

## Johns Hopkins Medicine - eForm A

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### 1. Abstract

- a. Provide no more than a one page research abstract briefly stating the problem, the research hypothesis, and the importance of the research.

The overall goal of the study is to counter the effects of increased N-methyl-D-aspartate (NMDA)/glutamate receptors in MeCP2-mutation-positive Rett syndrome (RTT) subjects by use of a placebo-controlled trial of dextromethorphan (DM), an agent known to block the NMDA receptor channels. The drug dose of DM will be 5mg/kg/day as this dose showed optimal results in our previous dose-effect study. We anticipate improved cognition and temperament, and reduced seizure frequency.

RTT is a neurodevelopmental disorder with devastating consequences to both brain and systemic neurons. Despite a prominent 50% reduction in the number of synapses, postmortem brain autoradiographic studies demonstrate a striking and disproportionate increase in the number of glutamate/NMDA (N-methyl-D-aspartate) subtype of receptors in the prefrontal cortex, especially in younger patients. DM blocks NMDA receptor channels, which improve some clinical manifestations of RTT (eg, cognitive and behavioral deficits, seizures) by reducing the excitotoxic damage and preventing the degree of intellectual disability over the long term. Presently, there is no effective therapy or definitive treatment for RTT, other than palliative care, therefore, our overall specific aim in this biologically-based study is to test the efficacy of DM vs placebo in RTT to block the disproportionate numbers of glutamate/NMDA receptors in the brain and alleviate symptoms. This study holds promise for improved quality of life for girls with RTT, by helping to reduce the degree of functional impairment in this population. Moreover, this study would lend itself to a mode of therapy for other epileptic conditions also associated with glutamatergic excitotoxicity.

Measurement of cytokines and glutamate in serum and released from peripheral blood mononuclear cells (PBMC): Much of the experimental work has been conducted on the *Mecp2* null mouse. Although the model has provided great insight into the disease, there are distinct differences between the murine model and the human disease. For example, the human disease in many is caused by several mutations, and not only the absence of *MECP2*. Many mutated forms of *MECP2* display some functionality (Gonzales ML and LaSalle JM, The role of MeCP2 in brain development and neurodevelopmental disorders. Current Psych Reports 2010:12:127-34). Multiple mutations described in children with Rett display varying phenotypes in females. The disease phenotype is severe in the male mouse model, however, in the human disease, males carrying mutated *MECP2* usually are few, not frequently recognized, are extremely ill, and therefore not well studied.

Two studies have been recently published suggesting the involvement of the innate immune response in RTT. In one, hippocampal neurons displayed abnormal dendritic morphology in the presence of conditioned media from cultures of *Mecp2*-null microglia. Glutamate was

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identified as the mediator causing the damage [2]. (Maezawa, I. and Jin, L. W. (2010) Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. *J Neurosci* 30, 5346-56). In the second study, investigators found that *Mecp2* null mice that received a graft of bone marrow cells from wild-type mice survived longer and displayed significant improvements in respiratory function and motor control. Because the benefits of wild-type microglia were diminished by blocking their phagocytic ability, the importance of phagocytic activity in RTT was suggested [3] (Derecki, N. C., Cronk, J. C., Lu, Z., Xu, E., Abbott, S. B., Guyenet, P. G., Kipnis, J. (2012) Wild-type microglia arrest pathology in a mouse model of Rett syndrome. *Nature* 484, 105-9). To determine the relevance of these findings in human monocytes, we developed a model to study *Mecp2* deficiency by transducing peripheral blood mononuclear cells (PBMC) with lentiviral shRNA against MECP2.

**2. Objectives** (include all primary and secondary objectives)

*Primary outcome:* Improve cognition. Cognitive skills will be measured by neuropsychological tests (Mullen Scales of Early Learning).

*Secondary outcome:* Improvement in behavior will be measured by: a) Vineland Adaptive Behavior Scales (VABS); b) behavior and temperament dysregulation will be measured by the Ghuman-Folstein Screen for Social Interaction (SSI).

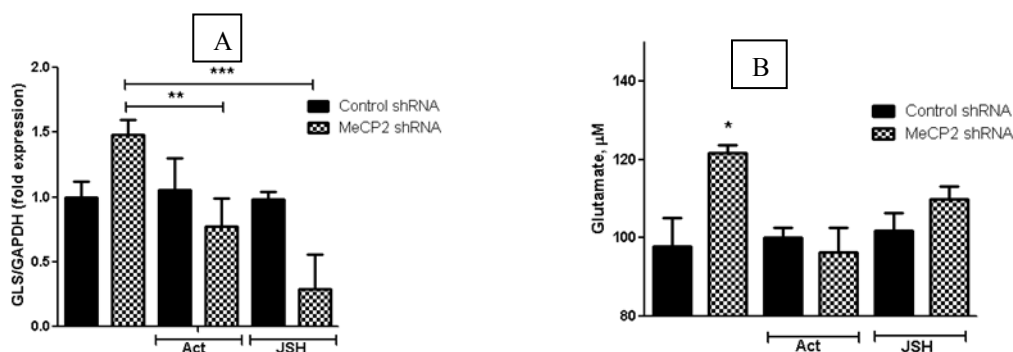
*Exploratory outcome measures:* a) Seizure frequency will be measured by a seizure diary and interim evaluations by neurologists. As commonly used anticonvulsants are not known to change EEG patterns in RTT patients, those on anticonvulsant medications prescribed by their neurologists will continue on the same medications and doses throughout the study; b) Rett Syndrome Behavior Questionnaire (RSBQ); c) Pediatric Quality of Life Inventory (PedsQL version 4). d) Measure cytokines and glutamate in serum released from peripheral blood mononuclear cells (PBMC) in children with Rett Syndrome and determine whether treating children with dextromethorphan modifies the cytokine and glutamate levels. Studies in Dr. Bressler's laboratory have shown elevated responses in human PBMC made MECP2 deficient with lentiviral shRNA against MECP2. We observed increased expression of glutaminase (GLS) mRNA and increased release of glutamate in MECP2 deficient PBMC compared to controls (fig 1). We also observed increased expression of TNF $\alpha$  and IL-6 mRNA (not shown). These responses were inhibited by drugs that block the NF- $\kappa$ B signaling pathway.

**3. Background** (briefly describe pre-clinical and clinical data, current experience with procedures, drug or device, and any other relevant information to justify the research)

Our investigations in RTT as well as that of others demonstrate elevated brain glutamate and its NMDA receptors in children below 10 yrs of age and in the RTT mouse model prior to 7 weeks of age. Additionally, our preliminary therapeutic trial shows that DM, which blocks NMDA receptors, benefits RTT patients by ameliorating some symptoms and improving quality of life. In stage 2 of RTT (1- 4 years of age) acquired skills are lost, with onset of autistic-like features, stereotyped behaviors, and poor hand use accompanied by diffuse slowing and frontocentral spikes on EEG. Mental retardation (MR) and respiratory irregularities in the wake period become evident in stages 2 and 3 (5-10 yrs). In stage 3, patients appear to recover from the autistic tendencies but all other symptoms remain, with poor weight gain, gastrointestinal problems, vasomotor instability of extremities, and osteopenia, as concomitant findings. Interventions to reduce some aspects of the morbidity and mortality in stages 2 and 3 that could be related to the neuroexcitotoxicity seen in Rett syndrome require early intervention. Neuroexcitotoxic injury, as also shown in cultured neurons to elevated glutamate levels by Russell *et al.* and Maezawa and Jin, needs to be stabilized

to prevent progressive brain injury, thus reducing long term effects of mental retardation and ancillary deficits.

The results in Dr Bressler's experimental studies of PBMC shown in Fig below warrant evaluation of the effects of DM treatment as compared to those who are on placebo.



**Figure 1. GLS mRNA levels and glutamate release in MeCP2 deficient leukocytes.**

Glutaminase (GLS) mRNA was measured from total RNA isolated from PBMC transduced with either the MECP2 shRNA lentiviral vector or control. GLS mRNA was normalized to GAPDH mRNA (A). Similarly, glutamate released into the media was measured in transduced and control cultures (B). Some cultures were also treated with the NF- $\kappa$ B inhibitors Act and JSH. The data are expressed as a mean of triplicates of a representative experiment  $\pm$  S.E.M. \*  $p<0.05$ , \*\*  $p<0.01$  and \*\*\* $p<0.001$  using ANOVA with Dunnetts multiple comparisons post hoc test.

#### 4. Study Procedures

##### a. Study design, including the sequence and timing of study procedures

(distinguish research procedures from those that are part of routine care).

Sixty subjects with clinical features of RTT, with a known mutation in the *MECP2* gene, will be studied in this placebo controlled trial of DM in RTT. We will use a randomization scheme instituted by personnel not directly involved with the study subjects. The DM group will take 5mg/kg/day orally in 2 divided doses 12 hours apart. The pharmacists will dispense the DM or placebo to both the in- and out-patient study participants. The study population will consist of 30 subjects in the placebo group and 30 subjects in the DM group. We will randomize patients to dextromethorphan or placebo using a computer-generated randomization sequence with varying block sizes that is stratified for age (2-5 years vs. 6-10 years). Given the moderate sample size, using pre-stratification will further increase the probability for having balanced and comparable treatment groups. The statistician will generate the randomization list for the JHH Investigational Drug Service. By always keeping the randomization list separate and inaccessible to study investigators, we will ensure concealment of random allocation.

*Assessment of treatment efficacy:* Subjects will be followed at regular intervals during the study by a series of evaluations, as indicated below. All subjects in the study will be followed until the last recruited subject has completed 3 months of the trial. In light of the large number of RTT patients <10 years of age available for study, we believe we can recruit 60 subjects within the 3-year study period.

All procedures are for research purposes to assess the effectiveness of DM vs. placebo.

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Since DM may be slowly excreted in about 10-20% of the normal population, a pharmacokinetics (PK) study will be performed by administering one 2.5mg/kg oral dose of immediate release DM-to be given during the first hospital admission (after the baseline blood sample for PK testing is obtained) to determine the rate of metabolism of DM. Drug levels will be monitored twice by a blood test: a) at peak level (3 hours) and, b) at the lowest level (12 hours). Should a subject be found to be an intermediate or slow metabolizer of DM, she would be excluded from this study because a safe dose of DM could not be determined. The study drug or placebo would not be started until after results from the baseline visit PK testing are available to the study team about the DM's PK status of the participant. Following the PK study, the rapid metabolizers will be included in the 3-month trial, and will take DM at a dose of 5mg/kg/day or an equal volume of placebo, given in two divided doses, exactly 12 hours apart. At the end of the study the code is broken to determine whether DM shows more effectiveness than placebo.

The test procedures/evaluations/assessments per the study protocol will be performed during two 3-day inpatient admissions to the Johns Hopkins Pediatric Clinical Research Unit (PCRU). Subjects will be admitted as follows: 1) the initial admission will be prior to the initiation of the placebo-controlled DM trial, and 2) the second admission will be at the end of 3 months of the study. Any test result done as part of the study protocol that shows a deviation from normal, is to be repeated, as a clinically indicated test. In addition to the baseline and end of study admissions, all subjects will be required to have two interim evaluations at 2 weeks and at 1 month during this study (as noted below) either by us in our outpatient clinic or by their local physician, with no more than a two-week delay being permitted to obtain the required interim tests.

Upon discharge from the initial (baseline) PCRU admission, all study participants will be closely followed by the research associate or research nurse by at least weekly telephone interviews during the first month, and then at least once/month telephone interviews through the end of the 3-month study.

*Study Protocol procedures: (all test results/performances will be compared to subjects' baseline values):*

- 1) We will request parents to provide us with medical records or release of information from appropriate sources; i.e, MeCp2 gene test report, birth and developmental history, and neurological reports.
- 2) a) General physical/neurological examinations; (approximate time allotted is 1 hour);  
b) EKG (due to reports of prolonged QTC interval); (time allotted is ~20 minutes);  
c) Orthostatic blood pressure assessment (due to autonomic dysfunction); (time allotted is ~10 minutes).

These tests will be performed before initiation of DM or placebo during the baseline inpatient admission, and are to be repeated at the required two interim evaluations at 2 weeks and 1 month during the trial, and again during the 3-month end of study admission.

3) Blood tests:

**Initial, baseline admission:** a) Routine CBC with differential (2cc); b) comprehensive metabolic panel (CMP) and liver function tests (LFTs) (3 cc); c) DM pharmacokinetic assay (12cc divided for 3 draws at baseline, peak and lowest level); d) For those already getting blood drawn for CBC, CMP and DM level, we will collect an additional one cc of blood to obtain serum and peripheral blood mononuclear cells in pre treated children. The blood will be immediately transferred to Dr. Bressler's laboratory and processed. Serum and cells will be frozen and assays will be performed when sufficient numbers of samples are collected. Cytokines will be measured by ELISA and cellular responses will be examined by flow cytometry.

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- Interim evaluations at 2 weeks and 1 month:** a) Routine CBC with differential (2cc); b) CMP and LFT (3cc); c) DM level (6cc).
- 4) **End of study (3-month) admission:** a) Routine CBC with differential (2cc); b) CMP and LFT (3cc); c) DM level (6cc). d) For those already getting blood drawn for CBC, CMP and DM level, we will collect an additional one cc of blood to obtain serum and peripheral blood mononuclear cells in post treated children. The blood will be immediately transferred to Dr. Bressler's laboratory and processed. Serum and cells will be frozen and assays will be performed when sufficient numbers of samples are collected. Cytokines will be measured by ELISA and cellular responses will be examined by flow cytometry. Approximately 2cc blood for a serum pregnancy test will be obtained on the first day of admission (takes about 5 minutes) in those who are post-menarchal, which will be very few given the younger age of this study population (to be obtained before initiation of DM during the baseline admission and again during the end of study admission after 3 months). Parents will be informed about the need and rationale (ie, drug effect) for the pregnancy test. If they decline the pregnancy test because of any concerns, they will be unable to participate in this study.
  - 5) Neuropsychological testing (approximately 2 - 2 1/2 hours) by a neuropsychologist to assess cognitive status (Mullen Scales of Early Learning (MULLEN), Vineland Adaptive Behavior Scales (VABS), will be performed during the baseline admission and at the 3-month end of study admission.
  - 6) Neurobehavioral evaluation (approximately 1 hour) by a child psychiatrist to assess behavioral abnormalities. Parent will complete behavioral checklists/questionnaires: Ghuman-Folstein Screen for Social Interaction (SSI), Rett Syndrome Behavior Questionnaire (RSBQ) upon baseline admission and at the 3-month end of study admission.
  - 7) Nutritional assessment (approximately 45 minutes) for monitoring growth indices (height, weight, head circumference, body mass index) and nutritional intake. A 3-day food record will be sent to families prior to admission (see Oral Consent form).
  - 8) Quality of Life questionnaire, the Pediatric Quality of Life Inventory (Peds QL version 4) to assess the child's physical, emotional, and social well-being from the perspective of a parent or guardian, to be obtained at the baseline and 3-month end of study admissions.
  - 9) We will assess compliance including drug and/or placebo intake or compliance with the study protocol including the two required interim evaluations by at least weekly telephone intake phone calls by our research nurse or research associate during the first month of the study, and by at least monthly telephone intake phone calls until the end of the 3-month study.
  - 10) After hours pediatric resident coverage in the PCRU will be provided by the Neill team, the hospital's standard procedure.
  - 11) De-identified information will be shared with our research collaborators.
- b. Study duration and number of study visits required of research participants.  
We anticipate to complete the study within 3 years. The test procedures as part of the study protocol will be performed during two 3-day inpatient admissions to the pediatric clinical research unit (PCRU) in the Johns Hopkins Institute for Clinical and Translations Research (ICTR). Subjects will be admitted to the PCRU twice to assess changes in primary, secondary, and exploratory outcome variables. The baseline admission will be prior to initiation of the 3-month study and the second admission will be after 3 months of the trial.

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In addition, subjects will have two interim evaluations required at 2 weeks and 1 month during this study to be done either in our outpatient Clinic or by their local physician. The neurological evaluation will take about one half hour, the EKG will take about 20 minutes, and the blood draw will take about 15 minutes.

- c. Blinding, including justification for blinding or not blinding the trial, if applicable.  
The study is a double blind placebo-controlled drug trial to determine the effect of DM (5mg/kg/day) versus placebo.
- d. Justification of why participants will not receive routine care or will have current therapy stopped. N/A
- e. Justification for inclusion of a placebo or non-treatment group.  
The previous dose response study was considered by the FDA to be biased as all participants knew the dose of the medication; therefore, the FDA suggested a placebo controlled trial. Also the previous study has shown no side effects on any of the doses including the highest dose of 5mg/kg/day in 2 divided doses of DM in RTT subjects of similar ages.
- f. Definition of treatment failure or participant removal criteria.  
The drug or placebo will be discontinued and the subject will be removed from the study if severe side effects occur, such as type-1 diabetes, allergic skin rash, agitation, nystagmus, persistent nausea, vomiting, increased liver enzymes, slurred speech, ataxia, drowsiness, fatigue, dystonia, or urinary retention; or, if any of the blood parameters or EKG being assessed are found to be abnormal. Subjects would be removed from the study if parent shows poor compliance including drug and/or placebo intake or compliance with the study protocol including the two required interim evaluations.  
Following discontinuation for reasons unrelated to DM/placebo such as severe intercurrent illness that is not uncommon in RTT, we would consider restarting the trial of DM/placebo once the subject fully recovers and returns to baseline. Our plan would entail establishing a 3-month limit wherein no baseline evaluations would be repeated when restarting any subject on DM following discontinuation. However, if the time of recovery and return to baseline is more than 3 months, then we would repeat baseline evaluations, except the pharmacokinetics of DM- which would remain unchanged.
- g. Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.  
Clinical follow up will continue until the last subject has completed the 3-month study. Subjects will be followed clinically until the entire study period ends, even if a subject's participation in the study is ended prematurely for reasons such as: 1) If the family decides to withdraw from the study; 2) emergence of other diseases that may interfere with interpretation of test results; 3) severe intercurrent illness that is not uncommon in RTT; 4) if severe side effects occur, such as those noted below; 5) having more than a 2-week lapse in obtaining the required interim evaluations/labs; 6) not following the study protocol.

## 5. Inclusion/Exclusion Criteria

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Inclusion criteria: 1) those with classic or atypical RTT who have a proven mutation in the *MECP2* gene; 2) subjects must be between 2 years - 9.99 years of age.

Exclusion criteria: 1) those without an established mutation in the *MECP2* gene; 2) those with mutations in the *MECP2* gene but who have had brain resection or surgical intervention; for example, tumor, hydrocephalus, severe head trauma; or, an associated severe medical illnesses such as vasculopathies, malignancies, diabetes, thyroid dysfunction, etc; 3) those on medications that could interact with DM, e.g. MAO inhibitors, SSRI, sibutramine etc. to avoid a serotonin syndrome; quinidine and drugs metabolized by the CYP450 isoform CYP2D6 (e.g. amiodarone, haloperidol, propfenone, thioridazine); 4) those proven to be intermediate or slow metabolizers of DM; 5) those with reported adverse reactions to DM; 6) those whose pregnancy test is positive; 7) those showing poor compliance with any aspect of the study; 8) foster children.

## 6. **Drugs/ Substances/ Devices**

- a. The rationale for choosing the drug and dose or for choosing the device to be used.  
DM blocks NMDA/glutamate receptors, which are markedly increased in younger RTT patients and are hypothesized to cause excitotoxic epileptic encephalopathy.
- b. Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.  
Blocking NMDA receptors will prevent neuroexcitotoxic injury to brains, as in non-ketotic hyperglycinemia, where it has been given to neonates in a fairly high dose (5-35mg/kg), which was well-tolerated.
- c. Justification and safety information if non-FDA approved drugs without an IND will be administered. N/A

## 7. **Study Statistics**

- a. Primary outcome variable. Improvement in cognition.
- b. Secondary outcome variables. Improvement in behavior.  
*Exploratory outcome variables:* Reduced seizure frequency; improvement in the Quality of Life and Rett Syndrome Behavior questionnaires.
- c. Statistical plan including sample size justification and interim data analysis.

The data will be analyzed when all 60 participants (30 subjects in the placebo group and 30 subjects in the DM group) have completed the 3-month the trial.

Neuropsychological measures for Mullen and VABS: Subjects will be assigned to intervention in a randomized manner as described in randomization. The neuropsychological measures to establish relative cognitive and adaptive competence of the 2 groups to be comparable, age equivalent scores on the Mullen and VABS measures will be compared between groups using 2x2 repeated measures analysis of variance (ANOVA) with the between subjects factor being drug or placebo and within subjects factor being time. Changes in cognitive measures (Mullen and VABS) will be correlated with the behavior measures and seizure frequency.

We based our sample size on the primary outcome, which are the receptive language age equivalent scores on the Mullen scale. There is no established minimal important difference for the Mullen score that is widely accepted and would provide a reasonable effect estimate for sample size calculation. However, a distribution-based estimate of the minimal important difference can be derived from the standard errors of measurement (SEM) that have been

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reported for the Mullen subscales (chapter 6 of the Mullen scale) and 1 SEM provides an accepted approximation of the minimal important difference. To detect a 4 point difference (1 SEM) in the receptive language age equivalent scores on the Mullen scale between the groups and assuming a standard deviation of 5 points (based on our pilot data) we need a total sample size (both groups) of 50 patients at a significance level of 0.05 (two-sided) and a power of 80%. Assuming a dropout rate of around 15% the required sample size increases to 60 patients.

*Sample size calculation.* Measurement of cytokines and glutamate in serum and released from peripheral blood mononuclear cells: We estimate that 12 children in each group (placebo and drug treated) will be needed using an  $\alpha=0.05$  and a  $\text{power}=0.8$ .

We will randomize patients to dextromethorphan or placebo using a computer-generated randomization sequence with varying block sizes that is stratified for age (2-5 years vs. 6-10 years). Given the moderate sample size, using pre-stratification will further increase the probability for having balanced and comparable treatment groups. A Johns Hopkins statistician will generate the randomization lists. By always keeping the randomization list separate and inaccessible for study investigators, we will ensure concealment of random allocation.

d. Early stopping rules. None.

## 8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

*Dextromethorphan:* Potential major risks include reversible type-1 diabetes, and allergic skin rash. Potential minor risks include lightheadedness, nystagmus, agitation, persistent nausea and/or vomiting, increased liver enzymes, and slurred speech. Dextromethorphan may be associated with mild and infrequent occurrence of dizziness, drowsiness, urinary retention, ataxia, fatigue, and dystonia.

Infrequently, patients on other medications including antipsychotics, lithium, SSRI's, MAO inhibitors may experience psychosis, serotonin syndrome, and hyperthermia. Long-term studies have not been performed with DM, and there may be unknown long-term side effects.

*Blood draw:* The health risk of venipuncture (blood draw) is negligible. There is minimal discomfort from the needle during insertion, with a slight risk of bleeding, bruising, or infection following the blood draw. Subjects may be fearful and have discomfort during the blood draw.

*Blood pressure monitor:* Minimal discomfort may be experienced when the cuff is in place around the child's arm.

*Pregnancy Test and Result:* In post-menarchal subjects (which would be very few given the younger age of this study population), a serum pregnancy test will be performed on the first day of admission. Parents will be informed about the need and rationale (ie, drug effect) for the pregnancy test. If they decline the pregnancy test because of any concerns, they will be unable to participate in this study. If they agree, the risk of learning that their child's test is positive and subsequent removal from the study may cause emotional distress to the parents and family. A positive result will be discussed with the family with the necessary level of sensitivity.

*EKG:* Rarely, there may be a topical skin reaction to the electrode paste or slight discomfort during placement of electrodes.

*Behavioral and Psychological evaluations:* During the intake interviews used to gather information for the questionnaires or scales during these evaluations, parents may choose not to



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answer any question(s) that may cause them discomfort, which will be handled empathetically and compassionately.

*Nutrition Assessment:* During the interview with the nutritionist to gather information about the subjects' diet, food preferences, food allergies, etc., some question(s) may cause parents discomfort. Slight discomfort may occur when measuring body mass, head, weight, height.

*Interview Discomfort:* During interviews to obtain information for the intake/clinical questionnaires, the parent(s) may decline to answer any question that may cause them discomfort, which will be handled empathetically and compassionately.

*Risk of Disclosure of Information/Loss of Privacy:* There is a risk of disclosure of information/loss of privacy; however, many steps are taken to minimize this risk as described below.

*Disruption to work or school schedules:* Parents or participants may undergo disruption to their work and/or school schedules when committing the necessary time to undergo the two 3-day inpatient admissions and the two required outpatient interim evaluations during the 3-month drug trial.

b. Steps taken to minimize the risks.

All subjects will be by handled by experienced staff at both Johns Hopkins and the Kennedy Krieger Institute, who are well qualified to handle and address any discomfort or concerns. Subjects are carefully screened and monitored before and after all study procedures. Also, during the interim period between the initial and 3-month follow-up admissions, subject status is assessed by at least weekly telephone calls by the PI, research associate, or research nurse, during the first month, and at least once/month thereafter until the end of this 3-month drug trial. Moreover, interim evaluations performed by us or by subjects' local physicians at 2 weeks and 1 month will identify any abnormality(ies) during the drug trial, and any test showing a deviation from normal will be repeated as a clinically-indicated test that is not paid for by this study.

In addition, the following additional steps will be taken to minimize the risks:

*Dextromethorphan (DM):* Patients on known drugs that could interact with dextromethorphan, including those listed above, are excluded from participating in this study (see Exclusion Criteria). Withholding DM or placebo reverses the symptoms without residual effects. In addition, our research pharmacist provides each participant a *Dextromethorphan Patient Information Sheet*, explains to parents the administration and dosage at discharge when provided with the DM or placebo, and provides a contact phone number if they have any questions about DM.

*Blood draw:* Experienced personnel will draw the blood. Bleeding, bruising, or infection from needle insertion can be readily treated. Our staff is well qualified to address possible fear or discomfort during the blood draw.

*Blood pressure monitor:* Experienced personnel will place the cuff around the arm.

*Pregnancy Precautions:* In those very few post-menarchal subjects, a serum pregnancy test will be performed on the first day of admission; if positive, the subject will be removed from the study.

*EKG:* Any topical skin reaction can be readily treated.

*Behavioral and Psychological evaluations:* Parents are informed prior to the interviews that they can choose not to answer any questions that may make them feel uncomfortable or upset. The psychiatrist and the psychologist are well experienced, empathetic, and compassionate during the interviews.

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Nutrition Assessment: During the interview, parents can choose not to answer any question(s) that may make them feel uncomfortable or upset. An experienced nutritionist in the PCRU will perform the body measurements.

Interview discomfort: Parents are informed prior to the interviews that they can choose not to answer any questions that may make them feel uncomfortable or upset. The interviewers are well experienced, empathetic and compassionate during the interviews.

Risk of disclosure of information/loss of privacy: Many steps are taken to prevent the risk of disclosure of information/loss of privacy, such as deidentifying/coding information and samples; storing identifying data separate from deidentified data in locked offices with limited/restricted access; password-protected and access-restricted databases. Release of information is provided only with appropriate signed authorization by parent. The blood samples are for research purposes only, and not for distribution for sale or for selling any products derived from the blood.

Time commitment: We will be as flexible as possible with scheduling the inpatient admissions to accommodate the family's schedule.

c. Plan for reporting unanticipated problems or study deviations.

The DSMB, IRB, KKI's RCO, JHH-ICTR, and the FDA, will be immediately notified of adverse effects by a *Problem/Event Report Form* completed by the PI.

The Data Safety Monitoring Board (DSMB), IRB, KKI's Research Compliance Office, JHH-ICTR, and the FDA, will be immediately notified of protocol events by an *Problem/Event Report Form* completed by the PI.

The DSMB, which is updated every 2 months by the PI with a progress report, will be chaired by Dr. Bruce Shapiro, Professor, Department of Pediatrics, JHMI and Vice President of KKI Training Program; and will include Dr. Ali Fatemi, Asst. Professor of Neurology, JHMI; Dr. Harvey Singer, Professor of Neurology, Johns Hopkins University School of Medicine. They will meet with the PI and Research staff at least annually to discuss progress and adverse events. The DSMB will assess "reasonable safety" and make changes if and when appropriate upon notification of adverse effects by the PI. The DSMB will make recommendations for protocol and/or consent modifications, suspension, or early termination of the trial. The PI will then inform the IRB and FDA of any recommended changes or suggestions. The DSMB will not have contact with patients or families.

d. Legal risks such as the risks that would be associated with breach of confidentiality.

There are very few to no legal risks associated with breach of confidentiality, as all information is shared only between researchers participating in this study, parent(s), and those authorized by them. Information is deidentified when shared with other research collaborators.

e. Financial risks to the participants.

This study has sponsorship from the FDA # 004247-01 and by the JHH-ICTR. Monies are given to the Institute and not to investigators.

Both the initial and follow-up 3-day hospital admissions to the PCRU, the subject's meals, including all proposed test procedures/evaluations performed during those two inpatient admissions as part of the study protocol are paid for by this research study. In addition, the costs of the required two interim maintenance evaluations (ie, neurological eval, EKG, DM levels, CBC, CMP (electrolytes/ LFTs) to be done at 2 weeks and 1 month are paid for by this research study.

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If participants live more than 5 hours driving distance from Johns Hopkins, the study will reimburse round trip travel costs (up to \$500 for the subject and up to \$500 for one parent (up to a total of \$1,000 per visit)).

For those driving, reimbursement will be processed after discharge from the study, per Federal reimbursement rate for round trip mileage.

**Not paid for by us:** Any clinical/laboratory assessments required outside of this study's protocol, or those tests to be repeated as clinically-indicated tests.

## 9. Benefits

- a. Description of the probable benefits for the participant and for society.

**Participant:** There are no individual medical benefits for participating in this study.

Dextromethorphan may improve cognition and behavior or lessen or diminish seizures. The measurement of cytokines and glutamate in serum and released from peripheral blood mononuclear cells will determine whether treating children with dextromethorphan modifies the cytokine and glutamate levels, and reflect this process in brain. In addition, since the subject will be evaluated and followed by a multidisciplinary team who will address the associated neurological deficits that occur in RTT, the family will be advised on current treatment(s) available for such impairments, and would be informed in the future should new therapeutic interventions become available. All tests and evaluations as part of the research protocol are performed free of charge by experienced professionals involved in the study.

**Society:** Results of diagnostic test procedures/evaluations may provide understanding of the biological basis of RTT. Understanding the neurobiology of RTT will be valuable to other mental retardation syndromes such as autism and Fragile-X and, thus, help individuals suffering with these disorders as well. We are expected to publish results of this study in the medical literature to be available to the general public, so that larger populations of RTT patients may benefit if favorable results are noted, or avoid DM use in future if no efficacy is proven.

## 10. Payment and Remuneration

- a. Detail compensation for participants including possible total compensation, proposed bonus, and any proposed reductions or penalties for not completing the protocol.

None

## 11. Costs

- a. Detail costs of study procedure(s) or drug (s) or substance(s) to participants and identify who will pay for them.

The study pays for the cost of all test procedures/consultations/evaluations per the Study Protocol performed during the initial and follow-up 3-day inpatient admissions to JHH-PCRUC (required prior to initiating the drug trial and after 3 months of the study), as well as the cost of staying in the hospital during the study.

The cost of two required interim maintenance evaluations (ie, neurological eval, EKG, DM levels, CBC/CMP (electrolytes/ LFTs) at 2 weeks and 1 month will be covered by this study.

Dextromethorphan or placebo, in the assigned dosage, is provided to the subjects at no cost during this 3-month trial.

Date: April 12, 2013

Principal Investigator: Sakkubai Naidu

Application Number: NA\_00064949

Travel expenses for the subject (up to \$500 maximum) and one parent (up to \$500 maximum) for up to a total of \$1000 per visit to the JHH-PCRUI is also reimbursed by this study.

**Not paid for by this study:** Any test result showing a deviation from normal will be repeated as a clinically indicated test, the cost of which is not covered by this study. Any fee-for-service costs of clinical consultations performed outside of this research study are the financial responsibility of the subject's parent. Before additional charges are incurred, the parent will be informed of the approximate fee-for-service cost.