Adjunctive Withania Somnifera (Ashwagandha) for Persistent Symptoms in People with Schizophrenia

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1. Background

Withania somnifera extracts (WSE) or Ashwagandha is a medicinal herb that has been used in India for more than 3000 years. Animal studies indicate that WSE has both immunomodulatory and anti-inflammatory actions. It enhances type-1 immune response and cytokine production, modulates the production of acute phase reactants, and inhibits COX-2 and NF-kB inflammatory signaling pathways (Mishra, Singh et al. 2000, Bani, Gautam et al. 2006, Khan, Ahmad et al. 2006, Malik, Singh et al. 2007, Mulabagal, Subbaraju et al. 2009). Since immune-inflammatory dysregulation has been associated with symptom exacerbations in schizophrenia, Chengappa and co-workers (personal communication) recently completed a study in which schizophrenia patients experiencing a recent symptom exacerbation were randomly assigned to receive 500 mg bid of WSE or placebo. WSE treated patients demonstrated greater improvement in Positive and Negative Syndrome Scale (PANSS) total, negative, and general symptoms compared to placebo treated subjects. WSE also resulted in greater improvement on the Perceived Stress Scale (Cohen, Kamarck et al. 2000). The purpose of the proposed study is to replicate these encouraging findings using similar methods at sites other than the University of Pittsburgh. The selected sites are University of California, Los Angeles (UCLA) and the Maryland Psychiatric Research Center (MPRC).

2. Study Objectives

a. Primary Hypotheses We posit that WSE (Sensoril®) will reduce psychopathology in persons with schizophrenia

> ***Specific Aim 1*** *To determine whether a standardized extract of Withania somnifera will reduce psychopathology scores (PANSS total score) in persons with schizophrenia. A secondary aim is to determine whether WSE reduces measures of positive and negative symptoms (PANSS subscales) and stress scores on the Perceived Stress Scale (PSS).*

Specific Aim 2 *To determine whether improvement on WSE is associated with a decrease in proinflammatory markers.*

The analyses to support the two specific aims are described in the Statistical Analyses section.

3. Study Design

3.1 Study Overview This proposal is a 12-week, double-blind, randomized, placebo controlled study of a standardized extract of WSE (target dosage: 1000 mg/day) added to ongoing antipsychotic treatment in mild to moderately symptomatic patients with either schizophrenia or schizoaffective disorder. Treatment will begin on day 1 at 250 mg oral route, BID. (500 mg/day WSE or placebo) and titrated to 500 mg, po; BID (1000 mg/day) in week 2, for the rest of the study, unless tolerability issues dictate a lower dosage. Psychopathology and adverse events will be assessed at every visit. High sensitivity C-reactive protein (hs-CRP) and other inflammatory markers will be measured at baseline and end of the study.

3.2 Timelines, Visits, Assessments, Rating Scales, Study Medications, Laboratory and EKG and Adherence

After obtaining written informed consent, patients will be screened for eligibility and those who meet all the inclusion and exclusionary study criteria will be randomly assigned (in a 1:1 allocation ratio) to receive double-blind adjunctive treatment either with WSE or placebo for 12 weeks (Visits 2 through 8) see table below describing Schedule of Events. Participants will be seen at scheduled visits unless they

need to be seen sooner. The screening and consenting visits and visit 8 will last nearly 2 hours; the other visits will last about 60 minutes.

3.3 Recruitment Targets and Timelines

Task	Year 1					Year 2					Year 3				
Months	1	3	6	9	12	1	3	6	9	12	1	3	6	9	12
IND and IRB approval															
Start – up															
Enrollment of subjects															
Completion of study procedures															
Data Entry															
Analyses/reporting /writing															

3.4 Schedule of Events

Approximate Visit Intervals	V1	V2	V3	V4	V5	V6	V7	V8
			2 wks	4 wks	6 wks	8 wks	10 wks	12 wks
Informed Consent, Urine Drug Test, Pregnancy test, Psychiatric Diagnosis and History, Medical History	X							
Labs, EKG[1]	X							X
PANSS, CGI, PSS[2]	X	X	X	X		X		X
MCCB	X				X			X
BP, Pulse,Temperature Weight, Height[3]	X	X	X	X	X	X	X	X
Sensoril or Placebo		X	X	X	X	X	X	X
Treatment Emergent Adverse Events or Serious Adverse Events			X	X	X	X	X	X

Visit Comments, Pill Counts[4]	X	X	X	X	X	X	X	X

Visit 1 can be split up (if needed) into 2 visits for screening and spread over 1 to 4 weeks. Visit 2 is the randomization visit. Following randomization, visit intervals for V3 through V8 can range from ± 4 days of the expected between interval visits; exceptions on a case by case basis by the study team.

Visits 1 and 8 will last roughly 2 hours. Visits 2, 3, 4, 5, 6, and 7 will last roughly 1 hour.

At Visit 1 – Urine drug test for illicit substances will occur as will a serum pregnancy test for women in the reproductive age group.

[1] Labs: CBC + Diff, Chemistry 14? (including liver and renal functions, electrolytes, blood sugar, and lipid profile), urine analysis, and hs CRP and serum for cytokine panels

[1] EKG: standard EKG

[2] The Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression Scale (CGI), Perceived Stress Scale (PSS),

[3] Height at screening only, waist circumference and BMI at screening and visit 8 or end of study.

[4] Pill Counts – will start at visit 3, i.e., post randomization

3.5 Outcome Instruments

Psychopathology will be assessed using the Positive and Negative Syndrome Scale that measures the severity of psychotic and non-specific symptoms and is commonly used in

schizophrenia clinical trials, and a global assessment of the severity of illness (CGI) as well as change, i.e. improvement or worsening. The Perceived Stress Scale (PSS) will also be utilized.

Positive and Negative Syndrome Scale (PANSS): (Kay, Fiszbein et al. 1987) The PANSS is a psychiatric scale used for measuring symptom severity of patients with schizophrenia. It is widely used in clinical trials in schizophrenia. The scale measures the two main types of symptoms in schizophrenia; positive symptoms, which refer to an excess or distortion of normal functions (e.g., hallucinations and delusions), and negative symptoms, which represent a diminution or loss of normal functions (e.g. speech, activity, motivation, etc.). The PANSS is a relatively brief interview, requiring 20-30 minutes to administer. The interviewer will be trained to a standardized level of reliability.

Clinical Global Impression Scale: (Guy 1976) The Clinical Global Impression Scale (CGI) for Severity and Change subscales is an assessment of the clinical impression of the severity of illness and the change in severity of symptoms. A severity score of 1 indicates subject is normal, not ill, 2 indicates borderline mentally ill, 3 indicates mildly ill, 4 indicates moderately ill, 5 indicates markedly ill, 6 subject indicates is severely ill and 7 indicates the subject is among the most extremely ill. Improvement or worsening or no change is noted in the change subscale. The CGI is widely used in clinical trials in psychiatry.

Perceived Stress Scale (Cohen, Kamarck et al. 2000) The Perceived Stress Scale (PSS) was developed to measure of the degree to which situations in one's life are appraised as stressful. Psychological stress has been defined as the extent to which persons perceive (appraise) that their demands exceed their ability to cope. The PSS has become one of the most widely used

psychological instruments for measuring perceived stress. It has been used in studies assessing the stressfulness of situations, the effectiveness of stress-reducing interventions, and the extent to which there are associations between psychological stress and psychiatric and physical disorders. Based on studies, the PSS predicts both objective biological markers of stress and increased risk for disease among persons with higher perceived stress levels.

MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al, 2008) The MCCB is intended to provide a relatively brief evaluation of key cognitive domains relevant to schizophrenia and related disorders. It was designed as an outcome measure for use in schizophrenia clinical trials. The MCCB is comprised of 10 tests, which assess seven cognitive domains. The MCCB composite score is a standardized mean of the seven domain scores. T-scores are standardized to normative data, and have an estimated mean of 50 and SD of 10 in the general healthy population. The administration of the MCCB takes approximately 70 minutes. The MCCB will be administered at baseline, week 6 and week 12.

3.6 Psychotropic Medications

Medications prescribed for psychiatric symptoms (antipsychotic agents, anti-depressants, anxiolytic and hypnotic agents, lithium and anticonvulsants) are captured at entry to the study and changes are recorded at each visit. Changes in medications, such as increases or decreases in dosages, addition of a new agent, or stopping an agent will be coded for in the statistical analyses. Further details are provided in Section 7.1.a (Efficacy and Safety Assessments) and Section 9. (Statistical Methods/Data Analysis) and 9.1.c. Psychotropic Medication Endpoints.

3.7 Laboratory Measures

Laboratory parameters will include the Complete Blood Count (CBC) with differential, a standard Chemistry 14 panel or similar that includes hepatic and renal functions, electrolytes, blood sugar, and lipids). A urine analyses will provide safety data necessary for inclusion or exclusion of study subjects. Women in the reproductive age group will have blood drawn for a serum pregnancy test which is an exclusion criterion. A urine drug test will be performed at baseline for illicit substances and the results of this testing will also assist in excluding or including subjects. An EKG will be read by a qualified physician.

Bloods will be drawn at baseline and endpoint to allow for hs-CRP and measures of inflammatory and related markers. This will include 1 tube for the hs-CRP to be sent to Quest (or other) commercial laboratory. Two other tubes (EDTA) will be drawn, centrifuged and 2 aliquots from each tube will be stored on freezer tube (total 4 cytokines and inflammatory marker aliquots).

Serum or plasma hs-CRP will be measured. (Please see ***Schedule of Events***).

3.8 A review of medical conditions will be conducted by research staff including medical history and medications received for various medical conditions will be recorded. Vital signs will include blood pressure, pulse, height and weight. Laboratory tests and EKG, as noted in the Schedule of Events Table, will be conducted as part of the screening and safety assessments. Women of reproductive age will have a serum pregnancy test at screening. A psychiatric history using the SCID will be conducted, and if needed additional history from the treating clinicians and/or review of medical charts will be obtained to confirm the psychiatric diagnosis.

Once the subject is randomized, adherence will be monitored by pill counts during visits, i.e., the difference in the number of pills the participant should have taken during the visit interval versus those reported as taken by the participant. Participants will be counseled to bring back pill containers at each visit.

4. Participant Selection

4.1 Human Participant Involvement and Characteristics.

The total number of participants to be randomized will be 66, which will male and female adults with DSM 5 schizophrenia or schizoaffective disorder, of any race and between the ages of 18-64. We anticipate screening nearly 90 people in order to obtain 66 participants who meet the inclusion/exclusion criteria and provide evaluable data. This number is based on an anticipated screen failure rate of 25%. Participants will be research subjects from MPRC (N=33) and UCLA (N=33).

We intend to recruit participants whose symptoms are in the mild to moderate range, whose antipsychotic medications have been received for ≥ 4 weeks and who do not require imminent hospitalization. In addition, participants will have had a recent positive symptom exacerbation prior to study entry. **(Please see inclusion/exclusion criteria for details.)**

4.2 Sites and Method of Recruitment

The study sites will be the research programs at UCLA and the MPRC. As in past studies from these sites, the psychiatrists and clinicians will be provided information about the study. Potential research participants may be first identified by their physician/clinical care team. The clinician will discuss the research project with the potential participant**.**

For people who contact the research team directly in response to IRB-approved advertisements, a telephone screen is conducted. The nature of the questions asked in the research screening interview are fairly consistent with the standard operating procedures for determining the need for treatment for any person whether research eligible or not.

Recruitment will also be sought from the UCLA and MPRC registries of previous research participants who consented to be recontacted.

If the potential participant can provide informed and competent consent to participate in the study, and wishes to participate, the full consent document will be signed by the person, and the study physician or research staff will sign the certification statement on the consent at the time of obtaining the consent. Consenting will include a quiz prior to signing to ensure the participant understands all procedures.

Should any change in the risk/benefit ratio occur after the person has begun to participate in the study, the participant will be informed of the new information. Once the investigator is notified of the change, all participants will be told verbally about the new information. Once the consent has been revised to include the change and the document is approved by the IRB, the new consent form will be reviewed with each participant and signed by him/her if he or she wishes to continue to participate in the study.

The study protocol specifies two sets of criteria for participation: inclusion criteria, and exclusion criteria, which are listed below:

4.3 Inclusion Criteria

a) Adult males or females (18 to 64 years)

b) DSM 5 diagnosis of schizophrenia or schizoaffective disorder

c) Ability to provide informed written consent

d) PANSS total score ≥ 70 and at least 2 positive symptom subscale items (i.e., delusions, conceptual disorganization, hallucinatory behavior, excitement, grandiosity, suspiciousness/persecution, hostility and unusual thought content) scoring ≥ 4, **or** one of these items scoring ≥ 5, on a scale ranging from 1 = absent to 7 = extreme.

e) Receiving anti-psychotic medications for ≥ 4 weeks

f) evidence of a positive symptom exacerbation during the year prior to study entry. Evidence of an exacerbation may be identified from medical records; by a need to increase the individual's psychiatric medication; by a need for an increased level of care; or through a history from the participant's treating clinician.

g) For women of child bearing age, a negative serum pregnancy test at screening.

4.4 Exclusion Criteria

a) Testing positive for illicit substances (positivity to marijuana or opioids will be assessed on a case by case basis due to the long elimination half life in the urine of marijuana and the use of opioids for various pain disorders, , caffeine and nicotine are excepted)

b) Receiving pharmacological treatment for addictions (naltrexone, suboxone, acamprosate, others) will be reviewed on a case by case basis

c) Seriously unstable medical illnesses

d) Pregnant or breast feeding women

e) Known allergy or history of serious adverse event with WSE

f) Subjects who may require imminent hospitalization (examples: suicidal or aggressive behavior)

g) Currently receiving antibiotics, anti-viral, or anti-parasitic medications

h) Currently receiving immunosuppressive medications (e.g. oral scheduled corticosteroids, chemotherapy or transplantation or HIV/AIDs associated drugs).

5.0 Study Treatments

Study drug will be dispensed by the research pharmacies at UCLA and MPRC. All unused study medication and empty bottles will be returned at each office visit.

During each office visit, the participant will be asked about symptoms, any side effects and questions relating to his/her health in general. If any side-effects emerge, the dosage can be decreased or the titration can be stopped. Participants who cannot tolerate at least 500 mg/day of Sensoril® will be discontinued from the study. Participants who experience side-effects after reaching the higher dose (1000 mg/day) can be tapered to a minimum of 500 mg/day of Sensoril®.

Placebo capsules made of gelatin will be provided by Natreon Inc., who will also provide Sensoril® capsules, each of 250 mg strength. Details of the Sensoril® and placebo capsules can be found in the **section 5.4** (drug supplies)

5.1 Allocation to Treatment

Sensoril® (or placebo) will be administered using a random 1:1 assignment starting at a dose of 500 mg/day (1 capsule of 250 mg strength twice a day). Then the study treatment dosage will increase in the second week to the target of 1000 mg/day; (2 capsules of 250 mg strength twice a day) as an adjunct to existing antipsychotic treatment. The dose of 1000 mg per day (or less, i.e., a minimum of 500 mg if tolerability is an issue) will be continued for the rest of the study. The randomization numbers will be computer generated and the blinded assignments maintained in the pharmacies.

5.2 Breaking the Blind

Blinding will be maintained by the dispensing pharmacists at the UCLA and MPRC research pharmacies and so the clinician team and study participants will remain blind to treatment assignment throughout the tenure of the study.

If unintentionally or intentionally (see next paragraph) the blind is “broken,” data collection will be stopped at that point for that individual participant. A case by case basis for completion of study procedures (i.e., even if efficacy data collection is stopped, safety data collection will

continue), if the participant would prefer to complete the study and the investigative team sees no reason that may harm the participant or the study goals, study completion may be permissible based on discussion with the Data and Safety Monitoring Board (DSMB).

If unblinding is absolutely necessary, the study PI will contact the research pharmacist to obtain the treatment assignment information and notify the appropriate entities as soon as feasible. (e.g. DSMB, IRB)

After completion of the entire study and after the data bases are "locked" and statistical analyses have been completed, if participants would like to know their treatment assignment, the research team will provide them that information.

Statistical analyses of primary and secondary outcomes will be carried out "blind" to treatment assignment. The treatment assignment to Sensoril® or placebo will be revealed upon completion of the main statistical analyses.

5.3 Treatment Adherence/Study Compliance

Once the dispensing of the medication begins, adherence will be recorded by the study coordinator on log sheets in the research binder during each visit. Adherence will be based on discussions with participant and returned capsule counts. Participants who withdraw consent or whose clinical status worsens to prevent continuation in the study will be withdrawn and stabilized under standard clinical care conditions. There is no open continuation phase in this study. Participants will be counseled about this issue prior to initiating the study and advised

that Sensoril® may be obtained over the counter or via the internet through a general nutritional supplement outlet.

Participants who are found to be non-compliant with daily dosing of Sensoril®/placebo for a period of 7 consecutive days during the 12 week study period, or are otherwise found to be significantly non-compliant with study procedures and/or investigator instructions may be withdrawn from study participation.

If there are more dropouts than anticipated and other considerations (e.g. budget, etc.) permit more participants to be recruited, we may modify our protocol and consent and request the IRB to recruit more participants until we have 66 participants with evaluable primary efficacy data.

6. Drug Supplies

6.1. Formulation and Packaging

Placebo capsules made of gelatin will be provided by Natreon Inc., who will also provide Sensoril® capsules, each containing 250mg of standardized WSE extract. The inactive ingredients in the Sensoril® capsule will comprise microcrystalline cellulose NF 102 – 50 mg, croscarmellose Sodium NF – 10 mg, silicon Dioxide, Fumed NF (Cab-0-sil) – 3mg, magnesium sterate, NF – 3mg. The placebo capsules will only contain the inactive ingredients, and the fill weights of the Sensoril and placebo capsules will be the same; except the placebo capsules will **not** contain WSE extract. The placebo and Sensoril® capsules will be in coded hard-gelatin capsules, identical in color (opaque white), size, texture and shape. Based on our previous

clinical trial experience, to mask the smell, cloth pouches which are closed and contain Sensoril® powder will be placed alongside the placebo capsules and the smell will permeate the placebo capsules. The Sensoril® or Placebo capsules will have a “spice-like” odor. For individual participant dispensing-please see next section 5.4.b.

6.2. Drug Administration

The study medicine (Sensoril® or Placebo) will be prescribed as follows: 1 study capsule 250mg p.o. BID (total daily dose = 500mg) week 1; and 500mg p.o. BID (total daily dose = 1000mg) starting the second week to the end of the study. A lower dosage is permissible for tolerability issues; however, those participants unable to tolerate at least 500 mg/day will be discontinued from the study.

6.2. Concomitant Medications

Furthermore, medications for hypertension, diabetes mellitus and others for various medical conditions, can continue as usual. Physicians and clinicians involved in the routine psychiatric/medical care of the participant will continue to see them as usual.

Participants will be requested to let the research staff/prescribing study physician know prior to taking any new medication (including over-the-counter drugs and herbal supplements) during their participation in this clinical investigation.

7. Study Procedures

7.1. All participants will receive a **screening visit** to determine study eligibility. Diagnosis will be verified by history, chart review and discussion with the clinicians of the potential participant. The consent form will take into consideration that such permission to contact the treating clinician is warranted not only to affirm the diagnosis, but that lack of imminent suicidal or aggressive behavior or hospitalization can also be verified. The diagnosis of schizophrenia or schizoaffective disorder and comorbid Axis-I disorders will be affirmed using the SCID. Initially eligible participants will then have a review of their medical history, have their blood pressure, pulse, weight and height recorded; (including a serum pregnancy test for women of child-bearing potential), EKG and laboratory assessments.

The specifics of the inclusion and exclusion criteria are provided in the Participant Selection section.

Participants with **co-morbid disorders** will be allowed to participate provided the co-morbid condition was diagnosed more than 3 months ago, and no pharmacological treatments for these disorders were changed in the 3 month period prior to screening. This 3-month period will permit participants whose co-morbid conditions are stable but not the current focus of treatment to enter the study. Participants who have previously experienced serious side-effects with WSE will be excluded.

7.2. Study Treatments, Assessments and Follow up

After all screening procedures have been completed and reviewed and the participant remains eligible for the study, the participant will receive either Sensoril® or placebo at visit 2. The

participant will get one of these medicines by chance (like flipping a coin), and have a 1 in 2 chance of getting either Sensoril® or placebo. The medicine is packaged identically so that it is not possible to tell which is Sensoril® or which is placebo. The medicine will be given to the participant as capsules, and is taken by mouth. Starting at 1 capsule twice daily, the medicine (or placebo) will be increased to 2 capsules twice daily a week later, unless the participant is experiencing side effects. Neither the participant, or the doctor or the research staff will know if the participant is receiving Sensoril® or placebo.

If any tolerability issues emerge, further titration of study medicines can be stopped at a minimum 500 mg/day. If dosage has been increased to 1000 mg/day it can be decreased to 500 mg/day for side-effects.

Details of procedures at each visit (Visit 1 to 8) are described in the schedule of events (**3.2.c**.). Psychopathology ratings (details of the scales were described in **Section 3.2.d** earlier) are briefly mentioned in section 7.1.a. Safety assessments will include determination of side effects at each visit, and depending on the side effects, further clinical action will be undertaken. Laboratory measures at baseline and end point will provide more safety data as will careful monitoring of physical and psychological status at each visit.

If the participant would like to know the treatment assignment at the end of the data analyses and if a contact address or phone number is left with research staff, we will attempt to notify them of their treatment assignment during the study.

7.3. Efficacy and Safety Assessments

As noted in the Schedule of Events table, psychopathology (PANSS, CGI) and stress (PSS) scales will be assessed at every visit and will form the measurements required for affirming the primary efficacy outcomes, and will inform future studies about the size of the WSE treatment effects. These scales will also assist in monitoring psychopathology and stress as safety concerns.

Psychotropic Medications

These medications are listed in the database by name, daily dosage, start and stop dates. This will permit coding of increases (or decreases) in doses of existing medications, stopping of existing medications (e.g. stopping an existing antipsychotic and starting a new one), etc. Further discussion of the data analysis is provided in section 9.1.c – Psychotropic Medication End Points

Safety Assessments

During the screening process, a review of the medical history, recording of vital signs, laboratory and EKG assessments will provide safety data to the PI and research staff, and help them assess suitability of the subject for the study.

Side-effects will be monitored at each visit, by asking an open ended question and following up with the MPRC Side Effect Checklist. Based on the nature of the side effects, these may be attended to by either reducing the dosage, discontinuing study capsules, with follow-up

laboratory and/or physical examinations, or consultations, until resolution occurs. Recording of vital signs, the use of psychopathology and stress rating scales will further assist in evaluating safety of participating participants.

7.4. Data and Safety Monitoring Board (DSMB)

The MPRC has established a Data and Safety Monitoring Board (DSMB), which is comprised of two psychiatrists, a statistician, a pharmacist, and a community representative. The psychiatrists are experts in the clinical treatment of people with schizophrenia. None of the DSMB members reviewing this study will be investigators on the proposed project; however, Dr. McMahon serves as a non-voting member of the DSMB to assist in the monitoring of all MPRC trials. The DSMB will be charged with the following responsibilities: 1) to review the proposed protocol; 2) to monitor study progress and the occurrence of side effects/adverse events, and serious adverse events throughout the course of the study; and 3) to review with Dr. McMahon, the statistician for the project, the study data management system. The DSMB will review reports on protocol progress, side effects and adverse events at least once a year. In addition, all serious adverse events (SAEs) will be reported to the DSMB, Dr. Buchanan, and the University of Maryland School of Medicine IRB. Dr. Buchanan will receive all SAE reports within 24 hours of their occurrence. The DSMB and University of Maryland School of Medicine IRB will receive the reports within 48 hours. If the incidence of any side effect/adverse event is 25% or more or any SAE occurs in excess in either treatment group, then the DSMB will notify the P.I.s. The P.I.s and DSMB will determine whether possible protocol modifications are required to minimize the further occurrence of such events.

7.5. Adverse Event Reporting

Adverse Event Definitions

Adverse event. Any untoward medical occurrence in a clinical study; regardless of the causal relationship of the event with the investigational drug or study treatment(s).

Associated with the use of the investigational drug or study treatment(s). There is a reasonable possibility that the adverse event may have been caused by the investigational drug or study treatment(s).

Disability. A substantial disruption of a person's ability to conduct normal life functions.

Life-threatening adverse event. Any adverse event that places the participant, in the view of the investigator, at immediate risk of death from the event as it occurred (i.e., does not include an adverse event that, had it actually occurred in a more severe form, might have caused death).

Serious adverse event. SAEs will be defined as any adverse experience that is unexpected or: i) results in death; ii) results in persistent or significant disability/incapacity; iii) results in or prolongs an existing inpatient hospitalization (even if the hospitalization is a precautionary measure for observation); iv) is a congenital anomaly/birth defect in offspring of subjects taking the product regardless of time to diagnosis; v) is the result of an overdose, whether accidental or intentional; or vi) is a suicide attempt (but not suicidal ideation).

Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse event; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse event (e.g. for a preexisting condition not associated with a new adverse event or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse event.

Unexpected adverse event. Any adverse event, the frequency, specificity or severity of which is not consistent with the risk information described in the clinical protocol(s) or elsewhere in the current IND application, as amended.

7.6. Recording Requirements

All observed or volunteered adverse drug events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational drug or study treatment(s) will be recorded in the participants' case record forms. For all adverse events, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the event (i.e., whether the event should be classified as a serious adverse event) and; 2) an assessment of the casual relationship between the adverse event and the investigational drug or study treatment(s).

Adverse events or abnormal test findings felt to be associated with the investigational drug or study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding

resolves or stabilizes at a level acceptable to the investigator-sponsor (or, if applicable, the medical or dental director for the investigator-sponsor).

7.7. Abnormal Test Findings

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

The test finding is accompanied by clinical symptoms and of clinical significance.

The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy. Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.

The test finding leads to a change in study dosing or discontinuation of subject participation in the clinical study.

The test finding is considered an adverse event by the investigator-sponsor of the IND application.

7.8. Causality and Severity Assessment

The investigator-sponsor of the IND application will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the investigational drug or study treatment(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the investigator-sponsor's final determination of causality is "unknown and of questionable relationship to the investigational drug or study treatment(s)", the adverse event will be classified as associated with the use of the investigational drug or study treatment(s) for reporting purposes. If the investigator-sponsor's final determination of causality is "unknown but not related to the investigational drug or study treatment(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

7.9. Written IND Safety Reports

The investigator-sponsor will submit a written IND Safety Report (i.e., completed FDA Form 3500 A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be 1) associated with the investigational drug or study treatment(s); 2) serious; and 3) unexpected. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the investigator-sponsor's receipt of the respective adverse event information.

For each written IND Safety Report, the sponsor-investigator will identify all previously submitted IND Safety Reports that addressed a similar adverse event experience and will provide an analysis of the significance of newly reported adverse event in light of the previous, similar report(s).

Follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the relevant information is available. If the results of the sponsor-investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the investigator-sponsor will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

7.10. Telephoned IND Safety Reports

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the investigator-sponsor will notify the responsible review division of the FDA by telephone or facsimile transmission of any observed or volunteered adverse event that is 1) associated with the use of the investigational drug or study treatment(s); 2 fatal or life-threatening; and 3) unexpected.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the investigator-sponsor's initial receipt of the respective human adverse event information.

7.11. Reporting Adverse Events to the Responsible IRB

In accordance with applicable policies of UCLA and the University of Maryland Institutional Review Boards (IRB's), the investigator will report, to the IRB, any observed or volunteered adverse event that is determined to be 1) associated with the investigational drug or study treatment(s); 2) serious; and 3) unexpected. Adverse event reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable adverse events will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator's receipt of the respective information. Adverse events which are 1) associated with the investigational drug or study treatment(s); 2) fatal or life-threatening; and 3) unexpected will be reported to the IRB within 24 hours of the investigator's receipt of the respective information.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator will report the adverse event to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

7.12. Withdrawal of Participants Due to Adverse Events

If unanticipated and serious adverse events emerge and are considered related or possibly related to study drug, the study may be stopped and all currently participating participants will be contacted to stop taking the study drugs and complete any final study procedures (safety associated laboratory test, etc). The respective IRBs and FDA will be notified of such actions by the investigators at each site, and by the IND holder at Pittsburgh to the FDA.

Similarly, if the holder of the CTD, Natreon, Inc informs the IND holder or the PI/Co-I of unanticipated adverse events that have emerged and could place currently enrolled participants at greater risk for physical and psychological harm than anticipated then actions for stopping the study and follow-up as described above will be initiated by the PI and research staff in conjunction with the IND holder and other responsible entities (e.g. FDA).

8. Statistical Methods/Data Analysis

8.1 Primary Endpoint(s)

For *Specific Aim 1*, an intention-to-treat strategy will be used for analysis and participants will be analyzed as part of the group to which they were randomized. A linear mixed model will be used to examine changes in measures of psychopathology (PANSS Total, Positive and Negative Symptoms and General Psychopathology Subscales) and psychosocial stress (PSS). For the primary endpoint (PANSS Total Score), a model that includes visit, treatment, visit * treatment interaction as fixed effects with baseline PANSS total score as a covariate will be used. Model-estimated marginal means for scheduled

visits will be computed per treatment group to examine time to response. Linear mixed models use all available data, account for non-independence of repeated measures and provide unbiased estimates if missing data are present.

8.2. Secondary Endpoints

Analyses similar to those described above, will be used to examine changes in secondary endpoints, namely positive and negative symptoms (PANSS subscale scores) and psychosocial stress (PSS score). In order to examine whether improvements in PANSS (total and subscales) and PSS scores are associated with a decrease in inflammatory markers (*Specific Aim 2*), a 2-part analytic strategy will be employed. First, changes from baseline to endpoint in inflammatory markers in the two treatment groups will be examined using a linear mixed model or repeated-measures ANOVA. Subsequently, a linear regression models will be used to examine multicollinearity between predictors, specifically whether changes (improvement) in clinical measures (PANSS total and subscale scores and PSS scores) are predicted by changes (decrease) in the level of inflammatory markers (hsCRP).
Psychotropic medication endpoints will be divided into antipsychotic medication changes and all other psychotropic medication changes. Increases or decreases or no change will be coded categorically, e.g. 1 or 2 or 3. Starting a new antipsychotic or stopping an existing one will be coded categorically e.g. 1 or 2; and no change as 3. Similar categorizations will be made for other psychotropic agents, e.g. lithium and anticonvulsant mood stabilizers, antidepressant agents, anxiolytic and hypnotic agents. As some medication adjustments are done simultaneously, e.g. upward titration of one antipsychotic while downward titration of the existing antipsychotic, coding will be determined in research meetings and/or if required with DSMB input.

8.3. Definition(s): Analysis population(s)

Any participant who provides informed consent and completes screening procedures, and receives randomized study medication, and also comes in for at least one additional study visit will form the safety population for data analyses.

Any participant who was randomized and provided baseline plus at least one other visit where the PANSS and PSS scales assessments were reassessed will form the intent to treat population for efficacy analyses for the primary outcomes.

8.4 Statistical Analysis

Descriptive statistics (means, and SD for continuous data and frequency counts for categorical data) will be used to summarize baseline socio-demographic variables (age, race, ethnicity, gender, education etc.) and clinical variables (illness duration, illness severity, etc) for participants randomly assigned to WSE or placebo. The efficiency of randomization will be examined by comparing the baseline characteristics between the two treatment groups using independent t-tests or contingency statistics (chi-square or Fisher's test). If appropriate, variables that are significantly different between the two groups at baseline will be empirically identified as potential confounders and will be included as covariates in subsequent multivariate analyses. The consolidated standards of reporting clinical trials (CONSORT) will be utilized to describe the screening of eligible participants, random assignment to WSE or placebo, reasons for withdrawal, participants forming the efficacy population and those who complete the trial, etc (http://www.consort-statement.org).

8.5. Safety Analysis

Safety analyses of vital signs, laboratory measures, body weight etc. will be evaluated for the safety population, either as continuous or categorical variables (for instance – normal, abnormal etc). Further, the treatment-emergent adverse events will be grouped by organ-system and tabulated by treatment assignment, and rates between treatments will be compared using contingency statistics (chi-square or other Fishers Exact test) for categorical variables and T-tests for continuous variables.

8.6 Sample size calculation

In a previous 12-week, double-blind, placebo-controlled study that examined the efficacy of WSE (Sensoril®) for the treatment of symptom exacerbations in participants with schizophrenia (Chengappa et al, in press: J Clin Psychiatry), participants randomized to Sensoril® had a significantly greater reduction in PANSSS total score (effect size=0.83); the primary outcome measure in the current study. If we assume the same effect size, then a sample of 66 (33 in each group) will have a power of 0.95 to detect an effect size of 0.83 with a 1-tailed alpha set at 0.05 and power of 0.90 with a 2-tailed test of significance (see Table). The proposed sample size will allow for us to continue to have sufficient power (i.e., 0.80), even if a significant number of randomized participants fail to provide data for the proposed analyses.

Power	Sample (1-tailed)	Sample (2-tailed)
0.80	19 + 19 = 38	24 + 24 = 48
0.85	22 + 22 = 44	28 + 28 = 56
0.90	26 + 26 = 52	32 + 32 = 64

0.95	33 + 33 = 66	39 + 39 = 78

9. Quality Control and Quality Assurance

Independent monitoring of the clinical study for protocol and GCP compliance will be conducted periodically by qualified staff of the UCLA and University of Maryland Human Subject Protection Offices.

The investigator-sponsor and the University of Maryland and UCLA will permit direct access of the study monitors and appropriate regulatory authorities to the study data and to the corresponding source data and documents to verify the accuracy of this data.

Details of the Data and Safety Monitoring Committee are noted in section **7.4**.

All individuals (P.I. and research staff) involved with the study will attempt to meet weekly throughout the duration of the research study to discuss and monitor confidentiality, data, recruitment and adverse events, including clinical outcomes relating to the participants in the study. At the weekly research meetings, the P.I. and all study personnel will be apprised of new developments relating to the study participants. Any unanticipated problems and unanticipated, serious adverse events felt to be possibly related or related to the research interventions will be reported to the University of Maryland and UCLA IRB's in accordance with the IRB's reporting requirements. Adverse events and reports from the data and safety monitoring will be reported to the IRB sooner if it changes the risk to benefit ratio of the protocol. In accordance with IRB adverse event reporting these will be in addition to the yearly summaries. A summary of all the unanticipated adverse events observed during the course of the study in a twelve-month period will be reported to the IRB at the time of the annual review.

All research data is recorded on Case Report Forms that will be stored in a locked file cabinet in study personnel's office. The research forms will not include any form of identifying information. ID numbers rather than subject identifiers are used to record information on computer databases which are protected by firewalls and only authorized personnel with user id and passwords are permitted to enter and view the data.

10. Data Handling and Record-Keeping

10.1. Data recording/Case Report Forms A Case Report Form will be completed for each subject enrolled into the clinical study. The investigator-sponsor will review, approve and sign/date each completed CRF; the investigator-sponsor's signature serving as attestation of the investigator-sponsor's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic. We will make all efforts to collect all data points associated with this research. Research staff will utilize various sources, including electronic medical record information for verification purposes, when available.

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

Source Data

The data obtained from hospital record or laboratory or EKG reports or other relevant medical information done prior to or during the study will be considered source documents and kept in a file for each participant subject. Some of these data points – for example – laboratory reports and results, or vital signs, or body weight measurements will be recorded in the CRF binders, and in such instances these data will be source documents for participating subjects.

10.2. Record Maintenance and Retention

The investigator-sponsor will maintain records in accordance with Good Clinical Practice guidelines; to include:

- FDA correspondence related to the IND and clinical protocol, including copies of submitted Safety Reports and Annual Reports
- IRB correspondence (including approval notifications) related to the clinical protocol; including copies of adverse event reports and annual or interim reports
- Current and past versions of the IRB-approved clinical protocol and corresponding IRB-approved consent form(s) and, if applicable, subject recruitment advertisements
- Signed FDA Form 1572 Statements of Investigator (i.e., for the investigator-sponsor and all identified sub-investigators)
- Financial disclosure information (investigator-sponsor and clinical protocol sub-investigators)
- Curriculum vitae (investigator-sponsor and clinical protocol sub-investigators)
- Certificates of required training (e.g., human subject protections, Good Clinical Practice, etc.) for investigator-sponsor and listed sub-investigators
- Listing of printed names/signatures of investigator-sponsor and listed sub-investigators

- Normal value(s)/range(s) for medical/laboratory/technical procedures or tests included in the clinical protocol
- Laboratory certification information
- Instructions for on-site preparation and handling of the investigational drug(s), study treatment(s), and other study-related materials (i.e., if not addressed in the clinical protocol)
- Decoding procedures for blinded trials *(as described in the section "breaking the Blind")*
- Master randomization list
- Signed informed consent forms
- Completed Case Report Forms; signed and dated by investigator-sponsor
- Source Documents or certified copies of Source Documents
- Monitoring visit reports
- Copies of investigator-sponsor correspondence to sub-investigators, including notifications of safety information
- Subject screening and enrollment logs
- Subject identification code list
- Investigational drug accountability records, including documentation of drug disposal.
- Final clinical study report

Research data and documents will be stored in locked/secure areas accessible to investigators and research staff of the study. Research data and documents may also be stored on protected network drives, accessible only to researchers. The investigators and research staff involved with the study will make every effort to maintain the subjects' confidentiality. Much of the research data will be coded and kept without identifiers' to ensure subject confidentiality. To achieve this, all subjects will be assigned a sequential subject number, which will be used to mark Case Report Forms, subject folders or binders, and testing forms. Identifiable information, such as the linkage codes used to identify subjects with their subject IDs, will be kept in secured areas accessible only to the investigator and research staff involved with the study. Subject names or directly identifiable information will not appear on any reports, publications, or other disclosures of study outcomes. Should the subjects decide to withdraw from study participation, information previously collected from the subjects will continue to be maintained in the same manner as other research data collected for this study.

The investigator-sponsor will retain the specified records and reports for up to 2 years after the marketing application is approved for the investigational drug; or, if a marketing application is not submitted or approved for the investigational drug, until 2 years after investigations under the IND have been discontinued and the FDA so notified.

10.3 Data Sharing

As part of the conditions of the grant from SMRI which funds this study, we will submit de-identified subject data to the National Institute of Mental Health Data Archive (NDA), specifically the National Database for Clinical Trials Related to Mental Illness (NDCT). The types of data we will submit include the following: demographic data and data from diagnostic and symptom assessments.

11. Ethics

11.1 Institutional Review Board (IRB) Approval

The investigator-sponsor will obtain, from the University of Maryland and UCLA Institutional Review Boards (IRB's), prospective approval of the clinical protocol and corresponding informed consent form(s); modifications to the clinical protocol and corresponding informed consent forms, and advertisements (i.e., directed at potential research subjects) for study recruitment.

The only circumstance in which a deviation from the current IRB-approved clinical protocol/consent form(s) may be initiated in the absence of prospective IRB approval is to eliminate an apparent immediate hazard to the research subject(s). In such circumstances, the investigator-sponsor will promptly notify the IRB of the deviation.

The IRB's operate in compliance with FDA regulations at 21 CFR Parts 50 and 21 CFR 56, and in conformance with applicable International Conference on Harmonization (ICH) Guidelines on Good Clinical Practice (CGP).

In the event that one of the IRB's requires, as a condition of approval, substantial changes to a clinical protocol submitted under an FDA-accepted IND application, or in the event of an investigator-sponsor's decision to modify the previously accepted clinical protocol:

- for a Phase 1 clinical study: The investigator-sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to the Phase 1 clinical protocol that significantly affects the safety of the subjects. For changes that do not affect critical safety assessments, the revisions to the clinical protocol will be addressed in the Annual Report to the IND.

- for Phase 2 and 3 clinical studies: The investigator-sponsor will submit (i.e., in advance of implementing the change) a Protocol Amendment to the IND describing any change to a Phase 2 or Phase 3 protocol that significantly affects the safety of subjects, the scope of the investigation, or the scientific quality of the study. Examples of Phase 2 and 3 clinical protocol changes requiring the submission of a Protocol Amendment include:

- Any increase in drug dosage or duration of exposure of individual subjects to the investigational drug beyond that described in the current protocol, or any significant increase in the number of subjects under study.

- Any significant change in the design of the protocol (such as the addition or deletion of a control group).

- The addition of a new test or procedure that is intended to improve monitoring for, or reduce the risk of, a side effect or adverse event; or the dropping of a test intended to monitor the safety of the investigational drug.

11.2 Ethical and Scientific conduct of the Clinical Study

The clinical study will be conducted in accordance with the current IRB-approved clinical protocol; ICH Guidelines on GCP; and relevant policies, requirements, and regulations of the Universities and applicable federal agencies.

Subject Informed Consent

The investigator-sponsor will make certain that an appropriate informed consent process is in place to ensure that potential research subjects, or their authorized representatives, are fully informed about the nature and objectives of the clinical study, the potential risks and benefits of study participation, and their rights as research subjects. The investigator-sponsor, or a sub-investigator(s) designated by the investigator-sponsor, will obtain the written, signed informed consent of each subject, or the subject's authorized representative, prior to performing any study-specific procedures on the subject. The date and time that the subject or the subject's authorized representative, signs the informed consent form and a narrative of the issues discussed during the informed consent process will be documented in the subject's case history. The investigator-sponsor will retain the original copy of the signed informed consent form, and a copy will be provided to the subject, or to the subject's authorized representative.

The investigator-sponsor will make certain that appropriate processes and procedures are in place to ensure that ongoing questions and concerns of enrolled subjects are adequately addressed and that the subjects are informed of any new information that may affect their decision to continue participation in the clinical study. In the event of substantial changes to the clinical study or the risk-to-benefit ratio of study participation, the investigator-sponsor will obtain the informed consent of enrolled subjects for continued participation in the clinical study

11.3 Investigator-Sponsor Discontinuation Criteria

Criteria and procedures for terminating the participation of an individual study subject
A research subject will be terminated from study participation in the event that:

1. Subject fails to follow-up with study procedures and study visits despite reasonable efforts on the part of the research staff to contact him/her will be discontinued from the study.

2. Subject experiences a serious adverse event that is unanticipated and thought to be possibly related to study drug will be discontinued from the study.

3. An unanticipated and serious adverse event emerges and is considered related or possibly related to study drug, the study may be stopped and all currently participating subjects contacted to stop taking the study drugs and complete any final study procedures (safety associated laboratory test, etc). The DSMB, IRB and FDA will be notified on such actions.

4. A female subject becomes pregnant during study participation.

Note: Missing multiple doses of study treatment does not qualify as a reason for termination. Participants who miss multiple doses will be encouraged to resume study medication at the standard BID schedule. However, if it is determined that a participant's study drug treatment has been interrupted for greater than 7 consecutive days, that individual may be considered for removal from the study by the investigator-sponsor.

Termination procedures:

- Perform a final on-site or telephone assessment of adverse event occurrence.
- Request the return of any remaining study drug doses. If necessary, send a pre-paid envelope to the participant and include a request that the participant return the study drug as soon as possible.
- Complete study drug accountability record to account for any returned doses and the subsequent disposal of such doses in accordance with guidelines of the Investigational Drug Service

Subjects withdrawn due to a serious unexpected adverse event will only be replaced once full assessment of the event has taken place, in order to ensure the safety of additional subjects. Replacements will occur in this study only up to the limits allowed by availability of subjects' budget considerations.

11.4 Criteria and procedures for termination of the clinical investigation

Given the safety profile of Sensoril®, it is not anticipated that the entire clinical investigation will be discontinued for safety reasons. However, should this scenario occur, the IRB's and the FDA will be notified promptly of discontinuation of the entire clinical study, or any part of the study.

Respective protocol modifications will be submitted prospectively to the IRB's and to the FDA should discontinuation of the any part of the clinical study be required. In the case of discontinuation of the study, all enrolled research subjects and study sub-investigators will be contacted as soon as possible via telephone (or letter if necessary) to be informed of the reason (s) for discontinuation and any follow-up required to address potential adverse effects. Any participants who are currently within the 12 week process of taking the study drug will be instructed to stop treatment immediately. If necessary, follow-up appointments with the Medical Director/Prescribing Physician or other medical care will be provided. Follow up assessment or medical care will occur for as long as necessary, depending on the cause of discontinuation. In the case of discontinuation of only certain parts of the clinical study, the same procedures will be followed. All research subjects will be informed of the reasons(s) for discontinuation of certain parts of the research study, and, if appropriate, will be informed of any increased risk for continue participation. They will be fully informed and re-consented for participation in the study, if necessary.

1. If the DSMB recommends to the PI/research staff there are safety concerns (as described above), the study will be stopped and procedures similar to above will be followed.

2. If Natreon Inc. or other agencies advises the PI of any safety concerns, similar to above, then procedures similar to those described above will be followed.

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