: While study participants may not receive benefits from the proposed research other than monetary compensation, their participation may lead to a better understanding of Gulf War symptomatology. For those not currently treated, study participation may lead to referral for treatment upon completion (or withdrawal) of the study. In terms of benefit to others, knowledge gained from the study may help the evaluation and treatment of Gulf War Veterans with GWVI. No serious adverse events have been reported to date related to pregnenolone. Pregnenolone is available as a dietary supplement over-the-counter in the United States.

Data Management:

Monitoring: The database manager will review completed forms with the study coordinator and generate queries, which the study coordinator will resolve before data entry.

Data Monitoring/Quality Assurance: The study coordinator will check that all assessments have been completed and check all forms for accuracy and completeness prior to the termination of each session. The centralized database will provide information on which assessments are to be completed at each visit. In addition, the data entry procedures will notify those entering the data when information has been omitted. These procedures should greatly increase overall quality of the data. As described earlier, the data entry system itself forces data entry personnel to enter complete within range data or produce a reason, which is stored in the database.

Protocol Fidelity Monitoring: The data manager will continuously track adherence to the protocol, and will report any problems to the PIs and the statistician. Monitoring will include adherence to inclusion/exclusion criteria, completion of measures at each assessment point, following procedures for termination of patients from the protocol, and training and certification for all research personnel. The monitor and data manager will verify that valid informed consent has been obtained from all participants. Research personnel must log the date of informed consent, the staff member obtaining the informed consent, and that the participant has received a copy of the informed consent document. In addition, all participants must verify that they have provided informed consent and received a copy of the informed consent document. All these steps must be completed prior to entering any information about the participant into the database.

DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

In order to ensure the rigorous management of any conflict of interest issues in the data analysis:

1. All persons involved in this study will be completely blinded to treatment condition in this randomized controlled trial investigating adjunctive pregnenolone vs. placebo in Veterans with Gulf War Veterans' Illnesses for the duration of the study, including Dr. Marx, the study coordinator, and all staff in the research group. The randomization code will be known only to the VA pharmacist Jaime Brown, and the blind will be broken only in the event of a medical emergency.
2. Dr. Marx will not conduct any of the psychiatric assessments (cognitive rating scales, psychiatric scales, etc.). Psychiatric and cognitive assessments will be conducted by the clinical coordinator and/or other research staff only.
3. This project will involve independent PhD-level statisticians. Dr. Marx will be completely removed from the conduction of the statistical analyses. After clinical trial data are entered by research staff, double-checked, and cleaned, the database will be locked. No statistical analyses will be conducted prior to the locking of the database. Once the data are locked, a completely de-identified copy of the dataset (no PHI) will be sent to independent statisticians. Once the de-identified database is locked, the blind will then be broken by pharmacist Jamie Brown. Dr. Ryan Wagner will conduct the statistical analyses (the statistical analysis plan will be determined a priori), and this analysis will be confirmed by an independent statistician.
4. There will be a VA DSMB that will be overseeing this project.
5. Dr. Marx will promptly contact the Durham VA IRB Committee, the Durham VA R+D Committee, the VA DSMB, and the ACOS for Research at the Durham VA if a patent is issued or if a licensing agreement is negotiated.

POWER ANALYSIS: PHYSICAL COMPONENT OF THE SF-36 (Primary Endpoint).

Primary Endpoint Hypothesis: Gulf War Veterans with GWVI who were randomized to pregnenolone will demonstrate significantly greater improvements in the Physical Component Score of the SF-36 compared to Veterans randomized to placebo.

Numeric Results for Two-Sample T-Test

Null Hypothesis: Mean1=Mean2. Alternative Hypothesis: Mean1<>Mean2

The standard deviations were assumed to be equal. A prior investigation in Gulf War Veterans with GWVI determined mean baseline standard deviation in the SF-36 Physical Component Summary to be 7.5 (Donta et al 2003). We chose a more conservative SD estimate of 12.5 for this sample calculation. We utilized the baseline SF-36 score from the same investigation (Donta et al 2003) to estimate approximate baseline SF-36 scores in a Gulf War Veteran cohort with GWVI. Consistent with the literature in this area, we utilized a 7-point difference in the SF-36 as a clinically meaningful change from baseline for this sample size calculation (please see attached SF-36 scoring information in Appendix).

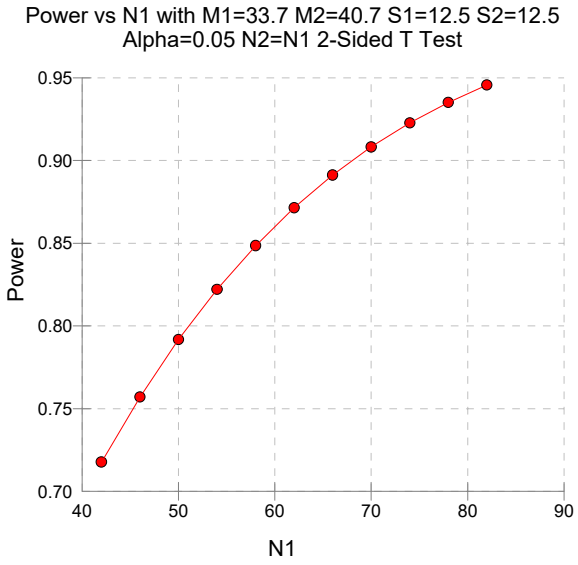
Power	N1	N2	Allocation Alpha	Beta	Mean1	Mean2	S1	S2
0.71774	42	42	0.05000	0.28226	33.7	40.7	12.5	12.5
0.75708	46	46	0.05000	0.24292	33.7	40.7	12.5	12.5
0.79175	50	50	0.05000	0.20825	33.7	40.7	12.5	12.5
0.82211	54	54	0.05000	0.17789	33.7	40.7	12.5	12.5
0.84855	58	58	0.05000	0.15145	33.7	40.7	12.5	12.5
0.87147	62	62	0.05000	0.12853	33.7	40.7	12.5	12.5
0.89124	66	66	0.05000	0.10876	33.7	40.7	12.5	12.5
0.90822	70	70	0.05000	0.09178	33.7	40.7	12.5	12.5
0.92276	74	74	0.05000	0.07724	33.7	40.7	12.5	12.5
0.93516	78	78	0.05000	0.06484	33.7	40.7	12.5	12.5
0.94569	82	82	0.05000	0.05431	33.7	40.7	12.5	12.5

Report Definitions

-Power is the probability of rejecting a false null hypothesis. Power should be close to one.
-N1 and N2 are the number of items sampled from each population; they should be small.
-Alpha is the probability of rejecting a true null hypothesis. It should be small.
-Beta is the probability of accepting a false null hypothesis. It should be small.
-Mean1 is the mean of populations 1 and 2 under the null hypothesis of equality.
-Mean2 is the mean of population 2 under the alternative hypothesis. Mean of population 1 is unchanged.
-S1 and S2 are the population standard deviations. They represent the variability in the populations.

Summary Statement

Group sample sizes of 70 and 70 will **achieve 90% power to detect a difference of 7 points** between the null hypothesis that both group means are 33.7 and the alternative hypothesis that mean of Group 2 is 40.7 with estimated standard deviations of 12.5 and 12.5 and with a significance level (alpha) of 0.05000 using a two-sided two-sample t-test. We thus have 90% power to detect a 7-point improvement in the SF-36.



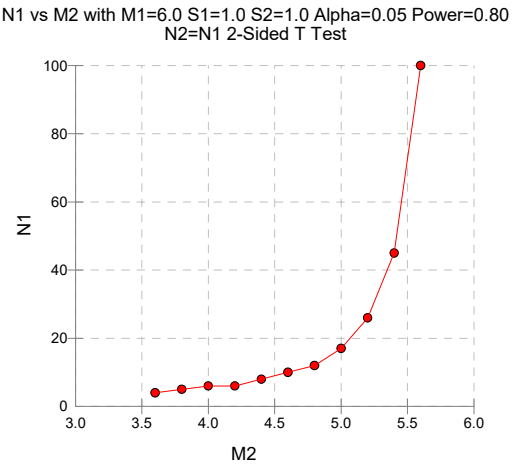
POWER ANALYSIS: PAIN SYMPTOMS (Secondary Endpoint)
Secondary Hypothesis #1: Treatment with pregnenolone will improve pain symptoms in Gulf War Veterans with GWVI.

The potential effect of pregnenolone on pain symptoms will be evaluated using several statistical methods. This clinical trial design will compare two randomly assigned groups (adjunctive pregnenolone or placebo) with respect to self-reported pain measured on a 1-10 pain scale. Inclusion criteria for the study require that all participants report pain severity of at least four. Given that pain often presents as long periods of low-level intensity pain punctuated by periods of acute exacerbation, it is likely that the mean pain level for selected participants will exceed four. Thus for power calculations, we assume a baseline mean pain level of six for each group. We assume a standard deviation of 1.0 (as we have little information to support any specific value in this population).

For the purpose of power calculations we assume that the mean pain levels of the two independent groups will be compared using a Two-Sample T-Test**,** Null Hypothesis: Mean1=Mean2. Alternative Hypothesis: Mean1<>Mean2. The standard deviations were assumed to be unknown and equal.

Assuming specified numbers of participants in each group (N) the mean differences detectable for at least 80% power and alpha=0.05 are shown in the following table and figure.

N1	Mean1	Mean2
4	6.0	3.6
5	6.0	3.8
6	6.0	4.0
6	6.0	4.2
8	6.0	4.4
10	6.0	4.6
12	6.0	4.8
17	6.0	5.0
26	6.0	5.2
45	6.0	5.4
100	6.0	5.6



Group sample sizes of 45 achieve at least 80% power to detect a difference of 0.6 between the null hypothesis that both group means are 6.0 and the alternative hypothesis that the mean of group 2 is 5.4 with estimated group standard deviations of 1.0 and 1.0 and with a significance level (alpha) of 0.05 using a two-sided two-sample t-test. We will have 70 Veterans per group, and should thus have **94% power to detect a difference in pain symptoms of 0.6** in the pregnenolone intervention compared to placebo.

POWER ANALYSIS: COGNITIVE SYMPTOMS (Secondary Endpoint)

Executive functioning as assessed by the Tower of London Test of the BACS cognitive assessment battery, Keefe et al.

The Tower of London component of the BACS cognitive assessment battery demonstrated improvements in the hypothesized direction in two of our prior pilot RCTs in Veterans with schizophrenia and mild TBI who had been randomized to pregnenolone (moderate effect sizes). We thus did power and sample size calculations for Tower of London executive functioning scores (secondary cognitive outcome measure) from estimates of means and standard deviations (SD) from preliminary data derived from our pilot RCT examining pregnenolone in OEF/OIF era Veterans with mild TBI. We conservatively utilized our pilot RCT in Veterans with TBI rather than our pilot RCT in Veterans with schizophrenia, as Veterans with schizophrenia demonstrated somewhat greater mean improvements in the Tower of London test (z-score change 0.61 greater than placebo). We used simplified models on change scores for power calculations as we do not know, and cannot reasonably estimate, the covariance parameters we would need to do these calculations in the context of the mixed models. We used correlations of 0.50 between all occasions, which is probably a conservative estimate (please see attached scoring information for BACS in Appendix).

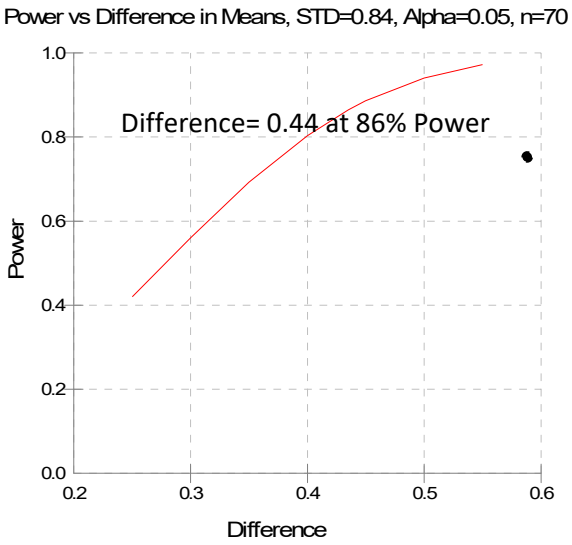
Secondary Hypothesis #2: Treatment with pregnenolone will improve executive functioning in Gulf War Veterans with GWVI, as assessed by the Tower of London test of the BACS.

Our preliminary data from our pilot RCT utilizing adjunctive pregnenolone in Veterans with mild TBI showed two estimates of standard deviation of change scores of 0.7546 in the placebo group and 0.9238 in the pregnenolone group. Our power analysis assumed a standard deviation consisting of the mean of these two groups = 0.8392, or approximately 0.84. Our preliminary data from our pilot RCT utilizing pregnenolone in OEF/OIF Veterans with mild TBI showed a difference between treatment and placebo of 0.435, or approximately 0.44. We did calculations for treatment differences ranging from 0.30 to 0.55 in the table below. To have 80% power to detect a difference of 0.44 between treatments (the difference in z-scores observed in Tower of London executive functioning scores in the pregnenolone group compared to the placebo group in our pilot RCT), we would require 61 subjects per group, or 122 subjects total. This may be a conservative estimate in that this assumes a correlation of 0.50 between baseline and endpoint, and the correlation is likely to be higher, and the mixed model is likely to have more power than the simpler model we used here. We will, however, have 70 subjects per group in the current investigation (in order to achieve 91% power for the primary hypothesis – that Veterans randomized to adjunctive pregnenolone will demonstrate significantly greater improvements in the SF-36 compared to Veterans receiving placebo), which will provide 86% power to detect a z-score change of 0.44 with a standard deviation of 0.84 for Secondary Hypothesis #1 as shown in the table and figure below:

Secondary Hypothesis #2: Tower of London Test of Executive Functioning

Power	N1	N2	Alpha	Beta	Mean Diff	Std Dev
0.559763	70	70	0.050000	0.440237	0.30	0.84
0.692311	70	70	0.050000	0.307689	0.35	0.84
0.803596	70	70	0.050000	0.196404	0.40	0.84
0.864561	**70**	**70**	**0.050000**	**0.135439**	**0.44**	**0.84**
0.886225	70	70	0.050000	0.113775	0.45	0.84
0.940490	70	70	0.050000	0.059510	0.50	0.84

0.972010	70	70	0.050000	0.027990	0.55	0.84



Summary Statement: 70 subjects per group will provide **86% power to test Secondary Hypothesis #2** that treatment with pregnenolone will improve executive functioning as assessed by the Tower of London test compared to placebo in Gulf War Veterans with GWVI.

Specific Aim 2: To conduct candidate biomarker investigations quantifying pregnenolone and pregnenolone metabolite levels (allopregnanolone, pregnanolone, androsterone, others) in serum samples at baseline, during treatment, and post-treatment with pregnenolone using mass spectrometry-based technologies, and to investigate potential genetic predictors of therapeutic response. These investigations will thus: a.) characterize the pharmacokinetics of pregnenolone and its metabolic profile - which could yield valuable dosing information for pregnenolone, characterize pharmacological windows of optimal therapeutic efficacy, and identify potential neurosteroid predictors of therapeutic response, and b.) examine single nucleotide polymorphisms (SNPs) of genes coding for enzymes involved in neurosteroid synthesis and metabolism. *Together these studies could contribute to the development of new neurosteroid interventional strategies that build on the current investigation and exhibit promise as pharmacological candidates in GWVI.*

We will thus determine if pregnenolone administration in Gulf War Veterans with GWVI increases downstream allopregnanolone and/or other GABAergic neurosteroid levels in serum post-treatment, representing potential mechanisms contributing to its therapeutic efficacy and candidate biomarkers for treatment response. Since little is currently known regarding the precise etiologies of possible neurosteroid dysregulation in CNS conditions, we will determine baseline serum neurosteroid profiles in Gulf War Veterans with GWVI enrolled this randomized controlled trial. We hypothesize that baseline neurosteroid levels will be inversely related to baseline functioning, cognitive symptoms, pain symptoms, fatigue, and overall psychological symptoms. In addition, we will identify the specific metabolism profiles of pregnenolone following eight weeks of treatment with this neurosteroid in order to characterize potential mechanisms of action via conversion to downstream GABAergic and sulfated metabolites. Our preliminary data suggest that the characterization of neurosteroid alterations post-treatment with pregnenolone may elucidate candidate biomarkers for therapeutic response to this intervention.

Statistical Analysis Plan

Preliminary Exploration: We will explore the data using descriptive statistics and graphical techniques prior to any hypothesis testing. For categorical variables, we will examine frequency distributions and where appropriate contingency tables and histograms. For continuous variables, we will examine frequency distributions and where appropriate stem-and-leaf plots and box-and-whisker plots. When appropriate, we will consider transformation. If

necessary, due to distributional considerations, we will consider a change of analysis method to a less parametric one. For time-to-event variables, we will use Kaplan-Meier plots to obtain a global picture of the patterns in each of the treatment groups.

General Modeling: Most of our hypotheses specify continuous response variables assessed longitudinally. For those models, we will use mixed models repeated measures models. There are three assessment points for the primary and secondary outcome measures (to be administered at Visits 2, 4, and 6; each study visit is two weeks apart). We will use an AR(1) covariance structure for the repeated measures. We will fit the models preliminarily using AR(1) covariance structures and using unstructured covariance structures, and use the difference between the two -2 log likelihood functions to construct a likelihood ratio test of the advantage of the unstructured covariance structure. If the unstructured covariance structure does not fit significantly better, we will use an AR(1) structure.

We will examine group differences in least squares means at each of the major time points (Weeks 0, 4, 8 of treatment post-randomization) as described in the plans for specific hypotheses. Distributional assumptions will be examined using residuals. We recognize that it is always possible that these mixed models may fail to converge or encounter difficulties based on their use of asymptotics. In that case, we will move to analyses that assume compound symmetry, and use a Huynh-Feldt correction for the extent to which compound symmetry fails to be met.

Missing data: The mixed models used to evaluate the continuous response variables are able to handle moderate amounts of missing data provided they are missing at random. We will examine the missing at random assumption by assessing differences between dropouts and completers on baseline as well as response variables up to point of premature withdrawal. If the missing at random assumption does not appear to be tenable, we will report the mixed models results but spend additional effort characterizing treatment effect at time of premature withdrawal.

Statistical Computing and Programming: All statistical computing using the current version of SAS.