Official Title: 31P-MRS and Resting State Functional Connectivity Analysis of the Effects of 5-hydroxytryptophan and Creatine for Antidepressant Augmentation in Patients With SSRI/SNRI-resistant Major Depressive Disorder

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Introductory Statement and Study Protocol

Introductory Statement

The proposed study is a single-center proof-of-concept study, targeting a volunteer sample of adults with MDD who have failed an adequate trial of an SSRI or SNRI. After screening (week -1) and baseline (week 0) assessments and baseline neuroimaging, 60 subjects (with recruitment of up to 75 subjects, allowing for 20% attrition) will be randomly assigned in 1:1:1:1 ratio to treatment for 8 weeks with either double placebo, 5-HTP 100mg twice daily + Cr-matched placebo, Cr 5g daily + 5-HTP-matched placebo, or 5-HTP 100mg twice daily + Cr 5g daily. The treatment phase will again be followed by neuroimaging (week 8) as well as two post-treatment assessments (weeks 10 and 12).

Study Protocol

Table 1. Eligibility Criteria

INCLUSION CRITERIA	RATIONALE
Adults age 25-40 years inclusive	Maximizing recruitment while minimizing effect of age-related degeneration on MRI findings
Current diagnosis of MDD identified by the SCID-5	Confirmation of target clinical condition
Current HAM-D_{17} score of $\geq$ 16	Confirmation symptoms at least moderately severe
Adequate adherence to any FDA approved SSRI or SNRI for at least 8 weeks	Confirmation of treatment-resistance
Right-handed	Facilitate brain coregistration
Residing at $\geq$ 4000 ft for at least 12 weeks	Ensure homogeneity with respect to hypoxic exposure
EXCLUSION CRITERIA	RATIONALE
Any non-MDD and non-anxiety psychiatric diagnosis, as identified by the SCID-5	Minimize diagnostic heterogeneity and risk of adverse events
History of or current diagnosis of renal disease, such as chronic renal failure, acute renal failure or end stage renal disease	Some reports that creatine worsens renal disease
Current colitis or diverticulitis	Facilitate recognition of serotonin syndrome
History of or current pulmonary disease	Facilitate recognition of EMS, minimize variation in hypoxia between subjects
Current smoking	Minimize variation in hypoxia between subjects
History of cardiac disease or QTc > 500ms	Minimize risk of sudden cardiac death
History of fibromyalgia or any rheumatological condition	Facilitate recognition of EMS
History of or current seizure disorder	Minimize risk of seizure
Current serious suicide risk identified by the Columbia Severity Suicide Rating Scale	Minimize risk of suicide in the study
Current treatment with an antipsychotic, mood stabilizer, or non-SSRI or SNRI antidepressant except for bupropion at FDA-approved doses or trazodone up to 200mg, or use of any supplements apart from standard multivitamins	Minimize risk of adverse drug-drug interaction and increase clinical homogeneity of group
Positive pregnancy test, pregnancy, failure to use adequate birth control method	Risks for fetus of interventions not adequately characterized
Previous diagnosis of serotonin syndrome or evidence of serotonin syndrome	Minimize risk of serotonin syndrome
Pre-existing eosinophilia (absolute eosinophil count $\geq$ 500/uL)	Facilitate recognition of EMS
Contraindications to MRI: ferromagnetic implants, implanted devices, claustrophobia	Maximize MRI safety
STUDY WITHDRAWAL CRITERIA	RATIONALE
Intolerable or clinically significant side effects to creatine or 5-HTP	Subject safety
Hospitalization for suicidal ideation/suicide attempt	Subject safety
Initiation of any excluded psychotropic medication (antipsychotic, mood stabilizer or antidepressant)	Minimize risk of adverse drug-drug interaction
Discontinuation of or inadequate adherence to FDA-approved SSRI/SNRI	This is an augmentation study
Initiation of any excluded serotonergic medications	Minimize risk of serotonin syndrome
Evidence of serotonin syndrome	Subject safety
Evidence of EMS	Subject safety
Incarceration	Research involving prisoners is generally prohibited

Positive pregnancy test, pregnancy	Subject safety

a. Inclusion and Exclusion Criteria: Inclusion and exclusion criteria are detailed in Table 1:

b. Study Withdrawal Criteria: A participant will be withdrawn from the study if he or she experiences intolerable or clinically significant side effects to the study intervention (including the development of serotonin syndrome or eosinophilia-myalgia syndrome), hospitalization for suicidal ideation or suicide attempt, develops a positive pregnancy test or gives other evidence of pregnancy, is incarcerated, initiates any excluded medication, or discontinues or changes the baseline antidepressant. In addition, the principal investigators retain the right to withdraw participants from the study without their permission, in the event they are unwilling or unable to maintain adherence to the research protocol.

c. Procedures: All procedures performed by study personnel are research-related. None of the study activities will be considered standard of care. There will be no cost to study subjects for their participation. Participants will be compensated for their time and travel. Table 2 outlines the schedule of study procedures for clinical participants. Study visits will be supervised by a board-certified/board-eligible psychiatrist and will be conducted either by a board-certified/board-eligible psychiatrist or a bachelor's level research assistant with training in the specific measures used. Physical examinations and laboratory interpretation will be conducted by a board-certified/board-eligible psychiatrist. Consent will be obtained before any study procedures are initiated. Potential participants will be informed of the study and offered a consent form to review. They will be encouraged to discuss study participation with their relatives. If a potential participant expresses interest in study participation, the informed consent process will be conducted. After the informed consent process, individuals will be offered time to consider study participation and to ask questions. Subjects will have the opportunity to discuss the study with a study team member in a setting free of coercion. The language of the informed consent form is written at a level easily understood by the subject and any questions asked by the subject will be answered honestly and without bias. A specific meeting time will be set up between a study team member and the participant where the entire informed consent document will be carefully explained in its entirety. The length of the meeting will be designed so there is the necessary amount of time for all questions to be answered. Subjects will be separately consented for blood sample storage for later genetic testing and will be allowed to opt-out of this procedure while still participating in the other aspects of the study.

To determine if an individual is eligible for study participation, a screening visit will be conducted. Initially, a HAM-D_{17} will be administered to determine if the patient exhibits depressive symptoms that are sufficiently severe for inclusion in the study. The duration of the patient's current residence and the altitude of that residence will be assessed. Next, the SCID-5 will be administered to confirm a diagnosis of a current major depressive episode. In addition, subjects will be screened for other DSM-5 disorders using the SCID-5 to assess exclusion criteria. Afterward, the MADRS, C-SSRS, BAI, YMRS, ATRQ, and CGI-S will be administered. All participants will receive a pregnancy test unless they report a history of menopause or hysterectomy. Study subjects will receive a baseline BUN/creatinine (to assess for renal insufficiency), CBC (to screen for pre-existing eosinophilia), measurement of whole blood serotonin, vitals including pulse oximetry, and a baseline focused physical. Subjects will be asked to be fasting for at least 3 hours prior to the blood draw. The blood draw will occur between 2 and 5pm to minimize circadian variation in serotonin levels. Subjects will be given an internet link and passcode to complete the Diet History Questionnaire II (DHQ-II) and asked to do this at home.

An EKG will be performed to evaluate the QT interval and to screen for other evidence of cardiac disease. They will also be screened for evidence of serotonin syndrome using the Hunter Criteria. A complete medical and psychiatric history (focused on illness duration, length of current

treatment, treatment history, and history of medical illnesses that preclude participation, e.g., renal disease, pulmonary disease, rheumatologic disease, diabetes, gastrointestinal disease, pregnancy, seizure disorder, serotonin syndrome) will be completed.

After the screening visit, participants will have a **baseline** visit after one week with the MRI happening ± 4 days from the baseline visit, prior to the start of treatment. At the baseline visit, the HAM-D_{17}, MADRS, C-SSRS, BAI, YMRS, and CGI-S will be administered. Vital signs will be checked and a physical exam performed if patient symptoms indicate a need. Whole blood serotonin will be drawn. Subjects will be asked to be fasting for at least 3 hours prior to the blood draw. The blood draw will occur between 2 and 5pm to minimize circadian variation in serotonin levels. Participants will be evaluated for serotonin syndrome using the Hunter Criteria. Baseline ^{31}P-MRS and resting state fcMRI will be completed. After initiation of treatment, **subjects will be reevaluated at 1, 2, 4, 6, and 8 weeks (±3 days)**. At each these visits, the HAM-D_{17}, MADRS, C-SSRS, BAI, YMRS, and CGI-S will be administered again. Subjects will be asked to be fasting for at least 3 hours prior to the blood draw. Whole blood serotonin will be drawn. The blood draw will occur between 2 and 5pm to minimize circadian variation in serotonin levels. Follow-up ^{31}P-MRS and resting state fMRI will be completed at week 8. Vital signs with pulse oximetry, focused physical exam if indicated, collection of urine for pregnancy screening if indicated, recording of concomitant medications, self-reported drug use, medication compliance assessment, study medication dispensing, and screening for treatment-related adverse effects will also be undertaken. Screening for treatment-related adverse effects will include application of the Hunter Criteria for serotonin syndrome[164] as well as evaluation for symptoms of eosinophilia-myalgia syndrome. If subjects exhibit symptoms of eosinophilia-myalgia syndrome, a CBC with automated differential will be obtained. Finally, participants will be seen for follow up visits at 2 weeks and 4 weeks (±3 days) after completion of 5-HTP/Cr supplementation. Measure at these visits will include the HAM-D_{17}, MADRS, C-SSRS, BAI, YMRS, CGI, DESS, vitals with pulse oximetry if indicated, focused physical exam if indicated, and evaluation for serotonin syndrome and EMS. Again, if symptoms of EMS are present, a CBC with automated differential will be obtained. After the screening visit, Participants can request study visits by videoconference with the exception of briefly dropping by the clinic for a blood draw and/or MRI as indicated in the schedule below.

Table 2. Study Procedures for Clinical Subjects

	Screening	Baseline	Treatment					Follow-up	
Week	-1	0	1	2	4	6	8	10	12
SCID-I	X								
HAM-D17	X	X	X	X	X	X	X	X	X
MADRS	X	X	X	X	X	X	X	X	X
C-SSRS	X	X	X	X	X	X	X	X	X
BAI	X	X	X	X	X	X	X	X	X
CGI-S	X	X	X	X	X	X	X	X	X
YMRS	X	X	X	X	X	X	X	X	X
DESS								X	X
Medical History	X								
Diet History Questionnaire II	X								
ATRQ	X								
Hunter criteria		X	X	X	X	X	X	X	X
Screening for EMS			X	X	X	X	X	X	X
Side-effect screening			X	X	X	X	X		
Pregnancy test**	X	X	X	X	X	X	X		
BUN/Creatinine	X								
CBC with differential	X		X*	X*	X*	X*	X*	X*	X*
Whole-blood 5-HT (fasting for > 3 hours; drawn between 2:00 and 5:00pm	X	X	X	X	X	X	X		
EKG	X								
Vitals including pulse-oximetry	X	X	X	X	X	X	X	X	X
Focused physical exam	X		X*	X*	X*	X*	X*	X*	X*
Review of current medications	X	X	X	X	X	X	X		
^{31}P-MRS		X					X		
Resting State fMRI		X					X		

* = only if clinically indicated by presence of adverse symptoms or symptoms suggestive of EMS or serotonin syndrome
** = all female subjects, unless reporting menopause, hysterectomy, tubal ligation or currently using an intrauterine device (IUD)

Once entered into the study, participants will be 1:1:1:1 randomized to receive either Cr+ placebo, 5-HTP+placebo, 5-HTP+Cr, or placebo+placebo. Block randomization using a SAS code, PROC PLAN with Block, will be used to ensure equal treatment allocation within each block. The study will be conducted as a double-blind trial, with neither participants nor research staff aware of participant assignment. Except in cases of medical emergency, the double-blind will not be "broken" until recruitment is closed and the final participant has completed 8 weeks of treatment and 4 weeks of follow-up. We anticipate an attrition rate of ~20% based on our pilot study (where attrition was 13%), and so will plan to recruit a total of 75 subjects, for a total recruitment of 60 subjects. The blind will be broken following the culmination of the study or at the request of a medical professional dealing with a medical emergency in a case in which it would help a study participant.

d. Study Drug Dosing: Cr doses will be 5g by mouth daily. 5-HTP doses will be 100mg BID. These doses are based those most commonly used in clinical trials or in historical practice. However, with natural products it is often unclear what the optimal doses are to balance safety and efficacy. For Cr, a dose of 5g was selected because this was the dose used effectively by Lyoo *et al.* (2012) and in our pilot study of 5-HTP + Cr**.** A 5-HTP dose of 100mg twice daily was selected because this is equivalent to the average total daily dose of 200mg given as a treatment option for depressed patients [89]. We have elected to provide patients with twice daily dosing because the half-life of 5-HTP is estimated to be 4.3$\pm$2.8h.[158] In previous studies, 200mg given as a single daily dose was well tolerated with few side effects reported.[89,159]

e. Statistical Design and Power

1. Power and Sample Size Determination: *Power to detect changes in PCr:tP between groups is our primary determinant of sample size in the R61 phase because of the centrality of spectroscopic findings in our hypotheses.* In another study, after 8-weeks of oralCr, adult female methamphetamine users had significantly increased PCr levels with a standardized effect size of

0.920.[126] In an open-label study in depressed adolescents,[124] PCr levels following Cr administration increased by 6.2%± 9.6%. In a dose-finding study of Cr compared to placebo, the effect size for differences in PCr levels between the group receiving 4 grams of Cr daily compared to placebo was 0.7. We hypothesize that supplementation with Cr with or without 5-HTP will increase frontal cortical PCr levels. ^{31}P-MRS neuroimaging will be performed at baseline, and repeated after 8 weeks of treatment with Cr + placebo, 5-HTP + placebo, or 5-HTP + Cr. To address our first hypothesis, a population-averaged generalized estimating equation (GEE) regression model is used for group comparison of the ΔPCr (PCrWEEK 8 - PCrBASELINE) associated with Cr + placebo, 5-HTP + placebo, or 5-HTP + Cr. We will control for age, gender, and tissue partial volume effects (i.e., gray matter, white matter and cerebrospinal fluid) for in vivo phosphorus metabolite measurements made at two time points. The GEE for each subject, i has the form $Yj(t)=Xj\ \beta + \varepsilon j$, where Yj and Xj represent measured metabolite (PCr or β-NTP) levels and design matrix of covariates, respectively. The error term follows a multivariate normal distribution: $\varepsilon_j \sim N(0, Vj)$, where Vj is the variance of metabolite levels. We assume that measures taken closer to each other in time are more highly correlated than those taken further apart. Therefore, the first-order autoregressive structure, AR(1) is assumed such as $cov[\varepsilon_j(t), \varepsilon j(t')] = \rho^{|t-t'|}Vj$ to model the dependence of the correlation on repeated measures from the same participant, where t is time variable and ρ is the correlation coefficient between the clinical symptom measures of two successive assessments. Assuming ρ =0.30, expected MDES = 5% across the groups, and harmonic mean SD = 10%, respectively, a sample size of 60 participants (15 per group; 4 groups) and 2 neuroimaging time points per participant has >80% power using the AR (1) GEE model at the 0.05 alpha level. In our open-label study, we experienced ~20% participant attrition. Imputation of missing data cannot be utilized for neuroimaging data. Therefore, anticipating a 20% attrition rate, we propose a target enrollment of 75 participants for the R61 phase.

2. Analysis of ^{31}P-MRS Data: To analyze study data, a raw Hamming filter will be applied before performing 2D fast Fourier transformation, and each free induction decay (FID) will be line-broadened with 10 Hz of apodization. After Fourier transformation and frequency shift correction, zero-/first-order phase correction and baseline correction with polynomial interpolation will be applied. Spatial filtering with a Hamming window function will be implemented to reduce signal contamination from neighboring voxels. The preprocessed ^{31}P-MRSI data will be fitted using jMRUI software[180] with the Advanced Method for Accurate, Robust and Efficient Spectral (AMARES) fitting algorithm.[181] Metabolites of interest include PCr, β-NTP, tP, and their ratios. From registered anatomical images, tissue segmentation will be performed using FSL (FMRIB's Software Library) software so that cerebrospinal fluid (CSF)-corrected metabolite concentrations as well as gray matter percentage in each voxel can be used as covariates in the analysis. Generalized estimating equations regression modeling will be used to evaluate group differences in metabolite levels controlling for age, sex, education level, and tissue partial volume effects (i.e. gray matter, white matter, CSF). Subjects with MDD will be compared to HCs at baseline and at follow-up. Sub-group analyses by gender will be included given evidence that Cr has been proposed as especially effective in women. Fisher's exact test will be used to compare groups on categorical variables.

3. Analysis of Resting State Data: As is seen in the small cohort of pilot data, we anticipate treatment groups to demonstrate increased global connectivity of the subgenual ACC after treatment, among other changes. A recent analysis determined that we can achieve single subject reproducibility of FC metrics of 0.16 Fisher-transformed correlation units.[161] If we conservatively set the effect size at 8% and SD at 9% of mean, a sample size of 60 depressed subjects would achieve >90% power to detect significant treatment effects with the repeated measures design compared to baseline. For FC analyses, 361 regions of interest (ROIs) comprising a parcellation of cortical gray matter[182] will be compared between placebo and active treatment subjects. We

will conduct a second level of analysis using eight networks delineated as follows and derived from Gordon *et al*.:[182] auditory, salience, frontoparietal, default, dorsal attention, ventral attention, sensorimotor, and visual. Two levels of analysis will be performed to balance multiple comparisons and granularity of spatial information. For each pair of 8 networks, mean BOLD time series will be extracted across the entire network and Fisher-transformed correlation coefficients will be calculated. An acceptable false discovery rate ($q < .05$) over all pairs of networks will be assumed to denote significant partial correlation with diagnosis. To evaluate which connections are anticorrelated, we will use an independent dataset for an unbiased estimate obtained from the S1200 Release of the Human Connectome Project (HCP) dataset. Group-wise ANOVA will be performed with false discovery rate correction for multiple comparisons across 361 x 361 connections tested for treatment group differences in FC before and after treatment compared to placebo group. Sub-group analyses by gender will be included given evidence that Cr has been proposed as especially effective in women. Subjects with MDD will be compared to HCs at baseline and at follow-up.

4. Analysis of 5-HT Levels: There is limited data available regarding the effect of twice-daily oral 5-HTP supplementation on whole blood 5-HT levels in subjects with MDD taking ADs. Estimates of whole blood (platelet) serotonin levels in MDD vary widely, including values from 119 $\pm$ 52.9 ng/ml (n=30)[183] to 169.5 $\pm$ 74.5 ng/ml (n=29)[184] to 203.2 $\pm$ 59.3 ng/ml (n=25),[185] suggesting that whole blood 5-HT levels average roughly 161 $\pm$ 62 ng/ml in subjects with MDD. A sample of 60 subjects with MDD allocated 1:1 between 5-HTP (5-HTP+Cr and 5-HTP+placebo) and placebo (Cr+placebo and placebo+placebo) would have, in a t-test of means, >70% power to detect an effect size of 0.8. This effect size would be equivalent to an increase in average 5-HT levels to roughly those seen in non-depressed subjects.[186] Although this is a relatively large effect size, it is consistent with findings related to the effect of oral tryptophan supplementation (at 50mg/kg/day in non-depressed men) which found an estimated effect size of 1.1.[187] This analysis assumes that creatine supplementation will not independently affect circulating serotonin levels, though there is no data regarding this to our knowledge. 5-HT levels will be analyzed using linear mixed models with repeated (before and after) measures, controlling for gender, age, group allocation, antidepressant use, oxygen saturation, and other confounds.

e. Measures

We plan to use the following instruments for data collection:

- Structured Clinical Interview for DSM-5 (SCID-5)
- Hamilton Depression Rating Scale (HAM-D)
- Montgomery-Asberg Depression Rating Scale (MADRS)
- Columbia Suicide Severity Rating Scale (C-SSRS)
- Clinical Global Impressions Scale (CGI) severity of illness subscale (CGI-S)
- Beck Anxiety Inventory (BAI
- Young Mania Rating Scale (YMRS)
- Discontinuation-Emergent Signs and Symptoms checklist (DESS)
- Serotonin Syndrome Screening-Hunter Criteria
- Eosinophilia-Myalgia Syndrome Screening
- Eosinophilia-Myalgia Participant Information Sheet
- Serotonin Syndrome Participant Information Sheet
- National Cancer Institute Diet History Questionnaire Version II (DHQ-II)
- Antidepressant Treatment Response Questionnaire (ATRQ)

f. Potential Risks: During the screening visit, participants may become emotionally upset when asked about their psychiatric history including suicide attempts, or physical and sexual abuse.

Participants may also experience discomfort when providing urine for pregnancy tests. It is possible that a participant's illness could worsen during the study. This could be related or unrelated to the study. If the participant's illness worsens to the point that they are a danger to herself or others, they will be referred for appropriate care. If the participant is hospitalized for worsening illness, they will be withdrawn from the study. Participants may experience gastrointestinal discomfort as a result of taking creatine or 5-HTP. Participants will be encouraged to take both medications with food to minimize this risk. Finally, as detailed above, supplementation with 5-HTP increases the risks of eosinophilia-myalgia syndrome and serotonin syndrome. Notably, in our pilot study, there were no serious adverse events attributable to the study drugs. Participants will be counseled about these risks, informed of the signs and symptoms of these conditions, provided with information sheets detailing the symptoms of these disorders in lay terms and describing steps to take for further evaluation if indicated, and will be screened for both conditions at each visit after the initiation of treatment.

MRI/MRS scans do not use ionizing radiation like x-rays or CT scans. Instead, magnetic fields and radio waves are used to take the pictures. There are no known risks related to MRI scans – other than the risk of injury when metallic objects are brought into the scanning room by mistake. Serious injury can occur during an MRI scan to persons who have:

- Cardiac (heart) pacemakers.
- Metal clips on blood vessels (also called stents).
- Artificial heart valves.
- Artificial arms, hands, legs, etc.
- Brain stimulator devices.
- Implanted drug pumps.
- Cochlear (ear) implants.
- Ocular (eye) implants or known metal fragments in eyes.
- Exposure to shrapnel or metal fillings
- Other metallic surgical parts.
- Orthodontic braces on the teeth.
- Body jewelry or piercings that cannot be removed for the scan.
- Certain tattoos with metallic ink
- Certain transdermal (skin) patches such as NicoDerm (nicotine for tobacco dependence),
- Transdermal scopolamine or Ortho-Evra (birth control)

If the participants have any such devices, or has had a surgery where metal devices were placed in their body, they cannot take part in the study unless cleared for MRI scanning by the surgeon who implanted the medical device(s).

Serious risks exist if ferromagnetic objects (things that stick to magnets) are brought into the scanning area. These items can become dangerous flying objects and are not allowed near the MRI scanner. The FDA has approved the 3T scanner for routine clinical studies. The FDA has decided that MRI machines of 8T or less do not pose a risk. Although the scans we are using in this study have no known risks, there could be ill effects that are delayed, such that they have not yet been recognized by the FDA. The brain scans do not cause pain. Apart from the scanner noise, the participant will not know the scan is taking place. Inside the scanner, some people experience claustrophobia (fear of being in small spaces), dizziness, headaches, or a metallic taste in the mouth. Some people experience double vision or see flashing lights. These symptoms are temporary, and will stop when the participant leaves the scanner. The participant may feel cramped inside the scanner. There is a mirror placed inside the scanner so the subject can see his or her face, and look out into the scanning room. The technologist will be able to hear the participant at all times. Very rarely, someone having an MRI scan feels a tingling in his or her

back. This is due to the magnetic field changing quickly during the scan. The precautions taken will avoid all the known risks related to MRI scans. The participant can stop the scan at any time.

Finally, there is a small risk that subjects' protected health information will be lost or misappropriated during the study.

g. Adequacy of Protection against Risks

h. Recruitment: Subjects will be recruited through radio advertisements broadcast on local radio networks. We will also contact eligible participants who have indicated an interest in research participation who have listed themselves in the Department of Psychiatry research recruitment database. Additionally, we will contact outpatient providers in the Department of Psychiatry to apprise them of the study so that they can refer patients (no referral fees will be offered), and these providers will be sent digital copies of the flyer via email. Clinical participants will be compensated a total of $235 in cash to account for travel expenses and time. Compensation will be given using the schedule below. Per-visit compensation is based on the estimated time for each visit (at least one hour) and is judged not to represent an undue inducement. Study participants may choose to skip being compensated at some visits and then be compensated in lump sums.

1. $25 - Screening
2. $65 - Baseline visit (includes $50 compensation for MRI)
3. $15 - Treatment phase visits (weeks 1, 2, 4, 6)
4. $65 – Treatment week 8 (includes $50 compensation for MRI)
5. $10 – Follow-up visits (weeks 10, 12) (total $20)

i. Informed Consent: Consent will be obtained before any study procedures are initiated. Potential participants will be informed of the study and offered a consent form to review. They will be encouraged to discuss study participation with their relatives. If a potential participant expresses interest in study participation, the informed consent process will be conducted. After the informed consent process, individuals will be offered time to consider study participation and to ask questions. Subjects will have the opportunity to discuss the study with a study team member in a setting free of coercion. The language of the informed consent form is written at a level easily understood by the subject and any questions asked by the subject will be answered honestly and free of bias. A specific meeting time will be set up between a study team member and the participant where the entire informed consent document will be carefully explained in its entirety. The length of the meeting will be designed so there is the necessary amount of time for all questions to be answered. Subjects will be separately consented for blood sample storage for later genetic testing and will be allowed to opt-out of this procedure while still participating in the other aspects of the study.

j. Screening for Serotonin Syndrome: Given the potential development of serotonin syndrome in patients taking multiple serotonergic medications (in this study, an SSRI plus 5-HTP), monitoring for serotonin syndrome is an important element of participant safety. There are no widely-used standardized scales for the assessment of serotonin syndrome, but algorithms for the detection and diagnosis of the condition exist [164]. As noted above, subjects will have vital signs performed at each visit. Participants will also be asked about symptoms of serotonin syndrome at each visit. In our pilot study, a physician completed a thorough physical exam for each patient to screen for these symptoms, but no cases of serotonin syndrome were required and this approach was deemed to be inefficient. Accordingly, in the currently proposed study, we will have a physician on call during each patient visit to complete a physical exam and further assessment for serotonin syndrome if there are vital sign abnormalities or symptoms (severe diarrhea or nausea, sweating, shakiness, stiffness, confusion) that are suggestive of serotonin syndrome. In such cases, the clinician will assess for ocular clonus, mydriasis, diaphoresis, rigidity,

hyperreflexia, and clonus, and will evaluate the likelihood of serotonin syndrome using the Hunter Criteria. Finally, at the baseline visit subjects will receive an information sheet describing signs and symptoms of serotonin syndrome in lay terms and providing instructions on how they should seek further evaluation if those symptoms are evident.

k. Screening for Eosinophilia-Myalgia Syndrome: No standardized measures for the detection of eosinophilia-myalgia syndrome (EMS) exist. In this study, participants will be informed about the symptoms of EMS, and will be provided with an information sheet describing the symptoms of EMS in lay terms and providing instructions on how they should seek further evaluation if those symptoms arise. They will be asked about symptoms that may be indicative of EMS (especially myalgias) at each treatment and follow-up visit. At the screening visit, a CBC with automated differential will be performed to screen for pre-existing eosinophilia. Although in principle participants could receive a complete blood count at each visit after the initiation of treatment to screen for the development of EMS, to our knowledge there is no published data to support this intervention and the presence of eosinophilia in the absence of clinical symptoms is not likely to be specific for EMS. In our pilot study, subjects received a physical exam at each visit to screen for symptoms of EMS, but this was found to be an inefficient measure. Accordingly, in the currently proposed study a physician will be on call during each patient visit to complete a physical exam and further assessment for EMS if there are vital sign abnormalities or symptoms (rash, other skin changes, hair loss, muscle aches, joint pain, shortness of breath) that are suggestive of EMS.

l. Adverse events

Unanticipated problems and adverse events that are related to the research, or which place participants at greater-then-expected risk, will be reported to the Institutional Review Board (IRB) within ten working days of the event. *Unanticipated problems* involving risk to participants or others are defined as any incident, experience or outcome that meets the following criteria:

- Unforeseen (not expected by the researcher or the research participant) given the research procedures and the subject population being studied;
- Related or probably related to participation in the research, or if the event or problem probably or definitely affects the safety, rights and welfare of current participants; and
- Suggests that the research places participants or others at a greater risk of harm (includes physical, psychological, economic or social harm) than was previously known or recognized.
- An *unexpected adverse event* is any adverse event occurring in one or more participants participating in a research protocol, whose nature, severity, or frequency is not consistent with either:
    - The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in the protocol related-documents (i.e. protocol, investigational brochure, consent form, or product labeling).
    - The expected natural progression of any underlying disease, illness or medical condition of the subject experiencing the adverse event.

The Principal Investigator will make the initial determination if an unexpected, adverse event is related or unrelated to the investigational drug or a clinical or research procedure. An adverse event is “related to the research” if in the opinion of Dr. Kious or Dr. Renshaw, it was more likely than not related to the investigational agent or intervention.

m. Potential Benefits of Proposed Research to Human Subjects and Others:

- A thorough physical and psychiatric evaluation
- Access to advanced neuroimaging procedures, which could reveal unanticipated structural abnormalities

- The study is designed to test the hypothesis that the combination of 5-HTP and creatine is effective for the treatment of major depressive disorder that has not responded to conventional treatments; accordingly, there is some possibility of clinical improvement for participants
- We also hope that the research will add to our understanding of the potential of the study interventions as treatments for major depressive disorder, as well as contributing to general understanding of the pathophysiology of that condition.

n. Importance of Knowledge to be Gained: The risks of adverse events related to study participation are judged to be small on the whole, and we believe that the procedures described above will mitigate them sufficiently. Even so, because of the social and personal impact of major depressive disorder, we believe that the risks posed by this study are reasonable.

o. Registration with Clinicaltrials.gov: A registration has been submitted to clinicaltrials.gov as required.