Study Title: Neurosteroid Intervention for PTSD in Iraq/Afghanistan-era Veterans

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Statistical Analysis Plan

Neurosteroid Intervention for PTSD in Iraq/Afghanistan-Era Veterans

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1 Overview

1.1 Synopsis

The PTSD-Pregnenolone study is a randomized, placebo-controlled, double-blind, single-center trial of treatment with pregnenolone or placebo to evaluate the safety, tolerability, and efficacy of pregnenolone in treating Iraq/Afghanistan-era Veterans with PTSD. The total study duration is 10 weeks. Following a 2-week placebo lead-in period, the study will enroll and randomize 90 participants to receive pregnenolone (n=45) or placebo (n=45) for 8 weeks (fixed escalating dose strategy). There is one follow-up phone call after the final study visit.

The primary endpoint will be the change from baseline to treatment week 8 in the Clinician-Administered PTSD Scale-5 (CAPS-5) total score, a measure of PTSD symptom severity. There are two key secondary endpoints, the change from baseline to treatment week 8 in co-occurring pain as measured by the Brief Pain Inventory (BPI), and the degree of depression as measured by the Hamilton Depression Inventory Scale (HAM-D). Safety and tolerability will be assessed through the evaluation of adverse events, laboratory measures, physical examinations, vital signs, 12-lead ECG measurements, and concomitant medications.

1.2 Study Objectives

Primary Objective

The primary objective of the study is to evaluate the effect of pregnenolone on safety, tolerability, and degree of reduction of PTSD symptoms in Iraq/Afghanistan-era Veterans with PTSD. In addition, two secondary endpoints will assess the effect of pregnenolone on co-occurring pain and depression.

Secondary Objectives

The secondary objective of the study is to quantify serum neurosteroid levels at baseline and post-treatment by highly sensitive and specific mass spectrometry-based techniques, to determine whether pregnenolone and downstream neurosteroid metabolites such as allopregnanolone (and/or other biomarker candidates such as inflammatory markers) are predictors of therapeutic response.

2 Study Design

2.1 Overview

Approximately 90 participants will be enrolled at a single study site, the Durham VA Healthcare System. All participants will take part in a 1-week placebo lead in period. Approximately half of the participants will then be randomized to receive the same regimen of pregnenolone, consisting of a single

dose of 50 mg BID x 2 weeks (100 mg daily), 150 mg BID x 2 weeks (300 mg daily), 250 mg BID x 4 weeks (500 mg daily). The other half of the participants will be randomized to receive a matching blinded placebo regimen.

Safety and efficacy measures will be collected at multiple visits and via phone calls throughout the study period. The specific timing of assessments is reflected in the Study Procedure Table within the study protocol.

2.2 Randomization

Participants will be randomized in a 1:1 ratio to pregnenolone or placebo. The randomization code is developed and held by the VA research pharmacist. Treatment assignments are not provided for use in statistical analysis until all data collection is finished and the clinical database has been finalized (locked).

2.3 Data Sources

The clinical study data and assessments are housed in a MIRECC data entry tool developed at the Durham VA Healthcare System.

Hillside adverse events data was source-gathered in the MIRECC data entry tool but processed and housed in a MS Access database.

Clinical laboratory measures of chemistry, hematology, urinalysis, and pregnancy and drug testing will be generated within the usual hospital lab processing and retrieved from the VA Clinical Data Warehouse.

Randomized treatment assignments (unblinded) will be provided by the VA pharmacist after data finalization.

Plasma concentrations of pregnenolone and other neurosteroids will be measured by a central core laboratory.

All raw data is extracted from its source and stored as SAS databases for analysis.

3 Analysis Populations

The following populations will be analyzed in this study:

Safety population: All randomized participants that received at least one dose of study medication.

Modified Intent-to-treat population (mITT): All randomized participants that received at least one dose of study medication and at least one post-baseline visit.

4 Analysis Methodology

The study protocol summarizes the planned analyses, and some details are outlined below.

4.1 General Approaches

The statistical analysis will include a descriptive summary of all data. Continuous measures will be summarized by their mean, standard deviation, median, 25th percentile, and 75th percentile, as well as the number of patient observations for which the measure is recorded. Categorical measures will be presented as the number and percentage of participants per category, within non-missing observations. Graphical summaries will be provided for both efficacy and safety measures to understand distributional characteristics and patterns during the study progression.

Statistical analyses and graphics will be produced using SAS 9.4 (SAS Institute, Cary, NC).

4.2 Safety and Tolerability

The safety and tolerability of the experimental drug pregnenolone will be assessed by summarizing comparing an array of safety measures between the active and placebo arms within each cohort, and by displaying these measures for comparison between cohorts. The measures include vital signs, chemistry, hematology, treatment-emergent adverse events (AEs) and serious adverse events (SAEs), and 12-lead ECG findings (with focus on the QT interval) measures. The safety analyses will be conducted within the Safety population.

Adverse events will be presented if they are treatment-emergent. For the purposes of the analysis, treatment-emergent adverse events will be a new or worsening adverse event that occurs between the first dose of study drug and the end of study follow-up.

Clinical laboratory and ECG parameters will be listed, graphed over time, and descriptively summarized within each cohort and treatment arm. The descriptive summary will include the change from baseline at each time point as well as the maximum change from baseline in an undesirable direction during the study treatment period (increase, decrease, or both, as appropriate for each measure).

Similarly, blood pressure and heart rate will be presented as summary statistics within each cohort and treatment arm. The descriptive summary will include the maximum change in an undesirable direction during the study treatment period (increase, decrease, or both, as appropriate for each measure).

4.3 Efficacy: Analysis of the Primary and Secondary Endpoints

Tests of the primary and secondary endpoints will proceed through three stages: The initial stage will assess the distributional characteristics of the tested outcome to determine requisite regression form and

whether transformations are necessary. As a second step, a series of models will be estimated to assess linearity; in instances of non-linearity, measures will be transformed or quadratics will be added to models as necessary. Subsequent final models testing significance of the treatment condition will be as follows (assuming linearity): Hypotheses will be tested using LMM regression procedures using an ANOVA paradigm. The tested outcome will be regressed on a dichotomous treatment proxy (Preg=1; Placebo=0) variable, a second proxy variable denoting time (Weeks 2, 6 and 10), and an interaction term crossing treatment with time; models will also include the participant-level intercept as a random effect. Tests of the outcome measure will be based on a post-hoc least squares contrast of the difference between randomization levels at the end-of-study (Treatment week 8) time point. Hypotheses are structured to be unidimensional. Formally, support for the hypothesis will require rejection of the null hypothesis positing no difference between treatment groups in favor of the alternative (H0: βpregXseek10 = 0; H1: β pregXseek10 <> 0; < or > depending on the structure of the hypothesis).

The resulting mixed model repeated measures regression procedures will summarize the effect of treatment with pregnenolone on change from baseline was assessed using a model including the baseline value, a dichotomous variable denoting treatment arm, visit, and an interaction of treatment with visit. An unstructured covariance structure will likely be used with the Kenward-Roger approximation to adjust degrees of freedom. Least square mean (LSM) differences between treatment groups will be estimated, together with standard error (SE) and 95% confidence intervals (CI). The test of each hypothesis will be evaluated at the 2-sided alpha 0.05 level.

Efficacy analyses will be generated using participants in the modified intent-to-treat population.

4.4 Analysis of Serum Neurosteroids, Including Pregnenolone Levels

Serum neurosteroid levels at baseline and post-treatment will be analyzed to determine if pregnenolone and downstream neurosteroid metabolites are predictive of potential therapeutic response, and most especially the concurrent values of primary and secondary outcomes. The relationship between change from baseline in selected clinical outcomes and serum neurosteroid levels at treatment week 8 presented graphically alongside Spearman partial correlation coefficients at a minimum. This analysis is exploratory in nature and beyond the intended scope of this analysis plan.

5 Missing Data

While the protocol proposed imputation approaches for missing data, under the current plan only available data will be reported without applying data imputation methods unless a larger than anticipated amount of incomplete data emerges.

6 Conventions for Statistical Output

All statistical output will be displayed consistently, and will include the following:

a. Consistent naming of column headers
b. Consistent font throughout

c. Table title centered
d. Unless otherwise stated, each cell will summarize the available data
e. Subheaders beneath main entries will be indented