Effect of MMFS-202-302 on Cognitive Enhancement in Schizophrenia

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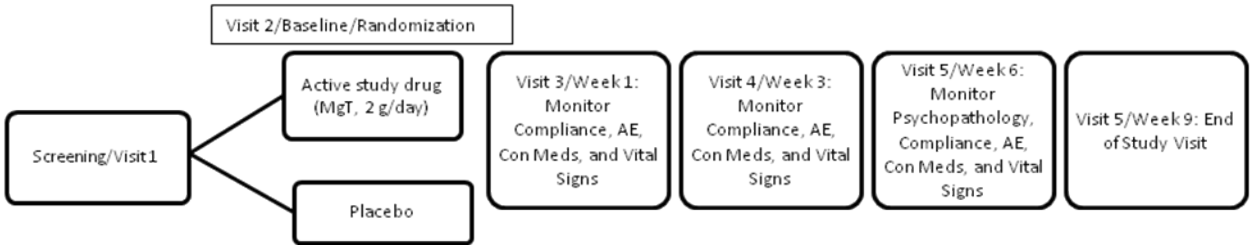
Study Schema



SUMMARY
The goals of this study are to study MMFS-202-302 in a double blind, randomized, placebo-controlled 9-week study of its effect on ameliorating cognitive deficits in 60 patients with schizophrenia or schizoaffective disorder with stable levels of positive symptoms. Secondary end points will include changes in functional measures, and positive and negative symptoms. One dose of MMFS-202-302 will be studied and compared with placebo as adjunctive treatment to atypical antipsychotic drug treatment. MMFS-202-302 is the clinical code describing the treatment formulation, combining the release profile of the compound and the dose of each tablet (i.e. 202 is a 0.5 g 6-hr release tablet and 303 is a 0.5 g 12-hr release tablet).

1.0 RATIONALE

1.1 Background and Significance

1.1.1 Cognitive impairment in schizophrenia and schizoaffective disorder

Cognitive deficits of variable severity are present in virtually all patients with schizophrenia and schizoaffective disorder at the time of first diagnosis, are often the major reasons for functional impairment, and respond only partially to treatment with antipsychotic drugs (APDs) [1]. Even this is disputed by some authorities in the field, who attribute improvement to practice effects. However, it is indisputable that many patients can be shown to improve beyond a practice effect size in specific cognitive domains, especially semantic memory, declarative memory, and speeded motor testing [2-4]. Such improvement can be demonstrated in 6-12-week studies, with additional improvement over a six-month total study period. The search for agents which can be used to treat cognitive impairment in schizophrenia (CIS) is of the highest priority for these reasons.

Of particular note, working memory (WM) is a particularly impaired process demonstrated across the illness spectrum [5]. Of all clinical symptomatology experienced, negative symptoms (NS) have shown to be the most intractable, resilient to treatment, and debilitating with regards to functional outcome. Notably NS, which include alogia, blunted affect, asociality, avolition, and anhedonia, are somewhat associated with WM performance; however, the precise nature of this relationship is unclear [6]. Investigation of the reward system has proven particularly insightful in this regard, especially given that abnormalities in reward processing are relevant to different aspects of SCZ cognition as well as non-cognitive aspects of NS [7], particularly anhedonia and avolition [8]. Current research indicates differential areas of preserved and impaired function in aspects of reward processing in schizophrenia subjects, including valuation of hedonic experience, decision making, and rapid versus slow reinforcement learning [7]. Critically, this evidence also suggests WM is an influential intermediate variable in the production of abnormal hedonic experience and decision making in schizophrenia, noting that subjects with better WM show less severe deficits in these aspects of reward processing [9]. This suggests that previously believed dissociable WM and affective functions, subserved respectively by dorsal and orbital prefrontal and infralimbic cortices, are both implicated in deficit behavior related to schizophrenia. Given this relationship, it remains unclear whether this occurs as a result of faulty neural circuitry or abnormalities related to shared dopaminergic inputs, or both. Furthermore, there is strong evidence that the psychobiological substrates of negative symptomatology are directly related to both reward [10] and WM [11] circuitry. These circuits, and thus functions, are known to be heavily regulated by both mesocortical

dopamine (DA) D1, mesolimbic D2 receptors [10, 12], and prefrontal NMDA and $GABA_A$ and $GABA_B$ receptors [13]. From a pharmacologic perspective, this presents an opportunity for treatment using novel agents that act upon these receptors.

1.1.2 Preclinical Rationale for Studying L-Threonic acid Magnesium salt (L-TAMS; clinical code: MMFS) to treat CIS, negative symptoms, and possible adjunct for psychosis

Preclinical Evidence:
L-threonic acid magnesium salt (L-TAMS - also known previously as magnesium L-threonate, MgT) has shown promise in PCP-induced animal models of schizophrenia as an adjuvant to clozapine in the amelioration of cognitive deficits [14]. It is hypothesized this occurs by L-TAMS enhancing the effectiveness of atypical antipsychotic NMDA and GABA actions, as Mg is a necessary co-factor in NMDA receptor response. In addition, recent data from Professor Xuechu Zhen's lab at Soochow University revealed that L-TAMS treatment improved PCP-induced social interaction in rats, an animal analogue widely studied model of NS. These promising findings prompt the investigation of this novel compound in the specific treatment of core cognitive and NS in schizophrenia, using both imaging modalities and clinical trial methodology. The value of these investigations is that precise neurobiological markers of potential change in NS can be identified.

Initial trials of putative cognitive enhancing drugs should collect data on all major domains of cognition, including working, semantic and episodic (declarative) memory, executive function, social cognition, attention and speed of processing as our knowledge in this area is limited. Findings from initial trials can then guide subsequent trials.

L-TAMS was also shown in a rodent study to improve novel object recognition (NOR) in subchronic phencyclidine-treated rats. This is a valuable model as a measure of declarative memory. Atypical APDs, including clozapine, have a similar effect on NOR in the PCP rodent model as we and others have shown. This model is based on NMDA hypofunction and dysfunction. They also improve other domains of cognition, including executive function and working memory. L-TAMS may do the same. Mg^{2+} is a necessary co-factor in NMDA receptor response, and L-Threonate is a critical component of L-TAMS for delivery of Mg^{2+} to the CNS and to neurons. The basis for the effect of the atypicals is enhanced DA and ACh efflux in cortex and hippocampus as shown by studies with microdialysis. Electrophysiological studies show that the atypicals restore NMDA and GABA currents in cortex and hippocampus, respectively. Importantly too, L-TAMS has been shown to improve working memory in rats [14].

Clinical Evidence:
Recent studies have shown that L-TAMS (clinical code MMFS) can also improve cognition in humans, particularly frontal cortex-mediated executive functions such as working memory. In a double-blind, placebo-controlled human trial (n=50), L-TAMS (MMFS-01) significantly improved *overall cognitive ability* in older adults with mild cognitive impairment (MCI) after 6 weeks of oral intake [15]. In that study, cognitive ability was assessed with a neuropsychological test battery (NTB; Cohen's d = 0.74, 0.91 at 12 weeks), which was comprised of tests of working memory (Digit Span), executive function (trail making test – part B; TMT-B), episodic memory (Face-Name association test), and attention (Eriksen Flanker Congruent/Incongruent test). Notably, performance on the *Digit Span test* was significantly improved at 6 weeks by MMFS (Cohen's d = 0.61, p = 0.023), and a trend remained evident at 12 weeks (Cohen's d = 0.30, p = 0.064), indicating that L-TAMS can improve working memory in humans. The current study will help to inform our understanding of the generalizability of these procognitive effects to different patient populations, specifically schizophrenia and schizoaffective disorder.

1.1.3 Rationale for an RCT of Cognitive Effects of L-TAMS (MMFS-202-302) in Schizophrenia and Schizoaffective Disorder

We propose to test the hypothesis that MMFS-202-302, the clinical formulation of the L-TAMS compound and dosage regimen, and a novel means to elevate intraneuronal Mg^{2+} levels in the brain, will improve one or more cognitive domains in patients with schizophrenia or schizoaffective disorder. This hypothesis is based upon data from Professor Guosong Liu's lab at Tsinghua University and Professor Xuechu Zhen's lab at Soochow University, that L-TAMS improves several

dimensions of cognition in rodents, particularly declarative memory as assessed by NOR and T-Maze, a measure of working memory. Professor Zhen reported that L-TAMS can augment the ability of sub-effective doses of the atypical APD clozapine to reverse the effects of subchronic PCP on NOR. We have found that other treatments which have a similar effect also improve other domains of cognition. We postulate that MMFS-202-302 will enhance the effectiveness of subeffective and/or clinical doses of other atypical APDs in addition to clozapine, as these drugs share many of the pharmacologic features of clozapine.

L-TAMS was also shown to improve PCP-induced social withdrawal, suggesting ability to improve negative symptoms. It blocked PCP-induced locomotor activity and impairment in PPI, suggesting ability to improve positive symptoms as well.

Patients receiving clozapine treatment will be favored for inclusion. L-TAMS has been shown to enhance the effect of clozapine to improve cognition and psychosis in PCP-treated rats. Patients receiving other atypical antipsychotic drugs that are widely used, i.e. risperidone, aripiprazole, quetiapine, lurasidone, or olanzapine will also be eligible. Patients with tardive dyskinesia will be excluded because of the evidence that tardive dyskinesia may indicate a treatment resistant form of cognitive impairment.

Additionally, this study will evaluate the potential WM enhancing and NS reduction effects of MMFS-202-302 among a group of individuals with schizophrenia disorder on a series of functional magnetic resonance imaging (fMRI) WM and reward tasks. We will evaluate whether MMFS-202-302 + APD vs. placebo + APD increases cognitive performance on a task of WM, and associated task-related increases in BOLD activation in the dorsolateral prefrontal cortex (DLPFC), and posterior parietal cortex (PPC). Additionally, we will assess whether MMFS-202-302 + APD vs. placebo + APD enhances reward circuitry (orbitomedial prefrontal cortex [OMPFC], anterior cingulate [ACC], nucleus accumbens [NAc], and ventral tegmental area [VTA]) and reward behaviors in the expression of NS. Positive findings will provide biomarker evidence for MMFS-202-302 effects on neural systems underlying these cognitive processes.

Finally, this study will measure brain network interactivity using electroencephalography (EEG) at baseline and end of study to examine any differences in activation or connectivity using learning and memory tests over the course of the trial. More information can be found in sections 5.0, 7.4, and 8.1 below.

2.0 OVERVIEW OF STUDY DESIGN

Patient population

All patients will meet criteria for DSM-V diagnosis of schizophrenia or schizoaffective disorder. At the time of enrollment, they will have been receiving a stable dose of an atypical antipsychotic drug for 2 months or longer and have stable positive symptoms, i.e. delusions or auditory hallucinations, at the screening visit. 60 patients will be enrolled, and randomized in a 1:1 ratio to one dose of MMFS-202-302 or placebo.

2.1 STUDY OBJECTIVES

1. To determine the effectiveness of 9 weeks' supplementation with MMFS-202-302 as augmentation of atypical antipsychotic medication, to improve working memory or other specific domains of cognitive function, e.g. attention, executive function, declarative memory, etc., in patients with schizophrenia or schizoaffective disorder. The primary outcome measure will be a main effect of group on the 9-wk-change-from-baseline working memory subscale of the MATRICS Cognitive Consensus Battery (MCCB). The MCCB consists of 10 cognitive tests covering seven domains. This will be augmented by additional neuropsychological measures of verbal fluency (FAS Phonemic Fluency), working memory (Brown-Peterson's Auditory Consonant Trigrams) and executive function (the Wisconsin Card Sorting Test). The MCCB composite score, and each of the additional cognitive domain subscales listed above will be secondary outcome measures.

2. To determine the effect of MMFS-202-302 on global function, as measured by the Clinical

Global Impressions Scale (CGI) assessment of change (CGI-C).

3. To determine the effect of MMFS-202-302 on negative symptoms of schizophrenia, as measured by the Brief Negative Symptom Scale (BNSS) and PANSS negative subscale, and imaging of brain reward networks (OMPFC, ACC, NAc, VTA).

4. To determine the effect of MMFS-202-302 on positive symptoms of schizophrenia, as measured by the PANSS positive subscale.

5. To determine the effect of MMFS-202-302 as augmentation of atypical antipsychotic medication on both the behavioral performance and activation patterns in schizophrenia patients during a WM neuroimaging paradigm. Specifically, investigation in whether improvement from baseline task performance occurs for the MMFS-202-302 + APD group, and if this improvement is associated with increases in WM-related brain region activation (DLPFC & PPC).

6. To evaluate whether MMFS-202-302 results in greater functional connectivity within identified reward and working memory circuits in subjects with schizophrenia.

7. To determine whether MMFS-202-302 stimulates an overall enhancement or restoration of typical structural brain abnormalities in schizophrenia, as measured by cortical volume and thickness of the neocortex.

8. To examine the effect of MMFS-202-302 on neural connectivity and activation in EEG assessment, using measurements of learning and memory.

3.0 CRITERIA FOR EVALUATION

Primary Efficacy Endpoint

- The primary efficacy endpoint will be the change from baseline to 9 weeks of MMFS treatment on the working memory MCCB subscale T-score, compared to placebo. The working memory subscale of the MCCB was selected as the primary outcome measure because working memory deficit is known to be a core feature of cognitive symptoms in schizophrenia and schizoaffective disorder patients, and contributes to deficits in other cognitive domains [16-18].Our previous preclinical data [14] and clinical trial data with MMFS in mild cognitive impairment [15] demonstrate that MMFS is capable of improving working memory.

Secondary Efficacy Endpoint

- Clinical Global Impression of Change (CGI-C) – this clinician-administered interview is important for demonstrating that improvements in cognition correspond to an overall functional improvement - an FDA requirement for approval of a cognition-enhancing drug for schizophrenia patients.

Tertiary/Exploratory Efficacy Endpoints

- MCCB composite score – a common efficacy endpoint in trials of drugs for cognition enhancement in schizophrenia and schizoaffective disorder, providing evidence of global cognitive improvement.
- MCCB individual subscales and supplementary cognitive tests – these subscales and tests will provide additional information regarding the efficacy of MMFS for improving specific cognitive domains in patients with schizophrenia or schizoaffective disorder. We believe that MMFS may improve other aspects of cognition in addition to working memory, especially those that involve frontal cortex function.
- A working memory composite score derived from the two MCCB working memory subscale tests along with the Brown-Peterson's Auditory Consonant Trigrams test. Deriving a composite score from 3 working memory tests may be superior to the MCCB working memory subscale, which is a composite comprised of only 2 working memory tests. This possibility will be tested to identify the best composite measure of working memory for future trials.
- Responder analysis – the number of subjects achieving a clinical significance cutoff of improving at least one half of a standard deviation (T-score change $\geq$ 5) on the working memory subscale of the MCCB, to evaluate the clinical significance of any MMFS treatment effects; an analogous approach will be employed for secondary outcomes.
- Positive and negative symptoms – Positive symptoms will be assessed with the PANSS positive

scale, and negative symptoms will be assessed with the PANSS negative scale and the BNSS. We hypothesize that MMFS-202-302 may significantly improve negative and positive symptoms, at least partially independently from its cognition enhancing effects.

Exploratory Subgroup Efficacy Analyses

Exploratory subgroup efficacy analyses will be carried out to inform enrichment of the study sample for future efficacy studies. MMFS is developed to improve cognition in patients with cognitive deficits, not as a general cognitive enhancer per se. Therefore, we plan to conduct subgroup analyses on the patients with cognitive deficits. Specifically, for the primary efficacy endpoint (MCCB working memory subscale), the data will be reanalyzed using ANCOVA as described above, restricted to the subgroup of subjects whose baseline score is at or below median for healthy age-matched norms (T-score of 50 or below on the working memory subscale). A similar analysis will be carried out using more conservative cutoffs of one half and one standard deviation (T-score of 45 and 40 or below), to further evaluate more severely deficient subsets of subjects. The purpose of these analyses is that we believe MMFS will be most efficacious for improving working memory in the subset of subjects who have working memory deficits upon entering the study. Analogous subgroup analyses will be carried out for the MCCB composite and other subscale scores, using the aforementioned cutoffs for the respective scores at baseline.

Safety Assessments

Safety will be evaluated with Fisher's exact tests, by comparing the following assessments between the MMFS-202-302 and Placebo groups: adverse events, laboratory test results, vital signs, body weight, and subjective remarks. The safety population will include all subjects who had at least one exposure to a study compound, and who had any subsequent encounter with the study staff.

4.0 PATIENT ELIGIBILITY

Subjects must meet all of the inclusion and exclusion criteria to be registered to the study. Study treatment may not begin until a subject is registered.

4.1 Inclusion Criteria

1. All patients must be capable of giving written informed consent.
2. Male or female subjects of any race; between 18 to 60 years of age, inclusive.
3. No hospitalization other than for evaluation in the past four months
4. Resides in a stable living situation, according to the investigator's judgment.
5. Diagnosis of schizophrenia or schizoaffective disorder of at least one year duration, as established by the SCID-I, and verified with medical records and/or confirmation of diagnosis by treating clinician. The illness is in a nonacute phase as determined by the subject's primary treating clinician
6. Current psychotropic drug treatment consists of monotherapy with an atypical antipsychotic drug.
7. No more than a mild level of EPS as determined by the Simpson Angus Scale (SAS) total score: ≤ 6
8. Not taking anticholinergic medication for EPS
9. No evidence of tardive dyskinesia
10. Subjects healthy enough to complete a 9-week clinical trial
11. Women of childbearing potential must have a negative pregnancy test at screening and baseline, and agree to use adequate protection (i.e. double barrier method) for birth control.
12. Able to complete cognition assessments in English
13. General intellectual abilities falling broadly within the average estimated IQ $\geq$ 80, as measured by the *Wide Range Achievement Test – 4th Edition (WRAT-IV)*.

4.2 Exclusion Criteria

1. Failure to perform screening or baseline examinations
2. Hospitalization within 8 weeks before screening, or change of antipsychotic medication or dose within 2 months prior to screening
3. Subjects who have participated in another clinical trial with an experimental medication within the past 2 months.
4. Patient has had cognitive battery similar to those used in this study within the last 12 months
5. Subjects with other DSM-V Axis I or Axis II primary diagnoses
6. Diagnosis of alcohol or substance abuse or dependence within the past 3 months,
7. Significant suicide risk as determined by the Columbia Suicide Severity Rating Scale (C-SSRS)

8. Subjects who plan to begin a new course of cognitive remediation therapy, or have been receiving cognitive remediation therapy for less than one year.
9. History of myocardial infarction, unstable angina, uncontrolled hypotension or hypertension within 3 months before screening.
10. Clinically significant abnormality on screening ECG
11. Alanine transaminase (ALT) or aspartate transaminase (AST) > 2.5 times the upper limit of normal (ULN)
12. History of stroke, brain tumor, head trauma with loss of consciousness, or other clinically significant neurological condition within 12 months before screening
13. Subjects with other uncontrolled medical conditions, in the opinion of the investigator
14. Polypharmacy with two or more antipsychotic drugs or mood stabilizers
15. Use of benzodiazepines
16. Individuals with kidney dysfunction will not be enrolled, as dysfunctional kidneys may have difficulty clearing the magnesium from the body
17. Individuals who are currently taking magnesium supplements

5.0 TREATMENT PLAN

5.1 Study Design

Design of Clinical Trial

After providing written informed consent and completing baseline assessments, patients with schizophrenia or schizoaffective disorder will be randomized to placebo or MMFS-202-302. We will make every effort to enroll subjects who have had a recent fMRI assessment as part of the Northwestern University Psychiatric Clinical Research Program. This would provide valuable pre-dose information regarding any structural abnormality. No patients with tardive dyskinesia will be admitted because of the evidence for its deleterious effect on cognition.

Patients will be recruited primarily from three mental health clinics in the Chicago area, at which the principal investigator been successfully recruiting for the last 28 months: the Stone Mental Health Center which is a part of the Northwestern Medicine system, Community Counseling Centers of Chicago (C4), and Clayton House, a residential treatment center. Other mental health facilities in the area will also be referral sources. We expect to recruit 4-5 patients per month for this study without difficulty. We expect the recruitment to be complete within 12 months or less after approval by the Northwestern University Institutional Review Board and study initiation.

All patients who are screened must provide written informed consent. Our lab has trained and certified coordinators and research assistants who will be assigned to this study as their primary responsibility. We have used the MATRICS cognitive battery in many studies over the last five years. Because of our 20- year interest in cognitive impairment in schizophrenia, there are a few other cognitive tests we collect on all of our research patients. These include a measure of phonemic verbal fluency (FAS), the Brown- Peterson Auditory Consonant Trigrams test of working memory (ACT), and the Wisconsin Card Sorting Test (WCST). We have experienced no difficulty in administering the MATRICS battery and this group of tests, which takes another 30 minutes to our patients.

We propose to study a total of 60 randomized participants, 30 receiving MMFS-202-302 and 30 randomized to placebo.

6.0 STUDY PROCEDURES

Study visits will include screening and 5 treatment visits (baseline, Weeks 1, 3, 6, and 9). Patients will be contacted by telephone at Weeks 2, 4, 5, 7, and 8 for monitoring of medication compliance, change in concomitant medication, and adverse events. Please see Appendix 1 – Schedule of Assessments.

At screening, the Structured Clinical Interview for Diagnosis for DSM Disorders (SCID) will be completed for confirmation of psychiatric diagnosis. A medical/psychiatric history will also be collected and a physical exam will be performed. Vital signs including weight, height, waist and hip measurement will be obtained at screening to record body mass index.

Subjects will be administered MMFS-202-302 at a fixed single dose of 2 grams (1 gram twice per day),

or placebo after the completion of the baseline assessments.

Methods:

A) Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) battery, including some supplemental cognitive assessments (FAS, ACT, WCST)
B) Positive and Negative Syndrome Scale (PANSS) for schizophrenia and schizoaffective disorder
C) MRI assessments:
    a. Structure: MPRAGE (T1), T2
    b. Function: Resting connectivity
    c. Function: Reward/aversion and attention: fMRI paradigm of IAPS (standardized images selected to be neutral, pleasurable, or arousing) and Spinner Task
    d. Function: Capacity working memory task
D) A set of cognitive psychological paradigms performed outside of the magnet following the MRI scan:
    a. Keypress task for an adaptation of the International Affective Picture Set (IAPS)
    b. A spinner wheel rating task of the reward/aversion associated with monetary gains/losses
E) Brief Negative Symptom Scale (BNSS)
F) Calgary Depression Scale for Schizophrenia
G) Clinical Global Impression of Severity (CGI-S) and Change (CGI-C)
H) Measurement of RBC Mg at baseline and end of study
I) EEG evaluation, measuring connectivity and activation using learning and memory tests

7.0 RANDOMIZATION METHOD

Those who meet the study inclusion criteria will be randomized into one of two treatment groups, one to receive adjunctive therapy of MMFS-202-302 and the other to receive placebo. Using SAS software, a computer-generated randomization sequences for each treatment arm will be assigned using permuted block method limiting imbalance in numbers between groups. To assign the randomization, the SAS procedure PROC PLAN will be used [19]. Until the trial is concluded, the randomization sequence will be blind to the study participants as well as research coordinators and raters. Randomization list will be provided by assigned statistician, Karu Jayathilake, and will be held by unblinded study drug manager at 680 N. Lakeshore Dr., Suite 1520; Chicago, IL 60611-7101.

8.0 STATISTICAL CONSIDERATIONS

8.1 Analysis of Clinical Data

Data will first be analyzed for efficacy on a per-protocol basis. The per-protocol population will include all subjects who complete all scheduled visits (+/- 5 days from planned visit time), have no protocol deviations that in the judgment of the principal investigator would invalidate their efficacy data, and achieve medication compliance of 80-120% (determined by pill counting; if pill packs are not available for confirmation, self-report of compliance will be used). Participants whose assessment falls +10 days or less from the scheduled visit will be included in per-protocol population, only if they were taking the study compound throughout. Data will secondarily be analyzed for efficacy on an intent-to-treat basis, with last observation carried forward (LOCF) for missing values or dropouts; subjects who did not receive any study compound will be excluded from the full analysis set, preserving the intent-to-treat principle. The primary analysis will be a univariate ANCOVA adjusting for baseline differences in the dependent measures of interest. We would also examine some variables categorically. The MATRICS working memory subscale T-score at 9 weeks will be the primary endpoint. Secondary endpoints will include the Clinical Global Impression of Change (CGI-C); the MCCB composite score; other individual cognitive domain subscale T-scores of the MCCB; a working memory composite score comprised of the 2 MCCB working memory subscale tests and the ACT test; and the PANSS positive and negative scales and BNSS. All endpoints will be evaluated as 9-weeks change from baseline, except for PANSS, which will be evaluated at both 6 and 9 weeks compared to baseline. Responder analyses will also be carried out using a chi-square test for the MCCB working memory subscale as a secondary endpoint evaluating clinical significance, using a cutoff of 0.5 standard deviation improvement (T-score improvement of $\geq$ 5 points). Finally, separate exploratory subgroup analyses will be performed on the MCCB working memory subscale T-score restricted to the subset of subjects who score 0, 0.5, or 1.0 SD or below the mean for normal age-matched normative data for that subscale. Analogous subgroup analyses will also be performed for the MCCB composite score, and the other MCCB subscale scores.

Alpha will be set at 0.05 for all analyses. The individual scores for each of the 6 other neuropsychological MCCB subscales, and supplementary cognitive tests (ACT, WCST, FAS), as well as the CGI-C measure of functional change will be evaluated to provide supporting information for primary and secondary measures.

Assumptions for the power analysis included random assignment into one group receiving placebo and one MMFS-202-302. The MCBB, as the primary instrument will be collected at baseline and at end point (9 weeks). Using univariate ANCOVA with baseline score as covariate, the time average difference in the response variable will be compared between groups to detect treatment difference. Based on Javitt et al, 2012, we assume the mean baseline working memory score will be 36.4, SD 11.7 for both groups. We assume the placebo group will show no improvement with an SD of change of 6.5, while the treatment group will improve a mean of 5 points with the same SD of change, a medium effect. We assume the correlation coefficient of repeated observation to be 0.5 and the covariance structure to be compound symmetry. The power calculation was conducted with alpha level of 0.05. Full results are available upon request. With a two-sided test, the analysis reveals that 28 subjects per group are required assuming 5 points of improvement of the treatment group compared to the placebo for the desired power of 80% with alpha level of 0.05. We plan to recruit 30 per group to account for drop outs. Thus, allowing for attrition up to 20%, this sample size will provide 74-83% power to detect the hypothesized medium treatment effect.

We will assess MMFS-202-302 effects on negative and positive symptoms as additional endpoints, as measured by the BNSS and PANSS. Further, we will analyze the effect on drug tolerability as determined by the following: body weight, body mass index (BMI), waist circumference, and movement rating scales.

8.2 *Analysis of Structural Imaging Data*

Structural MRI will be processed with the FreeSurfer (FS) image analysis suite (http://surfer.nmr.mgh.harvard.edu/) [20], followed by FS+LDDMM [21] for the high-dimensional mappings of the subcortical and cortical ROIs. These automated methods produce anatomic measures that are equivalent to measures made by experts, but with increased reproducibility and decreased manual effort [22]. Maps of longitudinal change are then computed by applying within-subject FS+LDDMM where the subject's baseline scan becomes a template and each of the follow-up scans becomes the target, which carry the baseline ROIs into follow-up scans. Subcortical ROI volumes at each time point are calculated as volumes occupied by the mapped subcortical ROI. Subcortical ROI shape at each time point will be computed using principal components analysis (PCA) represented by the associated subject scores [22]. Cortical thickness, volume and metric distortion (representing local volume and folding change) at each time point are provided by FS [23].

8.3 *Analysis of Functional Imaging Data*

Mixed effects models and analysis of variance will be used to evaluate behavioral performance on tasks administered in the scanner to characterize working memory performance and reward valuation across task conditions and to contrast performance between MMFS-202-302 vs. placebo treated groups. Event-related fMRI analyses will be carried out using SPM v8.0 (Statistical Parametric Mapping version 8.0 http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) in a MATLAB environment. A fixed effect analysis will be used to model activation associated during events of interest in each subjects' time series data. Activation maps from these analyses will be carried forward to higher-level mixed effects analyses to model each group's activation during the events of interest allowing for separate estimations of variances for each group. Analyses contrasting activation in the MMFS-202-302 vs. placebo groups will include both whole brain and a priori regions of interest (i.e., DLPFC, PPC, OMPFC, ACC, NAc).

8.4 *Analysis of EEG data*

EEG data will be analyzed using custom scripts designed by the laboratory of the PI and Co-I using MATLAB software (www.mathworks.com). Statistical analysis methods will be used that are standard for behavioral data in learning and memory experiments for EEG data. These include individual as well as group-level statistics that permit inferences regarding the population (e.g., ANOVA, MANCOVA, Causal Modeling, ICA, etc.). To address the hypotheses, we will identify EEG patterns of variation across experimental tasks and determine how these patterns change across the age groups listed above.

Analysis of clinical. imaging, and EEG data will be completed by Derin Cobia, PhD, Joel Voss, PhD, and

Karu Jayathilake, MS, all of whom are experts in biostatistics and analytics.

9.0 DETAILS OF PROCEDURES

9.1 Study Materials

9.1.1 Structured Clinical Interview for DSM-IV (SCID)

The SCID-CT is a short semi-structured interview. With an administration time of approximately 15 minutes, it was designed to meet the need for a short but accurate structured psychiatric interview for multicenter clinical trials and epidemiology. The SCID-CT will be administered by raters at the study site. The SCID-CT must be administered by a site study staff member listed on the site delegation of authority log with at least 2 years of experience with the population under study. The results of this assessment will be used to confirm the diagnosis of schizophrenia or schizoaffective disorder and rule out any exclusionary diagnosis. The SCID will be administered at the screening visit.

9.1.2 MATRICS Consensus Cognitive Battery (MCCB)

The MCCB includes 10 tests that assess 7 cognitive domains: speed of processing, attention/vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition. The tests will be administered together in one 60-to 90-minute session. For subjects who use nicotine, the MCCB will be performed at least 30 minutes after nicotine intake. Though not strictly required by the protocol, whenever possible, the MCCB should be administered at the same time of day (± 1 hour). The MCCB is a hybrid battery comprising multiple independently owned and published tests. Normative data for demographic corrections are derived from a single representative sample to which the tests were administered together as a unit, called “co-norming” [24]. The MCCB’s high test-retest reliability makes it a sensitive and accurate measure of cognitive change, and its minimal practice effects ensure that it is appropriate for repeat testing [25]. The MCCB was developed by the National Institute of Mental Health (NIMH) MATRICS initiative. The MATRICS was designed to stimulate the development of psychopharmacological agents to improve cognition in schizophrenia. The selection of tests included in the MCCB involved a broad-based interdisciplinary consensus process that is described in more detail on the MATRICS website (www.matrics.ucla.edu). Details of administration are included in the study-specific MCCB manual. The MCCB will be administered at baseline and end of study.

9.1.3 Positive and Negative Syndrome Scale (PANSS)

The PANSS is an interview-based measure of the severity of psychopathology in adults with psychotic disorders. The measure is comprised of 30 items and 3 scales: the Positive scale assesses hallucinations, delusions, and related symptoms; the Negative scale assesses emotional withdrawal, lack of motivation, and similar symptoms; and the General Psychopathology scale addresses other symptoms such as anxiety, somatic concern, and disorientation. An anchored Likert scale from 1-7, where values of 2 and above indicate the presence of progressively more severe symptoms, is used to score each item. Individual items are then summed to determine scores for the 3 scales, as well as a total score. A Composite scale score (Positive scale score - Negative scale score) can also be calculated to show the relative valence of positive and negative symptoms. Total time required for the PANSS interview and scoring is approximately 30-40 minutes. PANSS raters will be required to meet specific training and education criteria before they are certified to rate for this study. In addition, raters will receive specific training and education regarding all the following assessments prior to study initiation. The PANSS will be administered at baseline, week 6, and end of study.

9.1.4 fMRI Procedures

MRIs will be performed on all subjects that meet criteria for scanning (i.e. those that will fit in the scanner and do not have metal in their body). Scanning will be performed on a 3.0T Siemens TIM Trio MRI scanner with a UNIX based host computer (Sun Microsystems), actively shielded gradients, and echo-planar capability. Positioning calipers are used to fixate the head and reduce patient motion during the study. Once the MR technician and research personnel have position the subject comfortably in the scanner, the subject is then monitored with a wide angle and a telephoto camera. An auditory system provides two-way communication making it easier to explain the task to the subject. A slim line headphone set is used for maximum patient comfort. Visual stimuli are delivered to a custom rear projection screen placed inside the bore of the magnet approximately 24" from the subject. The subject views the stimuli via a mirror attached to the head coil that can be adjusted easily.

Structural imaging

1. T2 structural images: A series of T2-weighted images will be acquired for clinical neuroradiological examination and review for incidental findings.

2. T1 structural images: A series of T1-weighted images will be acquired to provide high resolution anatomic images of the brain for morphometric analysis and for aligning functional images for averaging data across subjects.

Functional imaging

Functional images will be acquired using echo-planar imaging. Acquisition parameters will be optimized according to task completed. Tasks will be performed for 8-12 minutes depending on the behavioral paradigm. Pauses are provided between tasks to allow subjects to rest and for providing instructions. Manual button press responses will be record using an MR compatible button box.

3. IAPS Decision-Making Task: For this task each subject is presented with a series of picture sets from the International Affective Picture System that present either positive, negative, or neutral stimuli. Participants will be asked to specifically quantify positive and negative preferences involving (i) decision-making regarding the valence of behavior, and (ii) judgments that determine the magnitude of reward valuation and aversion.

4. "Spinner" Monetary Game of Chance Task: For this task, a $50 endowment is provided, and the subjects were told that during the game, they might lose some or all of this stake, retain it, or increase it. They are presented one of three spinners, which are subdivided into three equal sectors—each labeled with a different monetary value. The image of an arrow rotates around the center of the spinner during the prospect phase, and then stops at one sector at the start of the "outcome" phase. The amount of money indicated on that sector would be added to or subtracted from the subject's total, but the cumulative winnings or losses are not displayed. The timing of the prospect and outcome phases made it possible to distinguish hemodynamic signals associated with anticipation from those associated with the experience of outcomes. During control trials, the display consists of a stationary fixation point. The design is in a single-trial format, and the trial sequence will be counter-balanced.

5. C
apacity Working Memory Task: This task is a spatial variant of the Sternberg Item Recognition paradigm, a paradigm which field experts have identified as promising for imaging biomarkers in clinical trials evaluating effects on working memory. During a brief encoding phase, subjects view an array of containing a variable number of items (3,5, or 7 items). They are to remember the location of items over a delay period of variable duration (2,4, or 6 sec) and then respond during response phase whether the presented probe appears in the location of one of the items presented in the array. Activation in the DLPCF and related regions during the delay period of correctly performed trails will be evaluated.

6. Resting State: During this task subjects will be asked to keep their eyes open and fixate a central crosshair.

fMRI task order will be pseudo randomized across groups to account for order effects. Start and stop time of the scan session will be recorded. MRI procedures take up to 2 hours to complete.

9.1.5 Brief Negative Symptom Scale

The Brief Negative Symptom Scale (BNSS): [26] was developed in response to the consensus conference and the NIMH MATRICS initiative on negative symptoms. It includes assessment of the 5 domains included in the Consensus Development Conference: blunted affect, alogia, asociality, avolition and multiple aspects of anhedonia (e.g., anticipatory pleasure and frequency of pleasurable activities). It has demonstrated strong inter-rater reliability, internal consistency, stability, and convergent/discriminant validity [27, 28]. The BNSS yields subscale scores for each domain, as well as a total global score.

9.1.6 Clinical Global Impressions Scale (CGI)

The Clinical Global Impressions (CGI) Severity and Change Scale will be used for repeated evaluations of global psychopathology. The CGI scale is widely used in schizophrenia research. The CGI-S is a single Likert scale rating severity of psychopathology on a scale of 1 (normal, not ill) to 7 (very severely ill). The CGI-C is a single Likert scale rating change of psychopathology during the trial on a scale of 1

(very much improved) to 7 (very much worse). The CGI will be completed at baseline and end of study.

9.1.7 Calgary Depression Scale for Schizophrenia

CDSS: The CDSS is a clinician-administered psychiatric symptom rating scale intended to measure the level, duration, and frequency of depressive symptoms in patients diagnosed with schizophrenia or schizoaffective disorder. The CDSS, developed at the University of Calgary [29], has good construct validity, internal reliability, and interrater reliability. The type and level of depression measured by this scale is also reported to have divergent validity from symptoms associated with schizophrenia and schizoaffective disorder. Clinicians administering this scale ask patients about their depressive symptoms since the last study visit. Each of the 9 items of the CDSS is scored between 0 (absent) and 3 (severe). The 9 items are then added together to yield a single total score in the range of 0 to 27. A score of 7 or higher is considered to be 85% sensitive to a diagnosis of major depressive disorder. For study eligibility, a CDSS score of ≤ 10 (minimal level of depression) will be required. The CDSS is used in this study as both a screening assessment and a safety assessment for depressive symptoms. The CDSS will be performed at baseline, and the final study visit.

9.1.8 Physical Examination

The physical exams must be performed by the PI or designee (or a licensed medical practitioner such as a physician's assistant or nurse practitioner) listed on the site delegation of authority log.
A complete physical examination includes the following assessments: general appearance, head, eyes, ears/nose/throat, neck, lymph nodes, skin, lungs, heart, abdomen, musculoskeletal, and neurologic evaluations. If the subject is discontinued for any reason during the treatment phase, every attempt should be made to perform a final physical examination.

9.1.9 Vital Signs

Arterial systolic and diastolic blood pressure and radial artery pulse rate will be measured while the patient is seated at the scheduled visits designated in the Schedule of Events in Appendix 1.

9.1.9.1 Height and Body Weight

Height will be measured at the screening visit. Patients will be measured without shoes. Body weight will be recorded at screening, baseline and every visit through the 9-week treatment phase.

9.1.9.2 Body Mass Index (BMI)

Body mass index (BMI) will be determined with the patient's height and weight at the screening visit and subsequent treatment visits. BMI must be calculated using the following formula: A person's (Weight in pounds divided by their height in inches squared) x 703.

9.1.9.3 Waist and Hip Circumference

Waist and hip circumference will be recorded at screening, baseline, and all study visits.

9.1.10 Electrocardiogram (12-Lead ECG)

The PI or a physician listed on the site delegation of authority log must review, initial, and date the report, which must be filed in the subject's study chart. Results will be captured in the subject's study chart, not in the electronic database. Clinically significant findings from the screening report must be captured in the medical history. Eligibility for study entry must be assessed by the principal investigator. Any clinically significant changes compared with baseline must be captured as AEs in the electronic database.
Subjects are to be supine for at least 5 minutes prior to ECG assessments. A central facility will be used in this study for interpretation and analysis of ECGs. All subjects will have standard resting 12-lead ECGs performed and interpreted. The time the ECG is performed will be recorded (using a 24-h clock). In addition, the time that the subject took their last dose of study medication (prior to the ECG) will be recorded on the CRF page.

9.1.11 Adverse Event (AE) Monitoring

The PI or a designee (eg, a licensed, qualified medical practitioner such as a physician's assistant or a nurse practitioner) listed on the site delegation of authority log must assess the severity and relationship to study medication(s) of all AEs. All observed or volunteered AEs regardless of treatment group or suspected causal relationship to the investigational product(s) will be recorded on the AE page(s) of the eCRF.

For all AEs, the investigator must pursue and obtain information adequate both to determine the outcome of the AE and to assess whether it meets the criteria for classification as an SAE requiring immediate notification to the Sponsor or its designated representative.
For all AEs, sufficient information should be obtained by the investigator to determine the causality of the AE. The investigator is required to assess causality and indicate that assessment on the eCRF. For AEs with a causal relationship to the investigational product, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator.
Adverse events (serious and non-serious) should be recorded on the CRF from the date the informed consent form (ICF) was signed until the end of their participation in the study, ie, the subject has discontinued or completed the study.

9.1.12 Concomitant Medications
Concomitant Medications will be documented using the Concomitant Medications form at Baseline and all study visits.

9.1.13 Study Medication Adherence
Participants will be asked about his/her medication adherence at each appointment. Study personnel will count and record the number of pills in the patient's study medication bottles and provide immediate feedback, reinforcing the behaviors of patients who appear to be taking medications as prescribed and problem-solving with those who appear not to be. Clinicians will review with patients the use of pill-minder boxes, as needed.

9.1.14 Laboratory Test Assessments
Northwestern Medicine Laboratory will be used for analysis of fasting lab assessments required during the study. Blood will be drawn from each patient at the screening visit, and at end of study as noted in the study Schedule of Events in Appendix 1. The fasting tests will include: Hematology and chemistry panels; pregnancy (women of childbearing potential only), serum creatinine, and urine drug screen (UDS).
Note: At screening and throughout study participation, a positive UDS or blood alcohol screen is not necessarily exclusionary and/or may not require a subject be withdrawn from the study. This decision will be per the discretion of the principal investigator.
A blood sample will be collected and analyzed for plasma Mg2+, Red blood cell Mg2+ (RBC-Mg) and total RBC cell count.

9.1.14.1 Genetic Testing
All subjects will be consented to obtain a blood sample for genetic studies to search for possible markers for response to MMFS-202-302 in participants with schizophrenia. DNA from patients already studied by the PI will be available for comparison.

9.1.15 EEG Assessments
Research participants will undergo EEG recording while participating in learning and memory tests at baseline and end of study visits. Prior to the experiment, research participants will be familiarized with EEG and prepared for the EEG recording session. Research staff will inform the research participants about the EEG procedures, and will ask the research participants to perform familiarization activities such as wearing the elastic caps that will be worn during the actual experiment (but without EEG recording) and showing the research participants informational videos and images of other individuals engaged in EEG recording.

After this familiarization process has finished, research participants will be prepared for EEG recording. This involves placing an elastic cap on the head of the research participant. Recording electrodes are embedded within the elastic cap, and these electrodes record the electrical activity of the research participants' brains non-invasively. The EEG apparatus is battery-powered, and poses no serious risk of physical harm to the research participant. Each recording electrode is filled with a non-toxic, hypoallergenic electrolytic gel so that the electrode makes contact with the participant's skin. The gel is injected using a blunt, plastic-tip syringe that poses minimal risk of skin abrasion because it is blunt and made of plastic. Electrodes are also affixed to the cheeks, temple area, and behind the ears using medical-grade tape that can be easily removed after the experiment. Participants then take part in the learning/memory testing during EEG recording. When the experiment is finished, the elastic cap is removed and participants are given the option to either wash the gel out of their hair in a sink provided in

the same laboratory (with help from the research staff as needed), or to simply leave and wash the gel out at home. Participants are then debriefed and dismissed. There are no known lasting physical ramifications of the EEG procedure. The only known risk to the research participant is the mild discomfort associated with wearing the EEG cap and possible frustration with the learning and memory tasks.
Research staff is trained to identify participant discomfort and frustration during the experiment. In response, research staff will adjust the EEG cap for comfort, introduce rest breaks as necessary, and provide positive feedback regarding performance when possible to reduce potential frustration.

9.2 Safety Scales

9.2.1 Abnormal Involuntary Movement Scale (AIMS)

The AIMS is a clinician-rated assessment of abnormal movements consisting of unobtrusive observation of the subject at rest (with shoes removed) and several questions or instructions directed toward the subject. Using a severity scale ranging from 0 (none) to 4 (severe), clinicians rate dyskinesia in several body regions, including the facial area, extremities, and trunk.

9.2.2 Barnes Akathisia Rating Scale (BAS)

The BAS is a rating scale geared toward assessment of neuroleptic-induced akathisia, though it can be used to measure akathisia associated with other drugs as well. [30, 31]. The BAS will be administered by a qualified rater at the site.

9.2.3 Simpson-Angus Scale (SAS)

The SAS is a clinician-rated assessment of neuroleptic-induced Parkinsonism consisting of 10 items. Items are anchor-based, rated on a 5-point scale and address rigidity, gait (bradykinesia), tremor, glabellar tap, and salivation [32]. The SAS will be administered by a qualified rater at the site.

9.2.4 Columbia Suicide Severity Rating Scale

The C-SSRS was developed by a team of researchers at Columbia University to address the need for standardized classification of suicide reports to assess suicide risk. This scale consists of a baseline evaluation that assesses the lifetime experience of the subject with suicide events and suicidal ideation and a post-baseline evaluation that focuses on suicidality since the last study visit. A baseline C-SSRS will be completed at the screening visit. The C-SSRS Since Last Visit form will be completed at all subsequent visits.

10.0 REMOVAL OF SUBJECTS FROM STUDY

Individuals can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

- Individual voluntarily withdraws from treatment (follow-up permitted);
- Individual withdraws consent (termination of treatment and follow-up);
- Individual is unable to comply with protocol requirements;
- Individual experiences toxicity that makes continuation in the protocol unsafe;
- Physician judges continuation on the study would not be in the patient's best interest;
- Individual becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event).

11.0 ADVERSE EVENTS

During the first week, common side effects are a slight drowsiness and a feeling of increased blood flow in the head. As with any mineral product, people with kidney deficiency should not take this product. Some individuals have reported dizziness or slight headaches when first taking MMFS-202-302.

11.1 Contraindications:

None

11.2 Special Warnings and Precautions for Use

None

11.3 Interaction with other medications

None.

11.4 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

All individuals experiencing an adverse event, regardless of its relationship to study drug/device, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

11.5 Definitions

11.5.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

11.5.2 Severity of Adverse Events

The severity of an AE is graded as follows:
Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
Moderate (grade 2): the event causes discomfort that affects normal daily activities.
Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
Life-threatening (grade 4): the patient was at risk of death at the time of the event.
Fatal (grade 5): the event caused death.

11.5.3 Serious Adverse Events

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

- Results in death.
  - If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

- Is life-threatening.
  - (the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours judged to be caused by study medication or procedures.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly/birth defect
- Is an important medical event

Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the

definition of "Serious Adverse Event."
For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

11.6 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event

Step 2: Grade the adverse event

Step 3: Determine whether the adverse event is related to the protocol therapy

Attribution categories are as follows:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.

Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information Section of this protocol;
- the GRAS dossier for the supplement;

11.7 Reporting Requirements for Adverse Events

Expedit

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.
- The investigator will inform the sponsor within 24 hours of notification of any serious adverse event by phone or fax, with follow-up with a written narrative of the event within 48 hours.
- The Northwestern University Institutional Review Board must be notified within 10 business days of "any unanticipated problems involving risk to subjects or others"

  The following events meet the definition of UPR:

  1. Any serious event (injuries, side effects, deaths or other problems), which in the opinion of the Principal Investigator was unanticipated, involved risk to subjects or others, and was possibly related to the research procedures.
  2. Any serious accidental or unintentional change to the IRB-approved protocol that alters the level of risk.
  3. Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject.
  4. Any new information (e.g., publication, safety monitoring report, updated sponsor safety report), interim result or other finding that indicates an unexpected change to the risk/benefit ratio for the research.
  5. Any breach in confidentiality that may involve risk to the subject or others.
  6. Any complaint of a subject that indicates an unanticipated risk or that cannot be resolved by the Principal Investigator.
- The FDA will be notified within 7 business days of any unexpected fatal or life-threatening adverse event with possible relationship to study drug, and 15 business days of any event that is considered: 1) serious, 2) unexpected, and 3) at least possibly related to study participation.

Routine Reporting

- All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission.

11.8 Unblinding Procedures

The blind should not be broken unless subject has completed all study procedures and the data has been locked. Emergency unblinding is expected to be infrequent.
Emergency unblinding should occur only if the principal investigator (or other treating physician) considers breaking the blind medically relevant. All subjects who have the blind broken will be discontinued from the study. Documentation of the reasons for unblinding, including date and time will be recorded in the source documents and the electronic case report form (eCRF).

12.0 STUDY AGENT AND DOSING

MMFS-202-302 will be provided by Neurocentria, with a a detailed Certificate of Analysis attesting to the contents of the product.

Neurocentria has provided the following list of ingredients for MMFS-202-302:

Product 1 – MMFS-202
Active ingredients: L-TAMS (500 mg – 6-hour release profile)
Inactive ingredients: polyvinyl pyrolidone, microcrystalline cellulose, silicon dioxide, talc, and magnesium stearate

Product 2 – MMFS-302
Active ingredients: L-TAMS (500 mg – 12-hour release profile)
Inactive ingredients: polyvinyl pyrolidone, microcrystalline cellulose, silicon dioxide, talc, and magnesium stearate

Product 3– Placebo
Inactive ingredients: talc, magnesium stearate, povidone K-90, colloidal silicon dioxide, microcrystalline cellulose, sodium carboxymethylcellulose, carbopol 974P, starcap 1500

The target dosage will be 2 g/day, administered as two 0.5 g tablets twice per day (2 -302 tablets in the morning and 2 - 202 tablets in the evening). The placebo comparator will be also given as 2 tablets twice per day, preserving the blind. The product should be taken with a full glass of water.

L-TAMS has been self-affirmed by an expert panel convened by its manufacturer Neurocentria as being produced using good manufacturing practices, and fulfilling the FDA's criteria for GRAS (generally regarded as safe) status. (AIDP, Inc. (2011, July 15). L-TAMS Self-Affirmed as GRAS [Press release]. Retrieved from http://www.magtein.com/press/AIDP-Magtein-7.15.pdf)

Study clinicians will be responsible for dispensing MMFS-202 and MMFS-302 at our research office. An unblinded study drug manager will receive and label and distribute the product for dispensing during the study.

After the study, subjects can obtain L-TAMS by purchase on the open market. Study clinicians, in cooperation with the subject and the subject's clinical treatment team, may develop a plan for continued L-TAMS treatment after the completion of the study. At the baseline and end of study visits, measures of safety and effectiveness will be administered and subjects will be evaluated for response and side effects to the treatment. To assess adherence to dosing of the agent, the study medication will be returned and counted.

Subjects will be instructed not to take any medications or supplements which contain any forms of magnesium compounds to avoid unsafe levels of magnesium. All new medications and supplements started during the study will be reviewed by the PI.

12.1 Return and Retention of Study Drug

The investigator will return used and unopened vials of study drug to the manufacturer at the end of the

trial. The clinical research manager will maintain copies of study drug shipping receipts, drug accountability records, and records of return or final disposal of study drug.

13.0 RISKS AND DISCOMFORTS

All efforts are made to minimize risks to subjects. Consistent with good clinical practice, safety will be monitored by the Principal Investigator, and will reflect the oversight of co-investigator study clinicians. Adverse events will be recorded and reported according to institutional policies. Risks of the study agent have been incorporated into the exclusionary criteria for this proposal.

Study Agent – L-TAMS: L-TAMS is considered a dietary supplement that the manufacturer has self-certified as fulfilling the FDA criteria for agents that are Generally Regarded as Safe. There are few risks to taking L-TAMS orally. Magnesium is known to cause gastrointestinal discomfort, nausea, vomiting, or diarrhea. Although occurrences are rare, very large amounts of magnesium might cause hypermagnesemia with symptoms including thirst, hypotension, drowsiness, confusion, loss of tendon reflexes, muscle weakness, respiratory depression, cardiac arrhythmias, coma, cardiac arrest, and death. There have been only two reports of death from hypermagnesemia (Therapeutic Research Faculty (2014). *Natural Medicines Comprehensive Database* Retrieved May 30, 2014, from http://naturaldatabase.therapeuticresearch.com/nd/Search.aspx?s=ND&cs=NDPTL~CP&pt=103&sh=7&i d=89669&ft=4#reactions).

MRI risks are minimal for subjects who are thoroughly screened and deemed safe for scanning. These include feelings of claustrophobic fear while being scanned, and minor physical discomfort because of remaining still throughout the scanning procedure.

EEG risks include the participant finding the tasks boring, tiring, and/or frustrating. If this happens, research staff will allow the participant to rest and then attempt continue. Participants might find the EEG cap to be uncomfortable. If this occurs, research staff will attempt to adjust the EEG cap so that it is more comfortable.

14.0 STUDY MANAGEMENT

14.1 Conflict of Interest

All investigators will follow the University conflict of interest policy.

14.2 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

14.3 Data Management and Monitoring/Auditing

A database in the RedCap system will be used for data entry and will be formatted to simulate the hard copy data entry forms. Once entered into this database, the data files can be extracted into Excel or SAS project databases, which are maintained on a networked directory under the direct supervision of the PI and biostatistician. Back-ups are routinely performed to assure the preservation of the database.

Data are checked for range, consistency, missing values, etc. During data analysis, combined datasets are created as needed. Data are maintained in the master system datasets using a source/derived data approach, where source datasets are maintained unmodified, while the derived datasets are modified as needed. Thus, alterations that prove to be incorrect are easily rectified. The confidentiality of all data is maintained using the HIPAA compliance standards of Northwestern University. No confidential information is posted to the web under any circumstances. All possible efforts are made to retain only the necessary information in all cases.

Regarding de-identification of the data set: No identifiable information for any subjects (i.e. name, initials or date of birth) will be collected in the analyzable RedCap database or directly linked to study data. Subjects will be coded only with study-specific unique ID numbers.

14.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

14.5 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, a IRB modification form must be completed within five (5) business days of making the change.

14.6 Other Protocol Deviations/Violations

All other planned deviations from the protocol must have prior approval by the Principal Investigator and the IRB. According to the IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

14.7 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

14.8 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the

last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

14.9 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

14.10 Benefits and Compensation

14.10.1 Potential Benefits

The possible benefits to the subjects who participate in this study are improvement in cognitive function, but there may be no direct benefit. Research staff could provide information from screening evaluations to the subject's clinical treatment team if requested by the subject. Findings from this study may benefit others. Participation will allow us to learn about the potential cognitive enhancing effects of MMFS-202-302 in patients with schizophrenia or schizoaffective disorder, which would inform further studies evaluating its use to treat cognitive deficits among various clinical populations.

14.11 Risk Benefit Assessment

The risks from the study procedures include:

Magnesium is known to cause gastrointestinal discomfort, nausea, vomiting, or diarrhea. Although occurrences are rare, very large amounts of magnesium might cause excessive magnesium levels in the blood (hypermagnesemia) with symptoms including thirst, hypotension, drowsiness, confusion, loss of tendon reflexes, muscle weakness, respiratory depression, cardiac arrhythmias, coma, cardiac arrest, and death. There have been only two reports of death from hypermagnesemia.

MRI risks:
Subjects may find some of the computer tasks and learning and memory tasks boring or frustrating, but may take as many breaks as they wish.

Subjects may be anxious or physically uncomfortable from lying in the MRI scanner, and it is also possible that subjects may feel claustrophobic. Direct communication with research staff is available during the scan and subjects can tell staff whenever they want the scan to be stopped or interrupted. For the MRI scan, we will screen subjects for any metal parts inside and outside the body.

The clinical interview questions may result in temporary discomfort when asking the subject about a potentially difficult time in the past or the psychiatric symptoms they experience in the case of psychiatric participants. There may be temporary discomfort/ bruising at the site of venipuncture.

It is believed the potential benefits outweigh the risks for this trial.

14.12 Costs and Payments

There are no costs to subjects for participation in this study. Subjects will be compensated at the rate of $40/visit for their completion of study procedures. If the MRI assessments are completed, subjects will receive an additional $20/ hour during the baseline and end of study visits. The total amount for MRI assessments will be prorated based upon the time and participation in the scanner. Subjects will have

the option to be paid either via a check that will be mailed to them or with a non-traceable prepaid debit card. The total amount for visit compensation will be prorated based on subject's time and participation. Up to
$20 per visit will also be provided to cover subjects' transportation and parking expenses.

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Appendix 1

Schedule of Assessments:

Study Day	**Screen**		**1**	**7 14 21 28 35**	**42**	**49**	**56**	**63**			
Event/Assessment											
Informed Consent	X										
Medical History & Demographics	X										
Physical Examination	X										
Electrocardiogram	X										X
Vital Signs	X	X	X		X			X			X
Clinical Safety Laboratory Tests	X										X
Measurement of Mg in blood samples	X										X
DNA blood sample	X										
Drug Screen	X										
Inclusion/Exclusion Criteria	X										
SCID-I	X										
Calgary Depression Scale for Schizophrenia		X									X
C-SSRS	X	X	X		X			X			X
AIMS		X									X
BARS		X									X
SAS		X									X
BNSS		X									X
MCCB extended battery		X									X
fMRI assessments		X									X
EEG assessments		X									X
CGI-S/C		X									X
PANSS		X						X			X
Adverse event monitoring		X	X	X	X	X	X	X	X	X	X
Concomitant medication		X	X	X	X	X	X	X	X	X	X
Medication management		X	X		X						X

Telephone Contact for Compliance Monitoring				**X**		**X**	**X**		**X**	**X**	