A Double-Blind Placebo-Controlled Trial of a Sulforaphane Nutraceutical to Reduce the Symptoms of Schizophrenia

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PROTOCOL

Title of Study: **A Double-Blind Placebo-Controlled Trial of a Sulforaphane Nutraceutical to Reduce the Symptoms of Schizophrenia**

Funding Source: Stanley Medical Research Institute

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1. INTRODUCTION AND OVERVIEW

We intend to explore the hypothesis that symptoms of schizophrenia may be reduced by the administration of a sulforaphane compound when used in addition to standard antipsychotic medications. Sulforaphane is a molecule that belongs to the isothiocyanate group of organosulfur compounds found in broccoli sprouts. Sulforaphane has potent anti-oxidant and anti-inflammatory activities (Juge, Mithen, & Traka 2007). A recent trial in adolescent and young adult males with autism spectrum disorder found that sulforaphane treatment as an add-on medication was associated with improved psychiatric symptoms (Singh et al. 2014). To date, there have not been any controlled studies of sulforaphane in persons with schizophrenia. The proposed trial is justified on the grounds that: 1) oxidative stress is associated with the pathophysiology of schizophrenia and sulforaphane has anti-oxidant and anti-inflammatory properties; 2) positive effects of sulforaphane have been found in autism, a disorder with some similarities to schizophrenia; and 3) the compound has relatively low toxicity and a low side effect profile. If this sulforaphane compound is found to be beneficial, it would provide a safe, inexpensive, and widely-available new modality for the adjunctive treatment of schizophrenia.

We will randomize N=64 participants with schizophrenia or schizoaffective disorder who have residual psychotic symptoms which are of at least moderate severity. The participants will receive the sulforaphane compound 6 tablets once per day (equivalent to ~ 100 micromoles (μmol) of sulforaphane) or placebo 6 tablets once per day, over the 18 weeks of the trial. We have selected the 18 week duration of the trial to maximize both the likelihood of obtaining a therapeutic effect and of retaining participants in the study. This is also similar to the duration of the active phase in the published autism trial (Singh et al. 2014).

The principal investigator and her research group are highly experienced in carrying out clinical trials of adjunctive treatments in persons with schizophrenia with 7 successfully-completed trials that have been published (Dickerson, Boronow, Stallings, Origoni, & Yolken 2003; Dickerson, Stallings, Boronow, Origoni, Sullens & Yolken 2009; Dickerson et al. 2009; Dickerson et al. 2014; Dickerson et al. 2009; Dickerson et al. 2011; Fenton, Dickerson, Boronow, Hibbeln & Knable 2001). The Investigational New Drug Application (IND 130136) was recently reviewed by the Food and Drug Administration and the investigator received a "may proceed" letter on April 20, 2016.

2. SCIENTIFIC BACKGROUND AND RATIONALE

Schizophrenia and Oxidative Stress

Schizophrenia is a pervasive human disease of unknown etiology. In recent years, evidence has accumulated indicating that some cases of schizophrenia are associated with oxidative stress (Boskovic, Vovk, Kores Plesnicar, & Grabnar 2011; Do, Conus, & Cuenod 2010; Saruwatari et al. 2013). Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Diminished anti-oxidants and/or increased production of reactive species will result in oxidative damage of cell lipids, proteins, enzymes, carbohydrates, and DNA (Boskovic et al. 2011). In schizophrenia, oxidative stress is also associated with immune activation (Pedrini et al. 2012). Oxidative damage has been suggested to be a common pathogenic process in this disorder which contributes to ongoing psychiatric symptoms and poor outcomes.

Sulforaphane

It is widely recognized that high consumption of plant-based diets reduces the risk of cancer and many other chronic diseases (Hardman 2014; Vanamala 2015). Evidence for the special protective role of cruciferous plants, such as broccoli, is very strong and has been ascribed largely to their high content of glucosinolates. Whereas glucosinolates are themselves not active protectors, they are converted to active compounds by both the coexisting plant enzyme, myrosinase, and by the microflora of the gastrointestinal (GI) tract to isothiocyanates which are extremely effective blockers of carcinogenesis (Shapiro et al. 2006). A widely studied example is the presence in broccoli sprouts of the glucosinolate, glucoraphanin, which is converted by myrosinase to the isothiocyanate, sulforaphane (Fahey, Zhang, & Talalay 1997; Fahey, Zalcmann, & Talalay 2001). Myrosinase is present in plant cells and is compartmentalized separately from the glucosinolates. Sulforaphane is metabolized rapidly by initial conjugation with glutathione, and successive steps of hydrolysis of the conjugates leading to ultimate formation of the N-acetyl-cysteine derivative. All these conjugates are dithiocarbamates and can be quantified by the cyclocondensation reaction developed in the Johns Hopkins laboratory of Dr. Jed Fahey, one of the study investigators (Ye et al. 2002). Sulforaphane crosses the blood brain barrier and has general but potent indirect anti-oxidant and anti-inflammatory activities which function systemically (Bahadoran, Mirmiran, & Azizi 2013; Dinkova-Kostova & Talalay 2008; Fahey & Kensler 2007; Fahey & Talalay 1999; Koo, Park, Kim, & Lee 2013).

The major mechanism by which sulforaphane protects cells was initially thought to be through Nrf2-mediated induction of phase 2 detoxification enzymes that elevate cell defense against oxidative damage and promote the removal of carcinogens. However, there may be additional mechanisms activated in response to sulforaphane including suppression of cytochrome P450 enzymes, induction of apoptotic pathways, suppression of cell cycle progression, inhibition of angiogenesis, and inhibition of inflammation (Fahey et al. 2015).

The compound which we will use in this trial is a sulforaphane compound which was developed by Nutramax Laboratories and marketed commercially as Avmacol®. The compound contains a precursor of sulforaphane, glucoraphanin, formulated with an enzyme, myrosinase,

which results in the generation of sulforaphane in the gastrointestinal tract. This compound is the only one available on the consumer market which co-delivers glucoraphanin and the enzyme, myrosinase, needed to hydrolyze it to its active form, sulforaphane, and which is shelf-stable at room temperature. This same compound is also being used in two other trials with psychiatric populations (A 6-month Randomized, Placebo Controlled Study to Evaluate Sulforaphane Add-on Effects In Treatment of First Onset and Early Stage Schizophrenia; Sulforaphane Treatment Of Children With Autism Spectrum Disorder).

Until now, many clinical studies targeting sulforaphane have utilized a hydrolyzed broccoli sprout extract prepared and standardized by the Cullman Chemoprotection Center at Johns Hopkins University. The use of the hydrolyzed broccoli sprout extract is burdensome in clinical trials because the sulforaphane is only moderately stable over time and the product must be kept frozen to maintain bioavailability; in addition, the percent of the dose excreted in urine as sulforaphane is relatively low. The cost, convenience, reproducibility, and potential for post-study continuance are all attractive features of a commercially available supplement compared to the made-for-clinical-trials-only broccoli sprout extract preparation.

It is of note that although Dr. Fahey first described and developed broccoli sprouts as a source of glucoraphanin, neither he, nor the other researchers at Johns Hopkins or Sheppard Pratt involved in this study are connected with Nutramax, the company that produces the Avmacol® product, nor will they benefit from the sales of any Nutramax product.

<u>Previous Clinical Trials of Sulforaphane Supplementation in Neuropsychiatric Populations</u>

A randomized controlled trial of broccoli sprout extracts was performed by Singh et al. (2014) in N=44 adolescent and adult males with autism spectrum disorders. The effects on behavior of daily oral doses of sulforaphane (50-150 μmol) for 18 weeks followed by 4 weeks without the treatment were quantified by behavioral measures. After the treatment period, those randomized to sulforaphane showed substantial improvements in social interaction, abnormal behavior, and verbal communication, whereas those assigned to placebo showed minimal change. After discontinuation of the sulforaphane, the actively-treated group reverted to baseline levels on the behavioral measures (though 10 of 26 who received active compound were not seen for the follow-up). The number of adverse events did not differ between the groups; one participant receiving the active compound experienced a single seizure during the trial and one participant experienced a single seizure several weeks after discontinuing treatment; both had a history of seizure disorder which is common in people with autism.

A second trial of broccoli sprout extracts in psychiatric patients was recently reported and was a small open-label trial of N=10 adult outpatients with schizophrenia, 7 of whom completed the trial (Shiina et al. 2015). Participants received a daily dose of 30 mg of the precursor, glucoraphanin (sulforaphane-glucosinolate), per day for 8 weeks. The primary outcome was a battery of computerized non-verbal tasks. Performance on one of the five cognitive tasks, the One Card Learning Task, improved in the trial but not the other tasks or ratings of psychiatric symptom severity. The sulforaphane compound was well tolerated. The very small sample size in this trial, the short treatment period, and the open-label design preclude any conclusions about the efficacy of the compound in schizophrenia. In addition, the study compound consisted of the

precursor, glucoraphanin, which was administered without the added enzyme, myrosinase, and likely had a conversion to sulforaphane which was 10% of that in the Singh et al. (2014) trial.

Preliminary Investigation to Determine Bioavailability of Study Compound

This preliminary investigation is currently being carried out by Dr. Fahey of the Johns Hopkins School of Medicine. In brief, 20 healthy adult volunteers will be recruited from previous studies and advertisements. Participants will be screened after consent based on whether they are able to comply with the dietary restrictions and medication exclusions. They will be asked to refrain from consuming cruciferous vegetables and condiments (e.g., mustard, horseradish, wasabi) that might contain glucosinolates or isothiocyanates for 3 days before and during the study. Participants will fast overnight and the following morning. They will then take 7 Avmacol® tablets orally; each tablet will contain 15 mg of glucoraphanin for an expected sulforaphane dose that should average 100-150 μmol. Participants will provide a pre-dosing urine sample, and the entire urine excreted during the first 8 hours and for the following 16 hours, in segregated collections. Total dithiocarbamate excretion in each urine sample (three per participant) and urine creatinine concentrations will be determined. Dithiocarbamates (DTC) are the ultimate metabolites of sulforaphane excreted in the urine, and are readily measured by techniques developed by the Cullman Chemoprotection Center (Ye et al. 2002). It is expected that the conversion of the oral dose to the excreted DTC will be about 40%, but any mean conversion of >10% (the mean bioavailability without added myrosinase) will be considered acceptable. Preliminary results from this study are that conversion is at least 35% (personal communication, JW Fahey, March 9, 2016).

Aims

The primary aim of the study is:

1. To evaluate the safety and efficacy of a sulforaphane compound for individuals with schizophrenia who have residual psychotic symptoms of at least moderate severity in a double blind trial. The primary outcome will be the severity of psychiatric symptoms as measured by the Positive and Negative Syndrome Scale (PANSS) (Kay, Fiszbein, & Opler 1987). We will also measure cognitive functioning with the MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al. 2008).

Secondary aims of the study are:

2. To study the effect of the sulforaphane compound in lowering several biomarkers of inflammation including C-Reactive Protein (CRP), heat shock protein 90, and other markers of inflammation and cell damage.
3. To investigate the association between the efficacy of sulforaphane therapy and initial and follow-up levels of these markers.

3. INFORMED CONSENT

The informed consent form will contain a full explanation of the possible risks, alternative treatment options, and availability of treatment in the case of injury, in accordance with the Federal Regulations as described in 21CFR50.

The principal investigator is responsible for obtaining written informed consent from any potential participant before performing any study specific tests or assessments required by the protocol. It is the responsibility of the principal investigator to document each participant's capacity to provide informed consent. The principal investigator may decide that an individual is not competent to give consent even though the treating source believes the participant to be competent, but not vice-versa. A copy of the signed document will be given to the participant (and providers as necessary); the original will be placed with the case report forms.

4. PARTICIPANT INCLUSION AND EXCLUSION CRITERIA AND RECRUITMENT

Participant Inclusion Criteria

- Capacity for written informed consent
- Age 18-65 years, inclusive
- Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) diagnosis of schizophrenia or schizoaffective disorder (APA 2013) as determined by the Structured Clinical Interview for DSM-5 Disorders (SCID-5) (First, Williams, Karg, & Spitzer 2015)
- Currently an outpatient at time of screening
- Residual psychotic symptoms of at least moderate severity as evidenced by a Positive and Negative Syndrome Scale (PANSS) total score of 60 or higher AND one or more of the following: one or more PANSS positive symptom scores of 4 or higher; OR containing at least three positive or negative items with scores of 3 or higher at the screening visit (Kay et al. 1987)
- Receiving antipsychotic medication for at least 8 weeks prior to enrolling in the study with no antipsychotic medication changes within the previous 21 days from visit 2 (week 0)
- Conformance to PORT Treatment Recommendation about Maintenance Antipsychotic Medication Dose (Buchanan et al. 2010)
- Proficient in the English language
- Participated previously in one of our screening studies (A Study of Individuals with Psychiatric Illness to Determine the Presence Of Antibodies to Infectious Agents and Immune Markers; A Study of Individuals with Schizophrenia or Schizoaffective Disorder to Determine the Presence of Antibodies to Infectious Agents and to Establish Eligibility for Individuals' Participation in Future Studies; A Study to Determine Antibodies to Infectious Agents and Eligibility for Future Studies in Recent Onset Psychosis). Note: Participation in one of our screening studies may also take place during the same time period as the screening visit for this trial.

Participant Exclusion Criteria

- Any clinically significant or unstable medical disorder as determined by the principal investigator and/or the study physician (e.g., HIV infection or other immunodeficiency condition (such as receiving chemotherapy), uncontrolled diabetes, congestive heart failure)
- DSM-5 diagnosis of intellectual disability or comparable diagnoses determined by previous versions of the DSM

- DSM-5 diagnosis of a moderate or severe substance use disorder, except for caffeine or tobacco, within the last three months prior to the screening visit. If the patient has a positive drug toxicity screen at the time of visit 1 (screening) further evaluation by the investigator will be done of the substance use to determine eligibility.
- Any current use of a broccoli supplement (e.g., Avmacol® or other health food broccoli supplement)
- Participated in any investigational drug trial in the past 30 days prior to the screening visit
- Pregnant, planning to become pregnant, or breastfeeding during the study period

Participants will be recruited from clinical programs at Sheppard Pratt and affiliated agencies. Participants may be drawn from individuals who participated in previous studies performed by our research group and who provided written permission to be re-contacted about future studies. Participants may also be recruited from other rehabilitation and treatment programs in central Maryland. Additionally, participants may be recruited from self-referrals through advertisements and/or word-of-mouth. All participants will provide written informed consent after the study procedures are explained to them.

5. STUDY DESIGN AND PROCEDURES

Study Overview

Over a two year period, we will randomize N=64 participants who have residual psychotic symptoms which are of at least moderate severity. Based on our experience performing similar clinical trials, we anticipate that we will evaluate up to 100 participants in order to randomize 64. We expect a drop-out rate of 15% after randomization. The trial will begin with a 2 week single-blind placebo run-in phase followed by a 16 week double-blind phase. During the placebo run-in phase, participants will receive placebo 6 tablets once per day. During the double-blind phase, participants will receive either the sulforaphane tablet 6 tablets once per day (equivalent to ~100 μmol of sulforaphane) or the placebo 6 tablets once per day. The primary outcome that will be assessed is the severity of psychiatric symptoms as measured by the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987). We will also measure cognitive functioning with the MATRICS Consensus Cognitive Battery (MCCB) (Nuechterlein et al. 2008). In addition, we will study the effect of the sulforaphane compound in lowering several biomarkers of inflammation including C-Reactive Protein (CRP), heat shock protein 90, and other markers of inflammation and cell damage. We will also investigate the association between the efficacy of sulforaphane therapy and initial and follow-up levels of these markers. For a detailed list of procedures and assessments at each study visit, see the Schedule of Assessments.

Study Compound

The active study compound and identical-appearing placebo will be prepared and provided by Nutramax Laboratories, Edgewood, MD, USA, the company that makes Avmacol®, a commercially-available over-the-counter compound (http://www.nutramaxstore.com/avmacolreg-60-tablets-p430.aspx). The active ingredient in Avmacol® is produced naturally by extracting the biologically inactive glucosinolate, glucoraphanin, from broccoli seeds, and compressing it in tablets with active myrosinase from broccoli sprouts. The ingestion of this compound leads to the

hydrolysis of glucoraphanin and the generation of sulforaphane within the gastrointestinal (GI) tract and the subsequent systemic absorption of the sulforaphane.

The dose per tablet is 16 mg of glucoraphanin or 37 μmol; 6 tablets per day should yield about 100 μmol of sulforaphane (Fahey et al. 2015). The study compound will be provided as a .375 punch size, round concave tablet, about the size of a regular (325 mg) enteric-coated aspirin. The compound will be taken orally, once per day. Once per day dosing is selected in order to optimize compliance.

The study compound will be stored in a freezer until time of dispensing for maximum assurance of potency even though Nutramax certifies it for 2 years shelf life at room temperature. At each visit during the treatment phase, the participant will receive a two week supply of the study compound that can then be stored at room temperature. Participants will be asked to take the study compound at the same time each day. Participants will not be instructed to alter their diet or to reduce their intake of vegetables as the amount of sulforaphane that is obtained from ingested vegetables is low and not expected to change the results of the study. Any participant who is taking a broccoli neutraceutical supplement will be excluded.

Concomitant Medications Allowed

Participants will continue taking previous psychotropic medications that they are prescribed. However, we will request, insofar as possible, that no changes take place in the type or dose of their psychotropic medications for the duration of the study. At each study visit, we will record all medications and doses that the participant is receiving, noting any changes. There are no medications that are disallowed in this trial.

Screening for Eligibility

Potential participants will be screened for eligibility based on a review of the psychiatric diagnosis or diagnoses, substance use history, medical history, and other criteria as listed in the study inclusion and exclusion criteria list. Information needed to determine eligibility will be obtained through careful review of the participant's medical record, previous study documents, discussion with the treatment team, and/or participant interview. After likely eligibility is established, the participant will be seen for the screening visit.

Screening Visit

The screening visit assessments will be completed within 1 to 14 days of the research staff obtaining the signed consent or until sufficient evidence exists to fully assess inclusion and exclusion criteria. The screening assessments include obtaining informed consent, symptom and SCID-5 (First et al. 2015) interviews, a detailed psychiatric history (obtained from available medical records, previous study documents, and participant self-report), a physical examination and medical history, and clinical laboratory tests as described in more detail below.

The clinical laboratory tests collected at the screening visit will include a complete blood count (CBC), complete metabolic panel (CMP), thyroid stimulating hormone (TSH), urinalysis, urine drug screen, and urine pregnancy test (if female and of child-bearing potential). Subsequent drug screening may be done at any time throughout the study at the discretion of the principal investigator. A complete blood count (CBC), complete metabolic panel (CMP), and urinalysis will be repeated at visit 11 (week 18).

Vital signs, including blood pressure, temperature, pulse, height, and weight will be collected at the screening visit and repeated at visit 7 (week 10) and visit 11 (week 18).

The following will be obtained, collected, and/or verified during the screening visit:

Diagnostic and Background Information:

- Primary DSM-5 Axis I diagnosis of schizophrenia or schizoaffective disorder (APA 2013) as determined by the SCID-5 (First et al. 2015)
- Background information including age, race, gender, participant and participant's parents' educational attainment, and psychiatric and medical history
- Assessment of past and recent suicidal behavior evaluated with a suicide screening assessment that is based on the Columbia-Suicide Severity Rating Scale (Posner et al. 2011)
- List of current medications (may be obtained from the participant's medical records)
- Co-occurring medical conditions (obtained through a brief review of systems and/or medical records), and vital signs
- Physical examination (unless one has been completed within the past 30 days and documented in medical records accessible to the research staff)
- Blood and urine samples for clinical laboratory tests as described above
- Written consent to contact outpatient treatment providers to inform them of the study and establish communication with them

Clinical Instrument:

- Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987), a rater-based scale used to assess and measure the severity of psychiatric symptoms over the past one week (unless previously obtained within the last one week)

The screening visit will take approximately two hours to complete and may be divided into separate sessions to minimize participant fatigue.

Research Laboratory Measures

Samples for these measures will be obtained at visit 2 (week 0), the start of the placebo run-in phase, and other time points as noted below.

- Blood sample (approximately 20 ml) to measure levels of immune markers and antibodies to infectious agents including high sensitivity CRP, TNF alpha, antibodies to food antigens such as casein and gliadin (Severance et al. 2012), and antibodies to NR2 (Dickerson, Stallings, Vaughan, Origoni, Khushalani, and Yolken 2012). Of note, CRP is a non-specific marker of inflammation which is elevated in many individuals with schizophrenia and has been found to be reduced by the administration of a sulforaphane nutraceutical (Bahadoran et al. 2013). These measures will also be performed at visit 7 (week 10) and visit 11 (week 18).
- Blood sample (approximately 8 ml) used to measure levels of biomarkers of inflammation including Keap1/Nrf2-induced markers of cytoprotection and of the heat shock response (in peripheral blood mononuclear cells), using real time PCR and protein gels (Western blots). These samples will be analyzed in the Johns Hopkins Cullman Chemoprotection

Center of Dr. Fahey. These measures will also be performed at visit 7 (week 10) and visit 11 (week 18).

- Blood sample (4-6 ml) for the measurement of the blood level of the antipsychotic medication. . These measures will be performed also at visit 11 (week 18).
- Throat swab samples for study of the oropharyngeal microbiome. These measures will be performed at visit 2 (week 0), visit 7 (week 10), and visit 11 (week 18)
- Urine sample for compliance and dose potency testing. Sulforaphane-glutathione dithiocarbamate (DTC) metabolites will be measured from urine provided by participants as biomarkers of compliance. These tests will also be performed at the Johns Hopkins Cullman Chemoprotection Center. These measures will also be performed at visit 7 (week 10) and visit 11 (week 18).

These blood, throat swab, and urine samples will be identified by participant number only when they are sent to the research laboratories at Johns Hopkins for analysis. The clinical investigators will be blind to these results of the participant's research laboratory tests during the course of the trial. The laboratory scientists will be blind to the participant's treatment assignment during the course of the trial.

Placebo Lead-In Phase

Participants will begin a 2 week single-blind placebo phase at visit 2 (week 0), within 7 to 30 days of completion of the screening visit. Visit 2 (week 0) will take approximately two to three hours to complete. Participants who complete the two week placebo run-in phase and continue to meet eligibility criteria will be randomized to a treatment arm at visit 3 (week 2).

Randomization and Double-Blind Treatment Phase

Double-blind randomization will occur at visit 3 (week 2). Participants will be randomized to receive either the sulforaphane compound or placebo tablets which are indistinguishable from the sulforaphane tablets for the 16 weeks of the double-blind phase. Symptom evaluations will be completed at bi-weekly study visits at which time participants will also be evaluated for adverse events. Visits 3 to 10 (weeks 2 to 16) will take approximately one hour each to complete. Visit 11 (week 18) will take approximately two to three hours to complete.

Randomization will assure that out of N=64 total participants, approximately half are assigned to each treatment arm. In addition, block randomization, based on initial PANSS total scores, will be used to assign participants to the two treatment groups to avoid differences in initial PANSS scores between the groups. Only the study data manager and the study pharmacist will be aware of participant assignment during the trial.

Treatment providers will be asked to maintain participants on the same psychotropic medication regime throughout the study. At each bi-weekly study visit, research staff will document any medication changes. Any medication changes that are made will be studied as a secondary outcome.

The study compound will be taken once per day and the dose will not be adjusted throughout the trial.

Visit Windows

Visit 2 (week 0) will take place 7 to 30 days after visit 1 (screening). Visits 3 to 11 (weeks 2 to 18) will take place 12 to 16 days after the previous visit. Visits may be divided into separate sessions to minimize participant fatigue.

Compliance

Pill counts and/or participant reports of compliance will be obtained. If pill counts or participant reports indicate the participant has not taken all of the prescribed study compound, the participant will be counseled about the importance of following directions. If the participant has missed more than 32 daily doses cumulatively throughout the study (>25% of study drug), the participant will be terminated from the study.

Compliance will also be confirmed with laboratory testing of urine samples provided by participants at 3 points during the trial (visits 2, 7, and 11). Participants will be told that these samples will be collected at random. These urine samples will be used to measure sulforaphane-glutathione metabolites in Dr. Fahey's laboratory.

Discontinuation

A participant may be discontinued from the study for any of the following reasons:

- If the participant fails to follow study directions (failure to keep appointments, follow directions or take the study compound as instructed)
- If the participant does not attend study visits, or is not in contact with research staff for 3 bi-weekly consecutive study visits
- If the participant has a positive drug screen, research staff will inform the participant that a repeat test will be done at the next visit; if the next test is positive then she/he will be removed from the study
- If it is the judgment of the study physician, in an effort to improve the participant's medical care, or if the participant develops a serious medical illness, becomes pregnant, or begins breast feeding
- If the participant has unexpected or serious side effects or serious medical complications

If a participant discontinues from the study, discontinuation assessments will be done at the discretion of the principal investigator.

A participant may withdraw from the study at any time at his/her own request.

Clinical Outcome Measures

- The primary outcome measurement will be the change in scores on the Positive and Negative Syndrome Scale (PANSS) (Kay et al. 1987) from the beginning to the end of the double-blind treatment phase, visits 3 to 11 (weeks 2 to 18). The PANSS contains 30 items that assess symptoms of schizophrenia including positive, negative and general symptom psychopathology. The PANSS was chosen because of its widespread use in clinical studies of psychosis and its demonstrated reliability in assessing psychopathology across diverse patient populations.

- Secondary outcomes will be changes in scores on the MATRICS Consensus Cognitive Battery (MCCB) (Neuchterlein et al. 2008). The MCCB is comprised of 7 cognitive domains and 10 related tests (Trail Making Test: Part A; Brief Assessment in Cognition in Schizophrenia: Symbol Coding; Hopkins Verbal Learning Test-Revised; Wechsler Memory Scale-Third Ed: Spatial Span; Letter-Number Sequencing; Neuropsychological Assessment Battery: Mazes; Brief Visuospatial Memory Test-Revised; Category Fluency: Animal Naming; Mayer-Salovey-Caruso Emotional Intelligence Test: Managing Emotions; and Continuous Performance Test-Identical Pairs).

6. SAFETY

Data Safety Monitoring Board

This committee will be composed of a group of three scientists who are not directly involved with the study and are qualified to serve in an advisory capacity in order to evaluate safety and efficacy of the trial. The safety monitoring board will convene before the study begins and, after the trial is underway, at regular intervals that they determine to be appropriate or at other times at the request of the study investigators.

Physical Examination and Clinical Laboratory Tests

A physical examination will be performed at the screening visit (unless one has been completed within the past 30 days and documented in medical records accessible to the research staff). Clinical laboratory tests including CBC, CMP, thyroid stimulating hormone (TSH), and urinalysis will also be performed. The study physician will determine the participant's medical eligibility for the study after reviewing the findings from the screening physical examination and laboratory tests.

In the event that the study physician determines that a participant's lab value is clinically significant, the participant will be made aware of the abnormal laboratory finding and will be referred for follow-up with his/her primary care physician or other appropriate medical care. Lab results may be sent to participant's primary care physician or other treatment providers. Copies of lab-work will be made available to participants upon their request. Clinically significant abnormal laboratory findings may be repeated to determine the participant's eligibility for the study.

In the case of serious medical illness and/or abnormal laboratory findings that emerge in the course of the study, the study physician may determine that a participant should be terminated or excluded from the study.

Pharmacy Oversight

The study compound will be kept in a locked freezer which is monitored daily for temperature by a computerized monitoring system. Supplement storage and audits will be performed quarterly by the Sheppard Pratt study pharmacist, Dr. Joshana Goga.

Minimizing Risk

Participants will be maintained on their regular psychotropic medications, with the intent to avoid any changes in dose or medication. Each participant will be instructed and expected to

continue treatment with his/her mental health providers and to remain on his/her prescribed psychotropic medications throughout the study. During the study, participants will be seen on a bi-weekly basis during scheduled visits and assessed for the presence of any adverse events.

If a participant reports psychiatric symptoms that raise concern about safety such as imminent, potentially-dangerous behavior, research staff will consult with the principal investigator and/or study physician and may contact the participant's current treatment providers. Permission to contact treatment providers will be obtained at the screening visit and updated as necessary throughout the study if providers change. Research staff will encourage participants to seek immediate help in these cases. Additionally, research staff may arrange for emergency assessment if a participant presents as a danger to self or others, in consultation with the principal investigator and/or the study physician. If safety cannot be assured, research staff will call 9-1-1.

Privacy and Confidentiality

Confidentiality will be protected by ensuring all research staff have been properly trained in confidentiality and human participant research procedures, coding all participant information when possible, and by securing participant files in locked filing cabinets or on secured databases with access available only to the research staff. Furthermore, data entered into a computer database will be stored on secured computers that will be password-protected with access available only to the research staff. Any screening information obtained from potential research participants who subsequently do not participate in the research study will be destroyed.

Data obtained in this study will be shared with the National Database for Clinical Research Related to Mental Illness (NDCT), which is required by the funding source. NDCT is a data repository run by the National Institute of Mental Health (NIMH) that allows researchers to collect and share de-identified data from studies related to mental illness. Other researchers may apply to obtain access to de-identified data from this study. The NIMH carefully analyzes each request in order to minimize risks of privacy. The information provided to NDCT may help researchers around the world treat future children and adults with mental illnesses so that they have better health outcomes. Participants will not be contacted directly about the study data shared during and after their research participation, and no protected health information will be entered into the NDCT.

Adverse Effects and Drug Interactions

Broccoli sprouts have been widely marketed throughout the world as a food source. Oral broccoli sprout preparations have been used in a number of human studies as described above to investigate the pharmacologic properties, safety, and biological effects of sulforaphane glucosinolates. Avmacol® supplements have been available to the public since 2013. The manufacturer reports that as of February 15, 2016, over 12,000 bottles have been distributed and there have been no reported adverse events from consumers in the general public.

Potential adverse effects that might be expected include mild GI symptoms such as indigestion, belching, or loose stools. These are the only symptoms that have been reported in the many clinical trials of glucoraphanin-rich or sulforaphane-rich broccoli sprout extracts (Alumkal et al. 2015; Cornblatt et al. 2007; Egner et al. 2011; Egner et al. 2014; Fahey, Talalay, & Kensler

2012; Heber et al. 2014; Kensler et al. 2012; Poulton et al. 2013; Riedl, Saxon, & Diaz-Sanchez 2009; Shapiro et al. 2006; Shapiro, Fahey, Wade, Stephenson, & Talalay 1998; Shapiro, Fahey, Wade, Stephenson, & Talalay 2001; Singh et al. 2014; Ye et al. 2002). True allergic reactions to broccoli (as opposed to intolerance or taste aversion) have never been reported. Sulforaphane might decrease how quickly the liver breaks down some medications because of the modulation of cytochrome enzymes such as P450; however, the extent of this effect is expected to be limited at the doses we are using. Moreover, the effects of sulforaphane on the modulation of drug metabolism/transport enzymes are varied and may serve to increase or to decrease the blood levels of medications that are metabolized by the liver (Fimognari, Lenzi, & Hrelia 2008). We will measure blood levels of antipsychotic medications at the beginning and end of the trial with tests performed by a local clinical laboratory.

It is of note that numerous other factors influence drug interactions in addition to P450 isoenzymes including age, gender, smoking, and genetic factors. Also, 30 to 40% of the participants in the previous Singh et al. (2014) study in adult autism were receiving antipsychotic medications; per the principal investigator of this study, clinical worsening was observed in only one subject and he turned out to be on placebo (A. Zimmerman, personal communication, Sept 4, 2015).

Adverse Events and Reporting

For the purposes of collecting and evaluating all information found during this trial, an adverse event is any undesirable or unexpected experience that occurs after informed consent has been obtained without regard to the possibility of a causal relationship, and without regard to treatment group assignment. A serious adverse event is any adverse event that: results in death; is life threatening; results in inpatient hospitalization or prolongation of existing hospitalization; results in a persistent or significant disability/incapacity; or results in congenital anomaly/birth defect. For all adverse events, research staff will question the participant regarding the occurrence and nature of the event and will document the information obtained. Adverse events shall be followed until they return to baseline or stabilize regardless of whether the participant has discontinued the study early or completed the final study visit, visit 11 (week 18); if occurring at the end of the study, the adverse events will be referred to the participant's primary care physician and/or other treatment provider for continued treatment and/or follow-up. All serious adverse events will be documented and reported to the Institutional Review Board (IRB) within the required time period. All adverse events will be summarized in a report at intervals requested by the IRB and the overseeing Data Safety Monitoring Board.

7. STATISTICAL ANALYSIS

Power calculations indicate that a sample size of N=64 has 80% power to find an effect size of d = .76 with an alpha =.05 and a two-tailed test of change between groups from screening to end of study.

Aim 1: Safety data will be analyzed using incidence density analysis, to compare rates of adverse events across groups, as well as survival analysis, to assess group differences in retention. PANSS total scores will be the primary efficacy endpoint. Repeated-measures regression models will be used to assess the main effects of group and time, plus the time by group interaction. This method is able to utilize all available data from participants with

incomplete follow-up, thus including participants who drop out of the study. (Based on our experience in previous trials with this population, we anticipate attrition of about 15% over the course of the trial.) A similar analytical approach will be used for the PANSS subscales and the MCCB outcome measures.

Aim 2: Repeated measures regression modeling will also be used to evaluate the effect of medication group, time, and their interaction on inflammatory biomarkers.

Aim 3: Treatment groups will be compared with respect to initial and follow-up levels of inflammatory biomarkers using *t*-tests. If groups are found to differ in this regard, the efficacy analyses will be repeated, adding the initial biomarker levels as covariates in the model.

8. COMPENSATION

Participants will receive $20 for completing visit 1 (screening) and $20 for completing visit 2 (week 0). For visits 3 to 10 (weeks 2 to 16), participants will receive $15 for each visit. At visit 11 (week 18), the ending visit, participants will receive $20 for completing the visit and an additional $40 completion bonus (for a total of $60). The maximum compensation received by a participant who completes the entire trial will be $220.

9. SCHEDULE OF ASSESSMENTS

	Screening 1-14 days	Week 0 Start Placebo	Week 2 Start Randomized Phase	Week 4	Week 6	Week 8	Week 10	Week 12	Week 14	Week 16	Week 18
Visit #	1	2	3	4	5	6	7	8	9	10	11
Visit Window Interval	1-14 Days	7 – 30 Days	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)	14 Days (+/- 2 days)
Inclusion/Exclusion	X										
Consent	X										
DSM-5 SCID	X										
Vital Signs	X						X				X
Psychiatric and Medical History	X										
Substance Use History	X										
Physical Exam	X										
CBC, CMP and TSH*	X										X*
Urinalysis including pregnancy* and drug screening*	X										X*
Immunological and infectious markers from blood samples		X					X				X
Throat swab samples		X					X				X
Markers of SUL metabolism from blood samples		X					X				X
Blood level of antipsychotic medication		X									X
SUL metabolites from urine samples		X					X				X
Med List	X	X	X	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X	X	X	X
Pill Report			X	X	X	X	X	X	X	X	X
PANSS	X	X	X	X	X	X	X	X	X	X	X
C-SSRS	X										
MCCB		X									X
Contact treatment providers	X										
Payment	$20	$20	$15	$15	$15	$15	$15	$15	$15	$15	$60

TSH, urine pregnancy, and urine drug screen only performed at Visit 1/Screening

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