A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, CROSS-OVER STUDY TO ASSESS THE SAFETY, TOLERABILITY, AND EFFICACY OF ORAL KETAMINE IN PATIENTS WITH RETT SYNDROME

INVESTIGATIONAL PRODUCT:	Ketamine
PROTOCOL NUMBER:	Ket-101-RSRT
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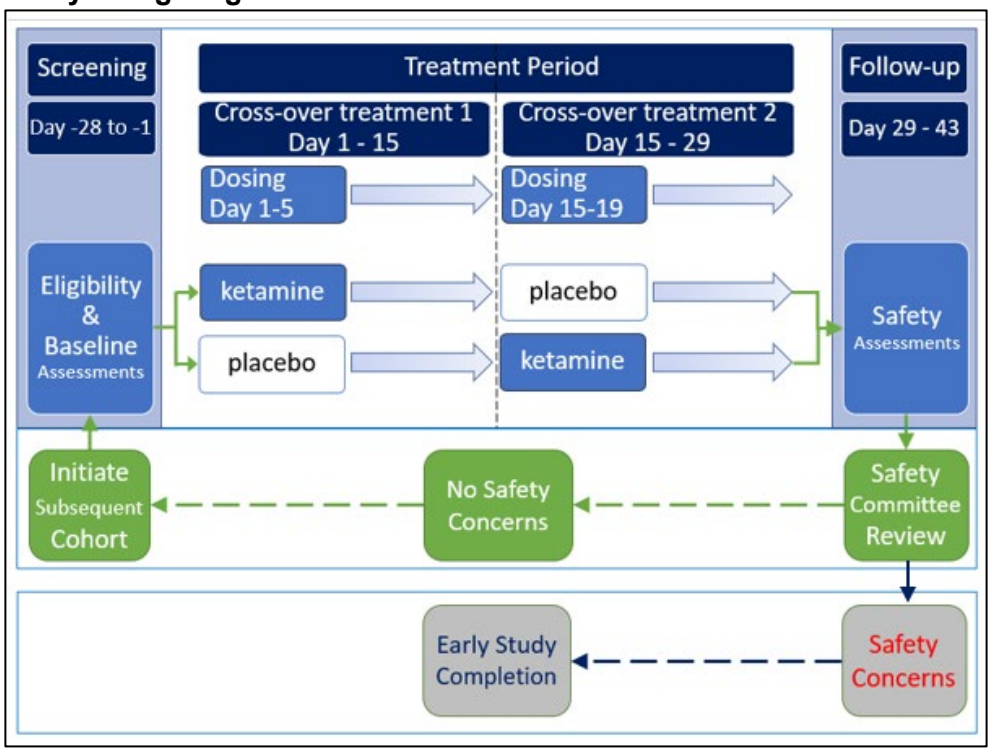
SPONSOR-INVESTIGATOR:	Jeffrey L. Neul, MD, PhD Vanderbilt University Kennedy Center
COLLABORATOR NAME / ADDRESS:	Rett Syndrome Research Trust 67 Under Cliff Rd, Trumbull, CT 06611

1. SYNOPSIS

Sponsor - Investigator	Jeffrey L. Neul, MD, PhD
Title of Study:	A double-blind, randomized, placebo-controlled, cross-over study to assess the safety, tolerability, and efficacy of oral ketamine in patients with Rett Syndrome
Study Phase:	IIa
Name of Investigational Drug; Route:	Ketamine; oral dosing
Study Centers:	multicenter study; approximately 7 sites
Study Duration:	Estimated first patient enrolled: October 2018 Estimated last patient completed: August 2021
Study Design:	Double-blind, randomized, placebo-controlled, cross-over study
Patient Population:	Female patients with Rett Syndrome aged 6 – 12 years, inclusive, who have not achieved menarche
Total Sample Size:	Approximately 48 patients are planned to be enrolled. For each of the 4 cohorts, approximately 12 patients will be enrolled.
Objectives:	To explore the safety, tolerability and efficacy of a 5-day regimen of ketamine administered to individuals with Rett syndrome. The primary objective is: • To assess the safety and tolerability of administration of oral ketamine The exploratory objectives are: • To assess the effect of oral ketamine on treating symptoms of Rett syndrome as measured by the change from baseline in: o Continuous biosensor data (activity, movement, heart rate, gait, sleep, posture, breathing regularity) o Rett syndrome severity as measured by the clinician Motor Behavioral Assessment (MBA) o Rett syndrome severity as measured by the parent Rett Syndrome Behavior Questionnaire (RSBQ) o Rett syndrome severity and improvement as measured by the clinician Clinical Global Impression Severity (CGI-S) and Improvement (CGI-I) Scales o Symptom severity as measured by the clinician Domain Likert Scale o Symptom severity as measured by the parent Domain Likert Scale o Sleep as measured by the parent Child Sleep Habits Questionnaire (CHSQ) o Rett Caregiver Inventory Assessment (RTT CIA)

	○ EEG signature compared to placebo
Methodology:	**Overview** This is a double-blind, randomized, placebo-controlled, multi-center, cross-over study to evaluate the safety, tolerability and efficacy of oral ketamine in patients 6 – 12 years of age, inclusive, who have not achieved menarche, diagnosed with Rett Syndrome with a confirmed *MECP2* mutation. The study consists of up to 4 cohorts initiated sequentially in ascending dose order (0.75 mg/kg BID, 1.5 mg/kg BID, 3 mg/kg BID, and 4.5 mg/kg BID). Each cohort will assess 1 dose of ketamine compared to placebo. After each cohort, an independent safety monitoring committee will determine if there are adequate safety data to support initiation of the subsequent dosing cohort. Patients may only participate in one cohort. A cohort within the study is divided into 3 periods: Screening, Cross-over Treatment, and Safety Follow-up. The Screening Period may last between 14 and 28 days prior to the initiation of study drug. The Cross-over Treatment period is a 4-week, double-blind, placebo-controlled, cross-over period to define safety and explore efficacy for the study. The Safety Follow-up Period is the final 2 weeks of the study to assess safety following Treatment Period completion. **Study Design Figure** 

	Screening and Baseline Parents, legally authorized representatives (LARs), or authorized caregivers will provide appropriately obtained informed consent, and where appropriate, patients will provide assent, prior to the completion of any screening procedures. At the Screening visit, patients will be assessed for initial eligibility and will take home biosensors for collection of daily in-home data prior to the Day 1 Randomization Visit. Eligible patients will return for the Randomization Visit on Day 1 for review of eligibility and additional baseline safety and efficacy assessments including a baseline EEG at select centers, physical examination, vital signs, ECG, as well as physician CGI-S, RSBQ, MBA, and an exploratory Clinician Rated Domain Likert Scale consisting of 8 domains (hand function, walking, verbal and non-verbal communication, comprehension, attention, behavior problems, mood). Caregiver rated scales including the CSHQ, RTT CIA, and an exploratory Parent Rated Domain Likert Scale of the same 8 clinician domains with the addition of a seizures domain, will also be completed. **Cross-Over Treatment Period** For each cohort, the double-blind, placebo-controlled, cross-over Treatment Period will last a total of 4 weeks starting with the first treatment on Day 1 and initiation of the alternate treatment 2 weeks later on Day 15. Each ordered cross-over dosing regimen comprises 5 days of BID dosing (treatment 1: Day 1-5, treatment 2: Day 15-19) and patient safety and efficacy evaluation for 14 days after dose initiation (Period 1: Day 15, Period 2: Day 29). After dosing completion and conclusion of the 4-week Treatment Period, patients will return on Day 29 for final efficacy assessments, safety assessments, and turn in the biosensors. **Safety Follow-up** A final Safety Follow-up phone call will occur on Day 43, 2 weeks after the Day 29 Visit and conclusion of the Treatment Period. **In-Clinic Assessments** At the clinic visits, safety and efficacy assessments will be performed by the clinician and the caregiver. Physical examination, EEG at select sites, vital signs, physician CGI-S or CGI-I, RSBQ, MBA, Clinician Rated Domain Likert Scale, CSHQ, RTT CIA, and Parent Rated Domain Likert Scale will be completed. **Dosing** Patients will receive the first oral dose of each 5-day dosing regimen in-clinic under observation. Tolerability of the in-clinic oral dose will be evaluated for two hours through assessment of vital signs and oxygen saturation at 15-minute intervals for the first hour, as well as monitoring of patient disposition. If well tolerated, subsequent doses will be administered in the home. The site will contact the caregiver each day of active dosing to confirm continued tolerability and to assess for emergent side effects.

	The patient and the clinical team will be blinded to treatment group assignments throughout the study. The pharmacist will be unblinded to ensure appropriate treatment assignment. **At-home Assessments** Over the following 2-week period after each of the observed in-clinic doses, caregivers will continuously capture biosensor data to assess patient activity, movement, heart rate, gait, sleep, posture, and breathing regularity. On Days 8 and 22, caregivers will report a Parent Domain Report Likert Scale and complete the CSHQ. The Parent Domain Likert Scale and the RTT CIA will also be completed on Day 43. **Safety Monitoring** Patient safety will be monitored throughout the study. Investigators will monitor safety and tolerability through a 2-hour observation and monitoring of in-clinic dosing of the first dose of each treatment, and through daily communication with the caregiver on at-home dosing days. In addition, study Investigators will form a medical monitoring committee and convene on a regular basis to discuss adverse events (AEs) and overall interpretation of tolerability. An independent safety monitoring committee will perform a safety assessment of each cohort with a subsequent ascending dose to assess if initiation of the subsequent cohort is supported. In the case of intolerability during the 5-day dosing period the patient will stop dosing at the assigned blinded dose and discontinue the study, and any adverse events will be followed until an acceptable clinical resolution is achieved. Patients who discontinue study drug due to an AE during the Treatment Period will return for the Day 29/Early Termination Visit 2 to 4 weeks after the last dose and receive a final Safety Follow-up phone call.
Duration of Study Participation:	Patients who complete the study will participate for approximately 8 to 10 weeks including Screening (2-4 weeks), Treatment (4 weeks), and Safety Follow-up (2 weeks).
Duration of Treatment	Patients will receive one 5-day treatment with ketamine
Reference Therapy, dosage and mode of administration	Matched placebo consisting of sterile water and masking flavor
Diagnosis and main criteria for inclusion	Patients eligible for enrollment must meet all of following inclusion criteria and none of the exclusion criteria. ***Inclusion*** 1. Prior to the conduct of any study-specific procedures, the patient must provide verbal assent to participate in the study (if developmentally appropriate), and the parent/caregiver/LAR must provide written informed consent. If the caregiver attending the clinic visits is not the parent or LAR, written consent must be obtained from the parent or LAR for the caregiver's participation in the study.

	2. Female patients 6 to 12 years of age, inclusive, at the time of consent, who have not achieved menarche. 3. Diagnosis of typical or atypical Rett Syndrome according to the revised Clinical Diagnostic Criteria[5] and presence of a disease causing *MECP2* genetic mutation 4. Ability to take oral liquid medications orally or through a feeding tube. 5. The patient's parent/caregiver/LAR must be able to understand the nature of the study and to allow for the completion of all study assessments. The same parent/caregiver/LAR must be capable of providing reliable information about the patient's condition, agree to oversee the administration of study drug, and accompany the patient to all clinic visits. ***Exclusion*** 1. Uncontrolled epilepsy, defined as caregiver report of unusual variability in seizure frequency that may warrant regimen change during the study to either untreated patients or patients on stable anti-seizure medication regimens. 2. Plans to initiate or change pharmacologic interventions during the course of the study or have not been on stable interventions for at least 4 weeks prior to Screening, or stable anti-seizure medication regimens (if applicable) for 12 weeks prior to the Randomization Visit. 3. Plans to initiate or change non-pharmacologic interventions (behavioral, educational or dietary) during the course of the study or have not been on stable interventions for at least 4 weeks prior to Screening. Typical school vacations are not considered modifications of stable interventions. 4. Patients who achieve menarche during study participation and prior to conclusion of the second dosing period of the cohort. 5. A history of prolonged QT syndrome or a Screening QTc interval with Fridericia's correction (QTcF) >460 for females less than 15 years or >470 for females over 15 years. 6. Have taken another investigational drug within the greater of 4 weeks of 5 half-lives prior to Screening or during the study. 7. Concurrent treatment with other NMDA receptor antagonists, treatments with known ketamine interactions, sedatives, barbiturates, benzodiazepines, narcotics, or opioids. 8. Concurrent treatment with strong CYP3A4 or CYP2B6 inhibitors dosed for systemic exposure, or for 4 weeks prior to initiating study drug. 9. Concurrent treatment with strong CYP3A4 or CYP2B6 inducers dosed for systemic exposure, or for 4 weeks prior to initiating study drug. 10. A history of hypersensitivity to ketamine. 11. A history of any condition that may be worsened by increased blood pressure or heart rate, bronchodilation, intraocular or intracranial pressure, or a history of porphyria.

	12. Patients with any condition that, in the opinion of the principal investigator, might interfere with the conduct of the study, confound interpretation of the study results, endanger their own well-being, or who may otherwise not be suitable for the study.
Primary Outcome Measure:	• Safety and Tolerability
Safety Outcome Measures:	• Adverse Events • Concomitant medications • Physical examination • Vital signs and oxygen saturation
Exploratory Efficacy Outcome Measures	• Biosensor data (activity, movement, heart rate, gait, sleep, breathing regularity and posture) • CGI-S and CGI-I • MBA • RSBQ • Clinician Rated Domain Likert Scale • Parent Rated Domain Likert Scale • CSHQ • RTT CIA • EEG

Statistical Methodology:	**Sample Size** Sample size determinations for this study are based on the primary outcome of safety and tolerability. For a dose-limiting adverse event with a 10% incidence rate, a sample size of 10 patients/dose level (10 patients/cohort) provides a 65% probability of observing at least 1 event at each dose level, and an 88% probability of observing at least 1 event by completion of Cohort 2. For measures of efficacy, there are no studies in the literature to provide reliable estimates of treatment effects of ketamine or the estimate of variance in symptom management for Rett Syndrome patients. Each dose level provides 80% power to detect a 1.00 standard deviation treatment difference with the 2-sided paired t-test with 0.05 Type I error. Assuming a discontinuation rate between 15 and 20%, approximately 12 patients will be enrolled in each cohort to provide 10 patients who complete the treatment period. The Safety Population will comprise all patients who take at least one dose of study medication. Safety data will be tabulated and summarized by treatment. Adverse events will be coded by MedDRA and concomitant medications will be summarized by WHODrug by ATCC classification and preferred term. By patient listings will be provided for serious adverse events, AEs leading to discontinuation, and deaths. Data collected in this study will be documented using summary tables, figures, and patient data listings. Data will be summarized by treatment and cross-over period. Continuous measures will be summarized descriptively (mean, standard deviation, median, minimum and maximum value) and categorical measures will be presented as number and percentage.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Table 1: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Definition
AE	Adverse Event
ATCC	Anatomical-Therapeutic-Chemical classification
BDNF	Brain-derived Neurotrophic Factor
BID	Twice daily dosing
BPM	Beats per minute
CFR	Code of Federal Regulations
CRO	Contract Research Organization
CSHQ	Children's Sleep Habits Questionnaire
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
CTCAE	Common terminology criteria for adverse events
CYP2B6	Cytochrome P450 2B6
CYP3A4	Cytochrome P450 3A4
eCRF	Electronic case report form
ECG	Electrocardiogram
EDC	Electronic data capture
EEG	Electroencephalogram
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICF	Informed consent form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
LAR	Legally authorized representative
LLN	Lower limit of normal
MDD	Major depressive disorder
MBA	Motor Behavior Assessment
MeCP2 / *MECP2*	Methyl-CpG-binding protein 2
MedDRA	Medical Dictionary for Regulatory Activities
mTOR	Mammalian Target of Rapamycin
NMDA	N-methyl-D-aspartate
OCD	Obsessive compulsive disorder
QT	QT interval
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate (Fridericia's method)
RSBQ	Rett Syndrome Behavior Questionnaire
RSRT	Rett Syndrome Research Trust
RTT	Rett Syndrome
RTT CIA	Rett Caregiver Burden Inventory Assessment

Abbreviation or Specialist Term	Definition
SOP	Standard Operating Practices
TEAE	Treatment-emergent adverse events
TID	Three times daily dosing
TPN	Total parenteral nutrition
TRD	Treatment-refractory depression
ULN	Upper limit of normal
WHODrug	World Health Organization Drug dictionary

2. INTRODUCTION

2.1. Overview of Rett Syndrome

Rett Syndrome is a severe and complex neurodevelopmental disorder resulting primarily from loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2). As a transcriptional regulatory protein, loss of MeCP2 results in dysregulation of more than 100 genes causing defects in synapse formation and neurotransmission. *MECP2* is encoded on the X chromosome and as an X-linked dominant disorder, mutations are typically inherited from a random spontaneous mutation in sperm thereby affecting females at an incidence of approximately 1 in 10,000 live female births.

A heterozygous mutation in *MECP2* disrupts MeCP2-dependent functions in half of the patient's cells due to X inactivation and the resulting expression of either the normal or mutant *MECP2* gene. The phenotype of the disorder can be further complicated by skewed X inactivation that may contribute to more mild or more severe phenotypes depending on the direction and percentage of skewed expression[6,7].

After a period of apparently normal development through 6 to 18 months of age, Rett patients develop a spectrum of symptoms that generally includes loss of acquired speech and purposeful hand movements, head growth deceleration, motor, respiratory and autonomic dysfunction, stereotypic hand movements and increased risk of seizures [8,9]. Additional symptoms include gastrointestinal distress, sleep dysfunction, scoliosis, and orthopedic issues, and full-time care is required. Currently, no cure for Rett Syndrome exists and individual symptom management as well as physical and occupational therapies are the standard of care.

2.2. Scientific Rationale for the Study

In Rett Syndrome patients, cognitive and behavioral abnormalities are consistent with forebrain hypofunction seen in animal models coupled with brainstem hyperexcitability causing paroxysmal respiratory and autonomic events. NMDA receptor antagonists with the ability to differentially affect the excitatory/inhibitory imbalance across the brain due to regional differences in glutamatergic circuitry may provide a potential therapeutic intervention. The non-selective NMDA receptor antagonist ketamine (2-O-chlorophenyl-2-methylamino cyclohexanone) has previously been shown to increase cortical network function[10] and decrease synaptic excitability in brainstem networks important for respiratory and autonomic control[11].

The therapeutic potential of ketamine for treatment of Rett syndrome is supported by several studies in mice. Treatment of *Mecp2* mutant mice with a sub-anesthetic dose of ketamine at 8mg/kg demonstrates acute effects on circuit function and reverses several mutant phenotypes including hypoactivity in forebrain circuits, abnormal sensorimotor gating[12], as well as apneic breathing (Figure 1).

Sub-anesthetic ketamine has demonstrated effects against chronic and neuropathic pain, anxiety, depression and treatment-resistant depression syndromes, bipolar disorder, and has shown positive effects on cognition in children, as described later. Ketamine rapidly stimulates dendritic growth and translation and expression of key synaptic proteins regulated by brain-derived neurotrophic factor (BDNF) and mTOR signaling[13], shown to be deficient in *Mecp2* mutants[14].

Figure 1: Ketamine reduces apneic breathing episodes in Mecp2$^{-/-}$ mice

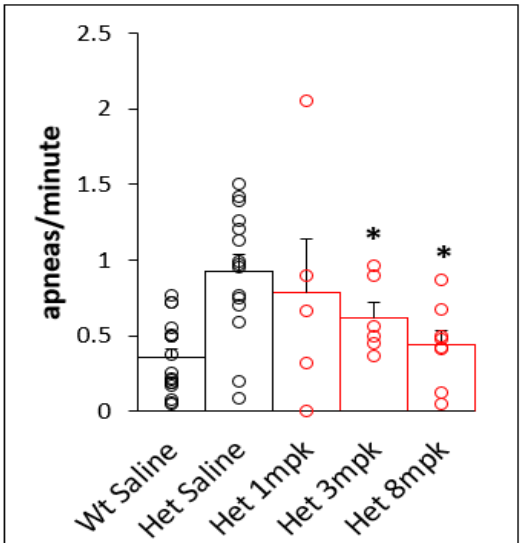


Figure 1. Dose-response effects of ketamine on apnea frequency in wildtype (Wt; black symbols) and heterozygous female *Mecp2* mutant mice (Het; red symbols) following single dosing, 90 min prior to plethysmographic recording of breathing (Katz et al., unpublished).

Table 2: Ketamine Bioavailability by Route of Administration

Administration Route	Relative Bioavailability	Dose Equivalence Correction Factor
Intravenous	100%	1
Oral	~20%	5
Intranasal	~44%	2.25
Intramuscular	~22%	4.65

Additionally, as shown in animal models of depression and stress,[15,16,17] ketamine has the potential to effect long-term synaptic repair by enhancing structural and functional connectivity. Supporting long-term effects of ketamine, more recent studies demonstrate prolonged efficacy with repeated dosing. For example, mice given 3 mg/kg intraperitoneal ketamine, once every 3 days for 4 weeks have reduced apneas even 24 hours after the last drug administration, indicating a durable treatment effect of repeated drug administration (Katz et al., unpublished). Clinical studies on the effects of sub-anesthetic ketamine in several neuropsychiatric indications also indicate that repeated dosing is likely to lead to more durable treatment effects than single doses. Together, these published and unpublished observations provide new insight into potential strategies for enhancing the efficacy of ketamine, including dosing regimen and route of administration.

The bioavailability of ketamine administered in various routes has been summarized[18] and shown in the table below. Due to extensive first-pass metabolism and approximately 20% oral bioavailability in humans, a starting oral dose of 0.75 mg/kg of ketamine is equivalent to an intravenous dose of approximately 0.15 mg/kg. The safety and efficacy of single oral doses up to 10 mg/kg[19] as well as repeated oral doses up to 1.5 mg/kg TID[20], has already been demonstrated for pediatric indications. The model of therapeutic intervention for this study is designed to assess an oral dosing regimen of ketamine within the sub-anesthetic range for tolerability and safety, and to explore potential efficacy of doses that theoretically should impact Rett syndrome symptomology.

In selecting the age range for this study, the ketamine label indicates that patients under the age of 3 years may be at an increased risk of neurotoxicity. Additionally, studies in rats indicate the sexually mature brain may be susceptible to excitotoxicity[21] that is not observed in younger animals[22]. Therefore, to ensure that patients enrolled in the study are not at increased risk for neurotoxic events, only patients between the ages of 6 to 12 years, inclusive, who have not achieved menarche, will be included.

2.3. Preclinical Information for Ketamine

The ketamine prescribing information[23] should be consulted for more detailed technical information, current discussion of nonclinical evaluations, and relevant information regarding the known safety profile of ketamine to date.

2.4. Clinical Information

Ketamine was initially developed for use as a general anesthetic and approved by the FDA in 1970 almost 50 years ago. Ketamine is now available generically through Hospira, Inc; Mylan Institutional; Par Pharmaceutical; and West-Ward Pharmaceuticals. The ketamine prescribing information[23] should be consulted for complete clinical information on ketamine to date.

Ketamine hydrochloride injection is indicated as the sole anesthetic agent for diagnostic and surgical procedures that do not require skeletal muscle relaxation and is best suited for short procedures but it can be used, with additional doses, for longer procedures. Ketamine hydrochloride is also indicated for the induction of anesthesia prior to the administration of other general anesthetic agents, and to supplement low-potency agents such as nitrous oxide. Routes of administration are intravenous or intramuscular injection.

Specific areas of application have included the following:

1. Debridement, painful dressings, and skin grafting in burn patients, as well as other superficial surgical procedures.
2. Neurodiagnostic procedures such as pneumonencephalograms, ventriculograms, myelograms, and lumbar punctures.
3. Diagnostic and operative procedures of the eye, ear, nose, and mouth, including dental extractions.
4. Diagnostic and operative procedures of the pharynx, larynx, or bronchial tree.
5. Sigmoidoscopy and minor surgery of the anus and rectum, and circumcision.
6. Extraperitoneal procedures used in gynecology such as dilatation and curettage.
7. Orthopedic procedures such as closed reductions, manipulations, femoral pinning, amputations, and biopsies.
8. As an anesthetic in poor-risk patients with depression of vital functions.
9. In procedures where the intramuscular route of administration is preferred.
10. In cardiac catheterization procedures.

2.4.1. Clinical Safety of IV Ketamine in Anesthesia

Ketamine has a wide margin of safety. Several instances of unintentional administration of overdoses of ketamine (up to ten times that usually required) have been followed by prolonged but complete recovery.

Ketamine has been studied in over 12,000 operative and diagnostic procedures, involving over 10,000 patients from 105 separate studies (ketamine prescribing information). During the course of these studies ketamine hydrochloride was administered as the sole agent, as induction for other general agents, or to supplement low-potency agents. Safety and effectiveness in pediatric patients below the age of 16 have not been established.

2.4.1.1. Adverse Events with IV Ketamine in Anesthesia

The following adverse reactions have been described when ketamine was used as indicated:

Cardiovascular: Blood pressure and pulse rate are frequently elevated following administration of ketamine alone. However, hypotension and bradycardia have been observed. Arrhythmia has also occurred.

Respiration: Although respiration is frequently stimulated, severe depression of respiration or apnea may occur following rapid intravenous administration of high doses of ketamine. Laryngospasms and other forms of airway obstruction have occurred during ketamine anesthesia.

Eye: Diplopia and nystagmus have been noted following ketamine administration. It also may cause a slight elevation in intraocular pressure measurement.

Genitourinary: In individuals with history of chronic ketamine use or abuse, lower urinary tract and bladder symptoms including dysuria, increased urinary frequency, urgency, urge incontinence, and hematuria have been reported. In addition, diagnostic studies performed to assess the cause of these symptoms have reported cystitis (including cystitis non-infective, cystitis interstitial, cystitis ulcerative, cystitis erosive and cystitis hemorrhagic) as well as hydronephrosis and reduced bladder capacity.

Neurological: In some patients, enhanced skeletal muscle tone may be manifested by tonic and clonic movements sometimes resembling seizures.

Gastrointestinal: Anorexia, nausea and vomiting have been observed; however, this is not usually severe and allows the great majority of patients to take liquids by mouth shortly after regaining consciousness.

General: Anaphylaxis. Local pain and exanthema at the injection site have infrequently been reported. Transient erythema and/or morbilliform rash have also been reported.

2.4.1.2. Emergence Reactions with IV Ketamine in Anesthesia

Emergence reactions have occurred in approximately 12% of patients when ketamine was used as indicated. The psychological manifestations vary in severity between pleasant dream-like states, vivid imagery, hallucinations, and emergence delirium, and in some cases accompanied by confusion, excitement, and irrational behavior which a few patients recall as an unpleasant experience. The duration ordinarily is no more than a few hours; in a few cases, however, recurrences have taken place up to 24 hours postoperatively. No residual psychological effects are known to have resulted from use of ketamine. Incidence of emergence phenomena is least

in the elderly (over 65 years of age) patient, are less frequent when the drug is given intramuscularly, and incidence is reduced as experience with the drug is gained. The literature suggests that pediatric patients are at lower risk for psychotomimetic side effects related to ketamine administration than adults[24].

The incidence of psychological manifestations during emergence, particularly dream-like observations and emergence delirium, may be reduced by using lower recommended dosages of ketamine in conjunction with intravenous diazepam during induction and maintenance of anesthesia. Also, these reactions may be reduced if verbal, tactile, and visual stimulation of the patient is minimized during the recovery period. This does not preclude the monitoring of vital signs. In order to terminate a severe emergence reaction, the use of a small hypnotic dose of a short-acting or ultra short-acting barbiturate may be required.

When ketamine is used on an outpatient basis, the patient should not be released until recovery from anesthesia is complete and then should be accompanied by a responsible adult.

2.4.2. Clinical Studies of Ketamine in Adults with Neuropsychiatric Conditions

Several neuropsychiatric conditions including bipolar disorder, depression syndromes, and obsessive compulsive disorder (OCD) are hypothesized to be caused by dysfunctional NMDA receptor signaling and have shown evidence of symptom improvement in adults when treated with ketamine. Though not all studies assessing ketamine have shown efficacy in every case, route of administration and duration of dosing, the studies described below support the therapeutic dose range under study and demonstrate the possibility for short- and long-term effects to be achieved in Rett syndrome.

Bipolar Disorder

The pathophysiology of bipolar disorder may be due to dysfunctional NMDA signaling, and ketamine as a non-competitive NMDA receptor antagonist was investigated for utility as a rapid onset anti-depressant in treatment-resistance bi-polar depression in a randomized trial of 18 patients[25]. The authors found that a single treatment with ketamine at a dose of 0.5 mg/kg over a 40-minute IV infusion time resulted in significant changes in depression and anxiety compared to placebo. Adverse events associated with ketamine included dissociation; feeling strange, weird or bizarre; dry mouth; tachycardia; and increased blood pressure. No serious adverse events and no significant changes in ECG, respiratory or laboratory values were reported[25].

Depression

NMDA receptor antagonists have also been evaluated in treating treatment-refractory depression (TRD) and major depressive disorder (MDD). Ketamine has been reported to lead to sustained improvements for up to 12 months in TRD in a case-series of 2 patients, where each received a 5-day regimen of intravenous ketamine and were titrated to an individual maximum tolerated dose lacking psychotomimetic effects but where both patients reported feeling “a bit heady”. Each patient received a 1-hour infusion of 27.5 mg or 30 mg, respectively, on each day of the 5-day treatment period[26].

In a randomized controlled cross-over trial in 18 patients with MDD, 50 mg of ketamine given intranasally over the span of 20 minutes demonstrated significant improvements in depressive symptoms through 48 hours post-dose, was associated with improvement of anxiety at 24 hours, and was well tolerated with small increases in psychotomimetic measures that typically resolved within 4 hours of dosing[27].

In March 2019, Spravato (esketamine) CIII nasal spray by Janssen Pharmaceuticals was approved for the treatment of TRD in adults taking an oral antidepressant. Esketamine, the S-enantiomer of racemic ketamine, was assessed intranasally in 1,709 adults in 6 clinical trials. The indicated treatment regimen includes a 2-hour in-clinic observation of dosing at 56 mg or 84 mg twice weekly for up to 4 weeks, followed by in-clinic maintenance at once weekly or once bi-weekly dosing. Side effects reported during clinical development at any dose at an incidence ≥5% and at least twice that of placebo were dissociation, dizziness, nausea, sedation, vertigo, hypoesthesia, anxiety, lethargy, blood pressure increased, vomiting, and feeling drunk[28].

Obsessive Compulsive Disorder

In a randomized controlled crossover trial of 15 patients receiving either placebo or ketamine dosed at 0.5 mg/kg over a 40-minute IV infusion, symptom relief of OCD met responder criteria and was statistically significant up to a week post-infusion[29], confirming shorter-term effects noted in a case report[30]. Additionally, in an open label trial of the same regimen in 10 patients with less severe OCD, acute relief of OCD symptoms was attained though no patients achieved responder status[31]. Of these, 59% of patients with comorbid depression demonstrated a significant and persistent improvement in depressive symptoms. In both studies, ketamine treatment was considered well tolerated. The most common adverse events reported were dissociative symptoms that resolved within 1-2 hours post-infusion and transient increases in blood pressure during infusion[29,30,31]. Two non-depressed patients in the open-label trial reported dysphoria, anxiety and passive suicidal ideation within the first 48 hours post-infusion[31].

2.4.3. Clinical Studies of Ketamine Dosed Orally in Adults

Oral ketamine has been assessed in adults in several neuropsychiatric conditions including palliative care settings for depression and anxiety, and analgesia. Chronic forms of pain have employed the highest doses and longest durations of oral ketamine in a clinical setting. Oral ketamine has been reported to have a more favorable side effect profile than parenteral ketamine, including reduced levels of psychotomimetic effects [32,33,34,35,36.]

Depression and Anxiety

Oral ketamine was assessed in 14 adults in hospice as a once-daily treatment at 0.5 mg/kg for 28 days for the treatment of depression and anxiety. Eight subjects completed the study. On days 14 and 28, anxiety and depression were found to be significantly improved. Adverse events reported were mild and included diarrhea, trouble sleeping and trouble sitting still. No changes in vital signs were reported and no serious adverse events occurred[37]. A case-series of 2 patients suffering from treatment-resistant depression with suicidal ideation received an ascending dose oral ketamine regimen of 0.5 mg/kg to 3 mg/kg and demonstrated sustained efficacy in relieving symptoms[38].

Pain

In a co-analgesic oncology palliative care setting, a study of 29 adults receiving an optimized sub-cutaneous infusion of ketamine for pain control demonstrated maintenance of efficacy upon switching to dose-equivalent oral administration TID for up to 9 days, at doses as high as 750 mg/day. A symptom score evaluation revealed side effects of slight (83%) or moderate (17%) insobriety, slight (86%) or moderate (14%) somnolence, and 1 report of hallucinations during sub-cutaneous administration, and no occurrences reported during oral administration[32].

A patient with complex regional pain syndrome type I was administered 30 mg TID that increased by 5 mg per week until achieving 60 mg 4 times a day and maintained the regimen for more than 2 years[39]. Another case-report documented a significant improvement in pain, allodynia and hyperalgesia in a patient with central post-stroke pain at a dose of 50 mg TID for 3 months[40].

2.4.4. Clinical Studies of Ketamine Dosed Orally in Children

Due to ketamine's well characterized safety profile, single sub-anesthetic doses of oral ketamine have been explored to aid surgical procedures in children, and more recently, repeat dosing of oral ketamine up to 14 days has been explored to assess the impact on chronic pain and cognitive function in children.

The effects of oral ketamine were evaluated in an open-label ascending dose cohort study in 12 children, aged 11 to 19 years, for assessment of chronic pain management and short- and long-term cognitive effects[20,41]. The oral doses evaluated were 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, and 1.5 mg/kg dosed TID over 14 days. Five of 12 patients (42%) demonstrated an improvement in pain score over the 14-day treatment period, with 2 patients reporting pain resolution[20]. Eleven of 12 patients (92%) demonstrated a significant and persistent improvement in executive functioning at 14 days as well as 3 months post-ketamine treatment[41]. Adverse events reported were generally mild and included short-term fatigue or somnolence after the first dose of oral ketamine, which resolved within 1–2 hours and did not recur upon repeat administrations. Delayed dysphoria (e.g., confusion, dizziness) was experienced by 7 of 12 participants and CTCAE v4.0 grade 2 dysuria without hematuria was experienced by 1 participant in the 1.5 mg/kg cohort. Grade 2 (moderate) anorexia and depressed level of consciousness were considered dose-limiting toxicities of the 14-day 1.5 mg/kg TID cohort[20].

In another double-blind, randomized, controlled study in 45 children aged 1-7 years, patients received a single dose of placebo, 3 mg/kg, or 6mg/kg oral ketamine prior to induction of anesthesia for a surgical procedure to assess ease of anesthesia induction and potential overall improvement of the patient's surgery experience[42]. Both 3 mg/kg and 6 mg/kg single doses of oral ketamine were considered well tolerated, with incidence of nystagmus statistically elevated from placebo. There were no reports of altered vital signs or surgery recovery, and no emergence phenomena were reported. Sedation occurred in 11 of 15 (73%) of patients who received 3 mg/kg and 100% of patients who received 6 mg/kg.

Another open-label study assessed a single 10 mg/kg dose of oral ketamine in 35 pediatric oncology patients aged 14 months to 17 years and demonstrated less distress related to surgical procedures than historical controls. The dose of 10 mg/kg oral ketamine resulted in 87% of patients attaining maximum levels of sedation at 45 minutes post dose with recovery within 2-4 hours. No cardiorespiratory adverse events related to ketamine were reported and no serious adverse events occurred. Emergence phenomena were experienced by 3 patients (9%) and included 2 patients (6%) that demonstrated mild confusion and disorientation, and 1 patient (3%) that demonstrated moderate agitation that resolved without intervention[19].

2.4.5. Clinical Studies of Ketamine Dosed Orally in Children with Rett Syndrome

To date, one open-label pilot study and one case report have indicated positive effects from oral ketamine treatment in Rett syndrome patients and support a controlled study to assess its therapeutic potential. Consistent with mouse model data, a multiple ascending dose pilot study in 4 Rett syndrome patients conducted by Case Western Reserve University and the Cleveland

Clinic demonstrated evidence for efficacy of single doses of intravenous ketamine on breathing regularity over the dose range tested (0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg, and 1.0 mg/kg) with profound effects observed at 10 hours post-dose (Katz, unpublished). All 4 doses of intravenous ketamine were considered well-tolerated.

In a case report called “The Scottish Patient” (*Welham et al.,* Presented at the 2017 British Pediatric Neurology Association Annual Meeting), repeated oral dosing of ketamine in a 10-year old Rett syndrome patient was described. In this case, the patient was given oral ketamine at 0.75 mg/kg BID for 5 days for refractory seizures that resulted in not only seizure resolution but also improvement in motor function, communication, and cognition lasting 8 weeks before seizures re-emerged. The patient was dosed with another 5-day regimen of ketamine at 0.75 mg/kg BID conferring additional seizure control and sustained improvements in Rett symptoms. In a 12-month review of the patient’s hospital admissions for seizures, an 80% reduction in hospital admission time was recorded in the 6 months after ketamine treatment compared to the 6 months prior to treatment.

3. ETHICS

The procedures set out in this study protocol, pertaining to the conduct, evaluation and documentation of this study, are designed to ensure that the Investigators abide by Good Clinical Practice (GCP), including but not limited to Title 21 Code of Federal Regulations (CFR) Parts 50, 56, and 312, and the International Conference on Harmonisation (ICH) guidelines and directives. Compliance with these regulations also constitutes compliance with the ethical principles described in the current revision of the Declaration of Helsinki and applicable local regulatory requirements and law.

The Investigator is responsible for protecting the rights, safety, and welfare of patients under their care, and for the control of the medication under investigation. All ethical, regulatory, and legal requirements must be met before the first patient is enrolled in the study.

3.1. Institutional Review Board

The Institutional Review Board (IRB) will meet all FDA requirements governing IRBs according to CFR, Title 21, Part 56. The Investigator (or designee) must submit this study protocol and any amendments, the approved informed consent form(s) (ICF), patient information sheets, patient recruitment materials, and other appropriate documents to the IRB for review and approval.

3.2. Written Informed Consent

Prior to performing any study-related activities under this protocol, including screening tests and assessments, written informed consent with the approved ICF must be obtained from the parent/legally authorized representative (hereafter referred to as caregiver) for the patient. Where possible, assent should be obtained from the patient.

The ICF, as specified by the clinical site's IRB, must follow the Protection of Human Patients regulations listed in the CFR, Title 21, Part 50.

All ICFs used in this study must be approved by the appropriate IRB. Amendments to the ICF must be approved by the presiding IRB before use.

4. STUDY OBJECTIVES

The overall objectives of the study are to assess the safety, tolerability, and efficacy of oral ketamine in patients with Rett syndrome.

4.1. Primary Objectives

The primary objective is:

- To assess the safety and tolerability of administration of a 5-day dosing regimen of oral ketamine in Rett syndrome patients.

4.2. Exploratory Objectives

The exploratory objectives are:

- To assess the change in continuous biosensor data (activity, movement, heart rate, gait, sleep, posture, breathing regularity) from baseline;
- To assess the change in Rett syndrome severity as measured by the Motor Behavior Assessment (MBA) from baseline;
- To assess the change in Rett syndrome severity as measured by the Rett Syndrome Behavior Questionnaire (RSBQ) from baseline;
- To assess the change in Rett syndrome severity and improvement as measured by the Clinical Global Impression of Severity (CGI-S) and Clinical Global Impression of Improvement (CGI-I) from baseline;
- To assess the change in symptom severity as measured by the clinician Domain Likert Scale from baseline;
- To assess the change in symptom severity as measured by the parent Domain Likert Scale from baseline;
- To assess the change in sleep from baseline as measured by the Child Sleep Habits Questionnaire (CSHQ)
- To assess the change in caregiver burden as measured by the Rett Caregiver Burden Inventory Assessment (RTT CIA) from baseline
- To assess the change in EEG signature between placebo and ketamine dosing

5. OVERALL STUDY DESIGN

5.1. Study Design

This is a double-blind, randomized, placebo-controlled, multi-center, cross-over study to evaluate the safety, tolerability, and efficacy of oral ketamine in patients with Rett syndrome in an ascending dose, 4-cohort design. The patient must have a documented *MECP2* mutation considered causative for the disorder. Patients will have a clinical evaluation involving physical examination and medical history review and require a definitive diagnosis of Rett syndrome based on the Revised Diagnostic Criteria[5].

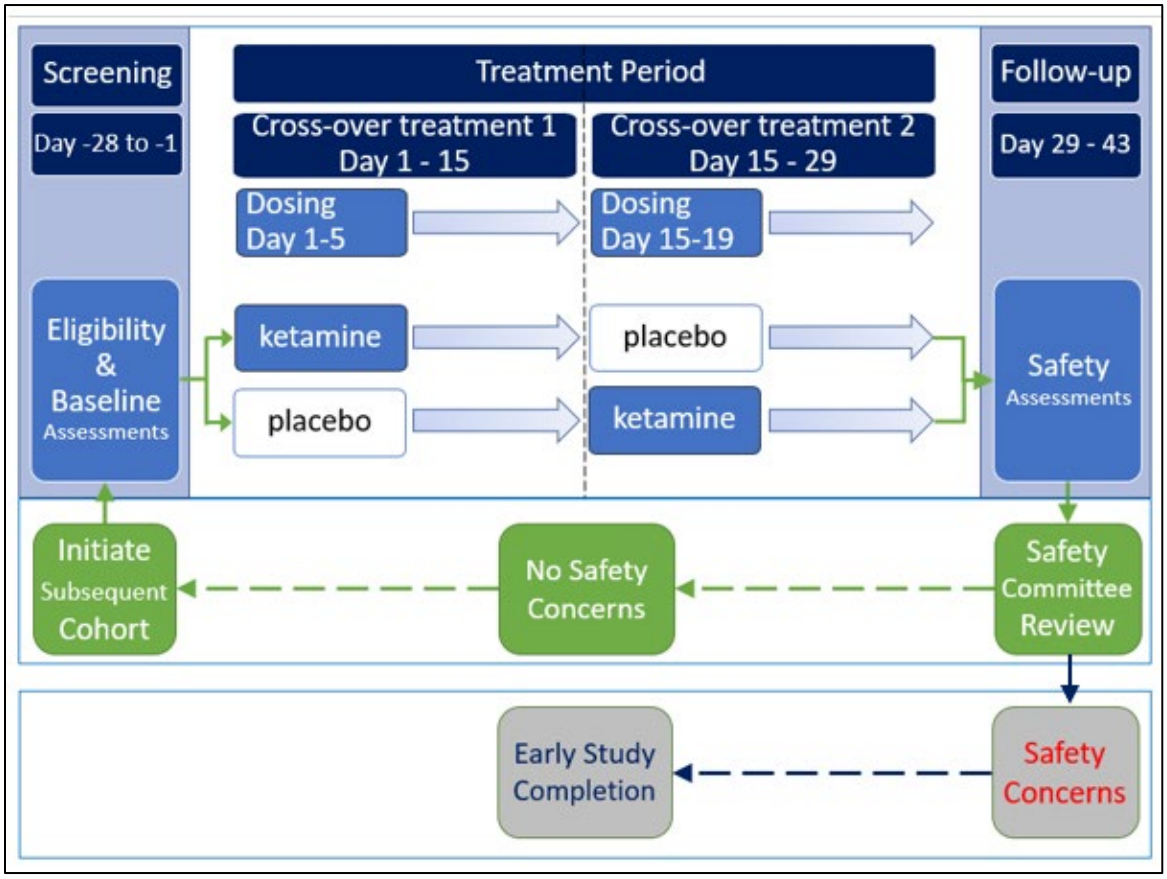
Patients will be female between the ages of 6 and 12, inclusive, who have not achieved menarche, and should not be treated concurrently with other NMDA receptor antagonists, or with other treatments with known ketamine interactions.

The study consists of up to 4 cohorts initiated sequentially in ascending dose order. Each cohort will assess 1 dose level of ketamine (0.75 mg/kg, 1.5 mg/kg BID, 3 mg/kg, or 4.5 mg/kg BID). At the conclusion of each cohort, an independent safety monitoring committee will determine if there are adequate safety data to support initiation of the subsequent cohort.

A cohort within the study is divided into 3 periods: Screening, Cross-over Treatment (comprising a regimented 2-treatment cross-over), and Post-Treatment Safety Follow-Up (Figure 2). The Screening Period will last between 14 and 28 days prior to the initiation of study drug. The 4-week Treatment Period is a double-blind, placebo-controlled period to define safety and explore efficacy for the study. The Post-Treatment Safety Follow-up Period is the final 2 weeks of each cohort to assess safety following treatment completion.

Each cohort is designed to assess 1 dose of ketamine compared to placebo. In the 2-treatment cross-over period patients will receive either placebo or ketamine in the first cross-over regimen and the alternate treatment in the second, based on their blinded randomization assignment. Patients may only participate in one cohort.

Figure 1: Study Design



5.1.1. Screening and Baseline

Caregivers will provide appropriately obtained informed consent, and where possible, patients will provide assent, prior to completing any screening procedures.

At the Screening visit, eligibility will be confirmed and patients will take home the biosensor systems for collection of daily in-home data prior to the Day 1 Randomization Visit. Eligible patients will return for the Randomization Visit on Day 1 for review of eligibility and additional baseline safety and efficacy assessments including an EEG at select centers, physical examination, vital signs, ECG, as well as physician CGI-S, MBA, and the Clinician Rated Domain Likert Scale consisting of 8 domains (hand function, walking, verbal and non-verbal communication, comprehension, attention, behavior problems, mood). Caregiver rated scales including the RSBQ, CSHQ, RTT CIA, and an exploratory Parent Rated Domain Likert Scale of the same 8 clinician domains with the addition of a seizures domain, will also be completed.

Due to the COVID-19 pandemic, the Screening visit may be conducted remotely via telehealth per institutional SOPs. In this case, safety assessments including vital signs may be collected off-site by qualified study personnel and ECG may be collected at Visit 2 to confirm eligibility prior to dosing.

5.1.2. Treatment Period and Safety Follow-Up Period

The double-blind, placebo-controlled, cross-over Treatment Period will last a total of 4 weeks, starting with the first treatment on Day 1 and initiation of the alternate treatment 2 weeks later on Day 15. Patients receiving ketamine in the first treatment will cross-over to receive placebo

in the second, and patients receiving placebo in the first treatment will cross-over to receive ketamine in the second.

Each ordered cross-over dosing regimen comprises 5 days of BID dosing (treatment 1: Day 1-5, treatment 2: Day 15-19) and patient safety and efficacy evaluation for 14 days after dose initiation (Period 1: Day 15, Period 2: Day 29).

After dosing completion and conclusion of the 4-week cross-over Treatment Period, patients will return on Day 29 for final safety and efficacy assessments. Due to the COVID-19 pandemic, Day 29 may be conducted remotely via telehealth per institutional SOPs. In this case, safety assessments including vital signs may be collected off-site by qualified study personnel.

Patients will be assessed for safety for an additional two weeks and a final Safety Follow-up phone call will occur on Day 43.

5.1.3. In-Clinic Assessments

At the clinic visits, safety and efficacy assessments will be performed by the clinician and the caregiver. Exploratory efficacy assessments including physician CGI-S or CGI-I, MBA, and Clinician Rated Domain Likert Scale should be performed prior to dosing to establish baseline and thereafter, to assess effects from the preceding treatment. Caregiver assessments including the Parent Rated Domain Likert Scale, RSBQ, RTT CIA, and CSHQ should also be performed.

Safety assessments including observation of patient disposition and vital signs should occur pre- and post-dose to assess tolerability of the in-clinic dose and inform continuation of the dosing regimen at home.

5.1.4. Dosing

Patients will receive the first oral dose of each 5-day dosing regimen in-clinic under observation. Tolerability of the first oral dose will be evaluated for two hours post dose in-clinic through monitoring of patient disposition and through assessment of vital signs at regular intervals. If well tolerated, subsequent doses of the 5-day dosing regimen will be administered in the home at approximately 12-hour intervals, with or without food. The site will contact the caregiver each day of active dosing to confirm continued tolerability and to assess for emergent side effects.

5.1.5. At-home Assessments

Over the following 2-week period after each of the observed in-clinic doses, caregivers will continuously capture biosensor data to assess patient activity, movement, heart rate, gait, sleep, posture, and breathing regularity. On Days 8 and 22, caregivers will report a Parent Domain Likert Scale and complete the CSHQ. The Parent Domain Likert Scale and the RTT CIA will also be completed on Day 43.

5.1.6. Safety Monitoring

Patient safety will be monitored throughout the study. Investigators will monitor safety and tolerability through observation and monitoring of in-clinic dosing of the first dose of each treatment, and through daily communication with the caregiver on at-home dosing days.

An independent safety monitoring committee will perform a safety assessment of each cohort with a subsequent ascending dose after the last patient has completed the final efficacy and safety assessment of the Treatment Period (Day 29). The safety committee will review accumulated safety data to assess if initiation of dosing for the subsequent cohort is supported.

In the case of treatment intolerability during the 5-day dosing period, the patient will stop treatment at the assigned blinded dose and discontinue the study, and any adverse events will be followed until an acceptable clinical resolution is achieved. Patients who discontinue study drug due to an adverse event (AE) during the Treatment Period will return for the Day 29/Early

Termination Visit two to four weeks after the last dose and receive a final Safety Follow-up phone call.

5.2. Number of Patients and Sites

Approximately 48 patients are planned to be enrolled (12 per cohort) in the study at approximately 7 sites.

5.3. Method of Treatment Assignment and Blinding

For each cohort, patients meeting all eligibility criteria will be randomized in a 1:1 ratio to receive placebo and ketamine, in either order. Randomization will not be stratified.

All study participants will be blinded to study drug assignment for the duration of the study. Packaging, administration, and flavor masking between placebo and ketamine will be identical. Pharmacists will remain unblinded and will keep confidential all treatment assignments.

5.4. Rationale for Study Design

Double-blind, randomized, placebo-controlled studies are considered optimal for obtaining unbiased estimates of the safety and efficacy of investigational treatments. The cross-over design allows for each patient to serve as her own control, which increases the ability to detect treatment differences. Analysis for carry-over effects is planned. The 4-week duration of the Treatment Period is anticipated to allow assessment of the safety and tolerability profile associated with a 5-day regimen of ketamine, and to explore potential efficacy.

6. SELECTION AND WITHDRAWAL OF PATIENTS

Patients eligible for enrollment in the study must meet all of the following inclusion criteria and none of the exclusion criteria.

6.1. Patient Inclusion Criteria

1. Prior to the conduct of any study-specific procedures, the patient must provide verbal assent to participate in the study (if developmentally appropriate), and the parent/caregiver/LAR must provide written informed consent. If the caregiver attending the clinic visits is not the parent or LAR, written consent must be obtained from the parent or LAR for the caregiver's participation in the study.
2. Female patients 6 to 12 years of age, inclusive, at the time of informed consent, who have not achieved menarche.
3. Diagnosis of typical or atypical Rett Syndrome according to the revised Clinical Diagnostic Criteria 2010[5] and presence of a disease-causing *MECP2* genetic mutation.
4. Ability to take liquid medications orally or through a feeding tube.
5. The patient's caregiver must be able to understand the nature of the study and to allow for the completion of all study assessments. The same caregiver must be capable of providing reliable information about the patient's condition, agree to oversee the administration of study drug, and accompany the patient to all clinic visits.

6.2. Patient Exclusion Criteria

1. Uncontrolled epilepsy, defined as caregiver report of unusual variability in seizure frequency that may warrant regimen change during the study to either untreated patients or patients on stable anti-seizure medication regimens.
2. Plans to initiate or change pharmacologic interventions during the course of the study or have not been on stable interventions for at least 4 weeks prior to Screening, or stable anti-seizure medication regimens (if applicable) for 12 weeks prior to the Randomization Visit.
3. Plans to initiate or change non-pharmacologic interventions (behavioral, educational or dietary) during the course of the study or have not been on stable interventions for at least 4 weeks prior to Screening. Typical school vacations are not considered modifications of stable interventions.
4. Patients who achieve menarche during study participation and prior to conclusion of the second dosing period of the cohort.
5. A history of prolonged QT syndrome or a Screening QTc interval with Fridericia's correction (QTcF) >460 for females less than 15 years or >470 for females over 15 years.
6. Have taken another investigational drug within the greater of 4 weeks of 5 half-lives prior to Screening or during the study.
7. Concurrent treatment with other NMDA receptor antagonists, treatments with known ketamine interactions, sedatives, barbiturates, benzodiazepines, narcotics, or opioids.

8. Concurrent treatment with strong CYP3A4 or CYP2B6 inhibitors dosed for systemic exposure, or for 4 weeks prior to initiating study drug.
9. Concurrent treatment with strong CYP3A4 or CYP2B6 inducers dosed for systemic exposure, or for 4 weeks prior to initiating study drug.
10. A history of hypersensitivity to ketamine.
11. A history of any condition that may be worsened by increased blood pressure or heart rate, bronchodilation, intraocular or intracranial pressure, or a history of porphyria.
12. Patients with any condition that, in the opinion of the principal investigator, might interfere with the conduct of the study, confound interpretation of the study results, endanger their own well-being, or who may otherwise not be suitable for the study.

6.3. Patient Withdrawal Criteria

All caregivers and patients are free to withdraw from participation in the study at any time, for any reason, and without prejudice. The Investigator may remove a patient from the study at any time and for any reason. Patients who withdraw or are withdrawn from the study will not be replaced.

6.3.1. Withdrawal Safety Criteria

At each in-clinic dosing, vital sign parameters will be assessed and patient disposition will also be monitored. Should intolerable adverse events emerge during the observation period, the patient should be withdrawn. Investigators will contact caregivers daily during dosing days to assess for adverse events and general patient tolerability.

Patients who appear to experience intolerable adverse events, emergent adverse events, or other adverse events considered to be related to study drug that may reflect an unfavorable risk-benefit profile should be withdrawn from the study at the discretion of the Investigator.

Patients who are withdrawn from the study should undergo an Early Termination Visit and subsequent Safety Follow-up Phone Call as appropriate per Section 8.3.2 Patient Withdrawal Procedures, and associated adverse events followed as described in Section 11 Adverse Events.

Caregivers should receive instructions to monitor the patient after at-home dose administration to assess for emergent adverse events or other signs of intolerability. Patients who discontinue the study due to intolerable adverse events should continue to be monitored as appropriate through an acceptable clinical resolution of the event.

6.3.2. Patient Withdrawal Procedures

A patient who prematurely discontinues study treatment/study participation should have all Day 29 assessments performed as an Early Termination Visit, as well as the Safety Follow-up Phone Call.

If a patient discontinues early from the study, the Investigator will record the reason(s) for early termination on the relevant electronic case report form (eCRF). The specific reason for the withdrawal should be documented on the eCRF.

Patients who experience an intolerable adverse event should be discontinued from the study. Adverse events resulting in patient early termination will be followed to the satisfactory

resolution and determination of outcome, as ascertained by the Investigator; See Section 11.1.2: Adverse Events. The data will be recorded on the appropriate eCRF.

6.4. Criteria for Study Termination

The study may be discontinued at any time for clinical or administrative reasons. If the Investigators or independent safety review committee determine that treatment with oral ketamine poses an unjustified risk to patients, the study may be terminated.

The Investigator will promptly notify the IRB of study termination or dose modification. Any study termination must be implemented by the Investigator in a time frame that is compatible with the patient's well-being.

7. DESCRIPTION OF STUDY TREATMENTS

7.1. Description of Treatments

For each cohort, patients will be randomized in a 1:1 ratio to 1 of 2 ordered cross-over treatment regimens as follows:

- Cohort 1
  - 0.75 mg/kg BID → placebo
  - placebo → 0.75 mg/kg BID
- Cohort 2
  - 1.5 mg/kg BID → placebo
  - placebo → 1.5 mg/kg BID
- Cohort 3
  - 3 mg/kg BID → placebo
  - placebo → 3 mg/kg BID
- Cohort 4
  - 4.5 mg/kg BID → placebo
  - placebo → 4.5 mg/kg BID

If the safety committee review of safety parameters for patients in each cohort indicates no safety concerns, the subsequent ascending dose cohort will be initiated.

7.2. Treatment Compliance

At each scheduled study visit after Randomization, the Investigator or designee will interview the caregiver regarding treatment compliance and compare the volume of dispensed versus returned study drug. Caregivers should strive for 100% compliance with the 5-day BID dosing schedule.

7.3. Study Drug Treatment Descriptions and Management

Please consult the full ketamine Prescribing Information and the description of study drug treatment requirements below for storage, handling, compounding, dispensing, accountability, returns and destruction.

7.3.1. Physical Description of Treatments

Treatments will include ketamine and matching placebo. Details regarding formulation and dosage are presented in Table 3.

Table 3: Treatments

Product Name:	Ketamine or placebo (sterile water)
Dosage Form:	Solution
Intended Manufacturer **Dosage Strength:**	50 mg/mL or 100 mg/mL ketamine concentrate
Route of Administration:	Oral
Physical Description:	Transparent or opaque liquid solution

7.3.2. Treatment Supplies, Labeling, and Storage

Each patient will receive a sufficient supply of ketamine solution or matching placebo solution based on patient weight in kilograms to complete the 5-day BID dosing regimen at the Day 1 Randomization Visit (cross-over treatment 1) and at the Day 15 Visit (cross-over treatment 2).

The pharmacy label for the ketamine or placebo treatments will minimally include Investigator name, address and telephone number, study treatment as “ketamine or placebo”, administration instructions (mLs per dose, frequency, and duration), and storage conditions. Additional information may be included on the label per Institution requirements.

Ketamine vials and stock solutions should be stored protected from light in the original package as stated on the ketamine label. Storage should occur in a secure, temperature-monitored, locked area, under the responsibility of the Investigator or other authorized individual until compounded and dispensed to the patient. Returned study drug should also be securely stored until full reconciliation is complete.

Special Note: Ketamine is considered a drug of abuse and extra care should be taken to monitor supplies and limit access.

Study drug bottles dispensed to patients should be stored at home in the original containers provided by the pharmacy protected from light according to the directions on the pharmacy label.

7.3.3. Study Drug Preparation

Treatment compounding by the pharmacist is required and may be performed according to the Institution’s standard operating procedures. Compounding materials include Ketamine stock concentrates, sterile water, and masking flavor. Treatment preparations should be based on the weight of the patient in kilograms on the day of the Visit. Placebo solutions should consist of sterile water and masking flavor and be indistinguishable from ketamine solutions. The same volume of placebo or ketamine solution should be prepared and administered when appropriate to maintain the blind.

Refer to the pharmacy manual for general guidance on compounding.

7.3.4. Study Drug Administration

Patients will take the first dose of each ketamine or placebo treatment in-clinic on Day 1 and Day 15 and caregivers will be instructed to administer subsequent doses approximately every 12 hours for the duration of the remaining 5-day dosing regimen.

7.3.5. Study Drug Return and Disposal

The Investigator and relevant site personnel will follow the Institutional standard operating procedure for study treatment disposal, and/or destruction.

7.3.6. Study Drug Accountability

To satisfy regulatory requirements regarding drug accountability, treatments will be reconciled in full. The unblinded pharmacist must maintain accurate records of the receipt of ketamine stock solutions, including the date and amount received, and disposition. Current dispensing records will also be maintained, including the date and amount of ketamine used to create patient solutions and the amount dispensed to each individual patient. Returned study drug records will be maintained and final study drug reconciliation will be recorded for each patient.

7.4. Concomitant Medications and Procedures

All medications, including over-the-counter therapies (e.g., vitamins, herbal, and nutritional supplements), taken at the time of the Screening Visit through the Follow-Up Visit will be recorded in the patient's source documentation and documented in the eCRF.

The following treatments are not permitted during the study:

- Use of other NMDA receptor antagonists, treatments with known ketamine interactions, sedatives, barbiturates, benzodiazepines, narcotics, or opioids;
- Use of strong CYP3A4 or CYP2B6 inhibitors dosed for systemic exposure, or for 4 weeks prior to initiating study drug;
- Use of strong CYP3A4 or CYP2B6 inducers dosed for systemic exposure, or for 4 weeks prior to initiating study drug;

8. STUDY ASSESSMENTS AND PROCEDURES

8.1. Demographic Characteristics and Medical History

Demographic characteristics will be collected at the Screening Visit including documentation of a causative *MECP2* genetic mutation, thorough medical history, and the patient's typical Rett Syndrome diagnosis according to Revised Diagnostic Criteria[5].

8.2. Actigraph

The ActiGraph wGT3X-BT is an FDA cleared Class II medical device intended to capture and record high resolution raw acceleration data that can be converted into activity and sleep measures.

In this study, patients will wear the ActiGraph xGT3X-BT on the wrist for continuous data collection during the Screening and Treatment Periods. Collected data will be uploaded at the Visit. Raw datasets collected will be explored for Rett syndrome-specific signatures in addition to pre-defined algorithms.

8.3. Hexoskin Smart Garment

The Hexoskin Smart Kit developed by Carre Technologies, Inc (Hexoskin), uses non-invasive biometric sensors embedded within machine-washable, breathable, light-weight fabric clothing to continuously track activity, sleep, heart and lung function. Specifically, hexoskin monitors ECG and heartbeat, heart rate variability, breathing rate and volume (L/min), activity intensity, acceleration, cadence, energy expenditure (kCal), position, and sleep.

In this study, patients will wear the Hexoskin Smart Garment for continuous data collection during the Screening and Treatment Periods. Since hexoskin Smart Garments are not indicated for children under the age of 13 or for patients with neurodevelopmental disorders, the raw datasets collected will be explored for Rett syndrome-specific signatures in addition to pre-defined algorithms.

8.4. Clinical Global Impression Scales

The Global Impression Scales are measures commonly used in clinical trials to allow integration of several sources of information into a single rating of the patient's condition[43]. The Clinical Global Impression Scales employ a 7-point Likert scale measuring either disease state severity or improvement after treatment.

In this study, the Clinical Global Impression Scale of Severity and Improvement will be performed by the Investigator. Assessment of Rett syndrome severity should consider the condition of the patient at the time of the assessment, while assessment of Rett syndrome improvement should consider the condition of the patient at the time of the assessment compared to Baseline. To reduce variability, one rater should perform the global impression scales for the patient for the duration of the study.

8.5. Rett Syndrome Behaviour Questionnaire

The Rett Syndrome Behaviour Questionnaire (RSBQ) is a 45-item measure to evaluate the behavioral and emotional features of Rett syndrome and to discriminate between patients with Rett Syndrome and idiopathic severe to profound intellectual disability[44].

8.6. Motor Behavioral Assessment

The Rett Syndrome Motor Behavioral Assessment (MBA) is a 40-item scale used to assess 3 subgroups in Rett syndrome patients: behavioral/social, orofacial/respiratory, and motor assessment/physical signs, in their current state and the worst ever state[45].

8.7. Parent and Clinician Rated Domain Likert Scales

In addition to the Global Impression Scales that assess the patient overall, an evaluation of individual symptom domains will also be explored. To assess potential changes in specific symptoms of a patient's condition, the Investigator will complete a 7-point Likert Scale for each of the following 8 domains: hand function, walking, verbal and non-verbal communication, comprehension, attention, behavior problems, mood. The caregiver will complete an exploratory Parent Rated Domain Likert Scale of the same 8 clinician domains (hand function, walking, verbal and non-verbal communication, comprehension, attention, behavior problems, mood) with the addition of a seizures domain.

8.8. Children's Sleep Habits Questionnaire

The Children's Sleep Habit's Questionnaire (CSHQ) was designed for children aged 4 through 12 years, to screen for the most common sleep problems in that age group, though it will be collected for all patients in this study[46]. The 35-item questionnaire will be completed by the caregiver.

8.9. Rett Caregiver Burden Inventory Assessment

The Rett Caregiver Burden Inventory Assessment (Rett CIA) was adapted from the Caregiver Burden Inventory created for Alzheimer disease to assess caregiver burden specifically for individuals with Rett Syndrome[47]. The RTT CIA consists of 26 questions across 4 domains (time dependency, physical burden, emotional burden, social burden) appropriate for the Rett Syndrome patient population and utilizes a 5-point Likert scale for answering each item: (1) I never feel this way, (2) I rarely feel this way, (3) I sometimes feel this way, (4) I quite frequently feel this way, (5) I nearly always feel this way.

8.10. Physical Examination

A physical examination will include an examination of all major organ systems. Caregivers should be instructed to notify the clinic of any signs of changes in the patient's physical or neurological state between visits for appropriate monitoring and follow-up.

8.11. Vital Signs, Weight, and Height

At each Visit, vital signs will be measured after the patient has been in a supine or semi-supine position for at least 5 minutes and will include body temperature, blood pressure, pulse rate, respiratory rate, and oxygen saturation.

Weight and height will be measured per Institution standard of care.

8.12. Electrocardiogram

A twelve-lead electrocardiogram will be performed at the Screening Visit or at Visit 2 to confirm eligibility prior to dosing after the patient has rested in a supine or semi-supine position for at least 5 minutes.

8.13. Electroencephalogram

For select sites performing an electroencephalogram (EEG), an electronic EEG recording will be collected for evaluation of NMDA receptor target engagement and subsequent changes in EEG signature expected with ketamine treatment.

9. ADVERSE EVENTS

9.1.1. Definition of Adverse Event

An adverse event is any untoward medical occurrence that may appear or worsen in a patient during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the patient's health, including laboratory test values, regardless of etiology. Any worsening (ie, any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an adverse event. A diagnosis or syndrome should be recorded on the adverse event page of the eCRF rather than the individual signs or symptoms of the diagnosis or syndrome.

9.1.2. Evaluation of Adverse Events

A qualified Investigator will evaluate all adverse events as to seriousness, severity/intensity, relationship to study drug, duration, action taken, and outcome.

9.1.2.1. Serious Adverse Event

A serious adverse event is an adverse event, as per Title 21 CFR 312.32 and ICH E2A.II.B that fulfills the following criteria:

- Is fatal (results in death);
- Is life-threatening (Note: the term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that could hypothetically have caused death had it been more severe);
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay);
- Results in persistent or significant disability/incapacity (a substantial disruption of the patient's ability to conduct normal life functions);
- Is a congenital anomaly/birth defect; or
- Constitutes an important medical event that may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed above.

Important medical events are defined as those occurrences that may not be immediately life-threatening or result in death, hospitalization, or disability, but may jeopardize the patient or require medical or surgical intervention to prevent one of the other outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization.

Events **not considered** to be serious adverse events are hospitalizations for:

- A procedure for protocol/disease-related investigations (e.g., sampling for laboratory, pharmacokinetic, and pharmacodynamic tests). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.

- Hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- A procedure that is planned (i.e., planned prior to the starting of treatment on study); must be documented in the source document and the eCRF. Hospitalization or prolonged hospitalization for a complication remains a reportable serious adverse event.
- An elective treatment of or an elective procedure for a pre-existing medical condition that does not worsen.
- Emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an adverse event is considered serious, the adverse event eCRF must be completed.

For each serious adverse event, the Investigator will provide information on severity, start and stop dates, relationship to investigational product, action taken regarding investigational product, and outcome.

9.1.2.2. Severity/Intensity

For both adverse events and serious adverse events, the Investigator must assess the severity/intensity of the event.

The National Cancer Institute-Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03 should be used to grade the severity/intensity of all events. If a CTCAE criterion does not exist, the Investigator should grade the severity according to the following criteria:

- Grade 1 (mild): does not interfere with the patient's usual function
- Grade 2 (moderate): interferes to some extent with patient's usual function
- Grade 3 (severe): interferes significantly with patient's usual function
- Grade 4 (life-threatening): results in a threat to life or in an incapacitating disability
- Grade 5 (death): results in death

9.1.2.3. Relationship to Study Drug

Relationship should be assessed and provided for every adverse event/serious adverse event based on currently available information. Relationship is to be reassessed and provided as additional information becomes available. Adverse events will be classified by the Investigator as definitely related, probably related, possibly related, unlikely related, or not related.

9.1.2.4. Duration

For all adverse events whether or not considered serious, the Investigator will provide a record of the start and stop dates of the event. If an event is unresolved at the end of the study it will be recorded as ongoing.

9.1.2.5. Action Taken

The Investigator will record the action taken with investigational product as a result of an adverse event or serious adverse event on the eCRF, as applicable, and record if concomitant and/or additional treatments were given for the event.

9.1.2.6. Outcome

The Investigator will record the outcome of adverse events on the eCRF, as applicable.

9.1.3. Follow-Up

Adverse events assessed as not related to study drug, including physical examination findings, must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the final study visit occurs, whichever comes first.

Adverse events assessed as related to study drug and serious adverse events will be followed for as long as necessary to adequately evaluate the patient's safety, or until the event stabilizes, is otherwise explained, death occurs, or the patient is lost to follow up.

9.1.4. Recording Adverse Events

All adverse events (regardless of seriousness or relationship to study drug) including those from the time informed consent is obtained through to the final study visit are to be recorded in the eCRF. Each individual adverse event is to be listed as a separate entry. The Investigator will provide information about dates of onset and resolution, seriousness, severity, action(s) taken, outcome, and relationship to the study drug. All adverse events should be documented in the patient's source documents.

9.1.5. Reporting Adverse Events

The Investigator must report all adverse events that occur during the study from the time written informed consent is given until the final study visit/phone call or early termination, regardless of their relationship to the study drug.

9.1.5.1. Reporting Serious Adverse Events

Serious adverse events will be reported from the time written informed consent is given through 30 days beyond the last dose of study drug. The Sponsor-Investigator will be responsible for reporting serious adverse events to the FDA, as required.

9.1.5.2. Reporting Urgent Safety Issues

The Sponsor-Investigator should be notified if the Investigator becomes aware of an actual or potential urgent safety issue.

Urgent safety issues should be treated as medically appropriate. An urgent safety issue may include: (1) issues with an investigational drug or comparators; (2) study procedures; (3) inter-current illness (including pandemic infections); (4) concomitant medications; (5) concurrent medical conditions; or (6) any other issues related to the safe conduct of the study or that pose a risk to study patients.

10. STATISTICAL METHODS

For statistical methods, treatment refers to placebo, ketamine 0.75 mg/kg, ketamine 1.5 mg/kg, ketamine 3.0 mg/kg, and ketamine 4.5 mg/kg.

10.1. Sample Size Rationale

Sample size determinations for this study are based on the primary outcome of safety and tolerability. For a dose-limiting adverse event with a 10% incidence rate, a sample size of 10 patients/dose level (10 patients/cohort) provides a 65% probability of observing at least 1 event at each dose level, and an 88% probability of observing at least 1 event by completion of Cohort 2.

For measures of efficacy, there are no studies in the literature to provide reliable estimates of treatment effects of ketamine or the estimate of variance in symptom management for Rett Syndrome patients. Each dose level provides 80% power to detect a 1.00 standard deviation treatment difference with the 2-sided paired t-test with 0.05 Type I error. Assuming a discontinuation rate between 15 and 20%, approximately 12 patients will be enrolled in each cohort to provide 10 patients who complete the treatment period.

10.2. Endpoints

10.2.1. Safety

Safety and tolerability of oral ketamine will be assessed by evaluating adverse events, vital signs, concomitant medications and physical examinations.

10.2.2. Efficacy

Efficacy is exploratory in this study. Endpoints will include the CGI-I score and published total and/or domain scores for the Clinical Domain Report, Parent Domain Report, RSBQ, MBA, RTT CIA, and CSHQ. Biosensor device algorithms and raw datasets will be explored.

Additional exploratory statistical methods to further assess for treatment effect will be described in the clinical study report and/or resulting publications, as deemed appropriate.

10.3. Analysis Populations

10.3.1. Treatment Period

The Safety Population will include all patients who receive study drug during the Treatment Period.

The Efficacy Population will include all patients who receive both assigned study drugs and have at least 1 post-treatment efficacy assessment for both assigned study drugs.

10.4. Analyses

Statistical analyses will be summarized in a statistical analysis plan prior to database lock. All data captured in the electronic data capture system (EDC) will be listed by patient.

Continuous measures will be summarized descriptively (mean, standard deviation, median, minimum value, and maximum value) and categorical measures will be presented as number and percentage.

10.4.1. Disposition and Baseline Characteristics

Disposition will be summarized by cohort and randomized sequence. The number and percentage of patients, who are randomized, treated, prematurely discontinued, and complete the study will be summarized.

Baseline characteristics will be summarized by cohort and randomized sequence.

The number of patients in each cohort will be summarized for each investigative site for the Treatment Period. Medical history will be coded with the Medical Dictionary for Regulatory Activities (MedDRA) terminology. Concomitant medications will be summarized by World Health Organization Drug Dictionary Anatomical-Therapeutic-Chemical classification (ATCC) and preferred term.

10.4.2. Efficacy

Unless otherwise indicated, treatment differences will be assessed with an analysis of variance model that includes sequence (placebo-ketamine, ketamine-placebo), treatment, and treatment by sequence interaction as fixed effects and patient nested within sequence as a random effect. Sequence will be tested using patient nested within sequence as the error term.

For daily biosensor data, change from the average value during Screening to the average value during treatment (Days 1 to 5 during first treatment and Days 15 to 19 during the second treatment) will be assessed for treatment differences. Additionally, change from the average value during Screening to the average value during the full treatment period (Days 1 to 14 during first treatment and Days 15 to 28 during the second treatment) will be assessed for treatment differences.

Change from pre-dose baseline (Day 1) to post-dose (Day 15 for first treatment and Day 29 for second treatment) will be assessed for treatment differences for total and domain scores of the Clinical Domain Report, Parent Domain Report, RSBQ, MBA, RTT CIA, and CSHQ. Additionally, change from pre-dose baseline (Day 1) to post-dose (Day 8 for first treatment and Day 22 for second treatment) will be assessed for treatment differences for total and domain scores of the Parent Domain Report and CSHQ, as well as Day 43 for the Parent Domain Report and the RTT CIA. As a secondary analysis, pre-dose baseline will be defined as day 1 of each treatment (Day 1 for first treatment and Day 15 for second treatment).

For the CGI-I, scores on Day 15 and Day 29 will be assessed for treatment differences with the analysis of variance. Additionally, each patient will be categorized as improved (at least minimal improvement per the CGI-I) or not improved. The McNemar test will be used to assess treatment differences in the proportion of patients who improve.

EEG analysis will be described separately.

10.4.3. Study Drug Exposure

Study drug exposure will be summarized for each treatment. The number of days on which study drug was dosed will be summarized.

10.4.4. Safety

All safety summaries will be descriptive with no statistical hypothesis testing and based on the Safety Population. Patients will be summarized according to the study drug received. All safety endpoints will be listed in by-patient data listings.

Adverse events will be coded using MedDRA and summarized by system organ class and preferred term. Clinically significant deteriorations in physical examination findings will be reported and summarized as adverse events.

Adverse Events

An adverse event reported after informed consent, but before the first dose of study drug (i.e., Day 1), will be considered a pre-treatment adverse event. Treatment-emergent adverse events (TEAEs) will be defined as any adverse event that occurs after administration of the first dose of study drug. The number and percentage of patients who report TEAEs will be summarized by treatment, system organ class, and preferred term.

Treatment emergent adverse events will also be summarized by intensity as well as relationship to study drug.

Patients who report the same preferred term on multiple occasions within a treatment will be counted once for the preferred term within the treatment: under the highest severity when summarized by severity and under the closest relationship to study drug when summarized by relationship. If a patient reports multiple preferred terms for a system organ class within a treatment, the patient will be counted only once for that system organ class within the treatment.

The number and percentage of patients who experience TEAEs will be summarized by treatment for the following:

- By system organ class and preferred term
- Severe TEAEs by system organ class, and preferred term
- Drug-related TEAEs by system organ class, and preferred term
- Serious adverse events by system organ class and preferred term
- Serious adverse events related to study drug, system organ class, and preferred term
- Adverse events resulting in discontinuation of study drug by system organ class and preferred term

By-patient listings will be provided for any deaths, serious adverse events, and adverse events leading to discontinuation of treatment.

Vital Signs

The mean change from baseline to each scheduled assessment will be summarized descriptively by treatment group for each vital sign variable specified in this protocol.

Baseline will be defined as the last vital sign value obtained before the first dose of study drug (Day 1 for first treatment and Day 15 for second treatment).

11. REGULATORY CONSIDERATIONS

11.1. Good Clinical Practice

The conduct, evaluation, and documentation of this study are designed to ensure that the study is conducted according to GCP, as described in ICH Guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki.

11.2. Protocol Amendments

Any permanent change to the protocol, whether it is an overall change or a change for specific study center(s), must be handled as a protocol amendment. The Investigator will not make any changes to this protocol without prior written consent from the Sponsor-Investigator and subsequent approval by the IRB.

11.3. Audits and Inspections

Regulatory authorities may audit the Investigator during or after the study.

12. DATA HANDLING AND RECORDKEEPING

12.1. Confidentiality

Submission of this protocol and any other necessary documentation to the IRB is expressly permitted. Authorized regulatory officials will be allowed full access to inspect and copy the records. The copied and inspected records will remain at the site and will not be transmitted or removed from the site.

12.2. Patient Data Protection

Prior to any testing under this protocol, including screening tests and assessments, candidates must also provide all authorizations required by local law (e.g., protected health information authorization).

The Investigator will protect individual patient information to the fullest extent possible during this study. Patients will be identified only by unique subject numbers in eCRFs and other datasets generated for this study.

12.3. Data Collection

Data obtained for analysis in this protocol will use an electronic data capture system for use by individuals who authorized to make or change entries. Data from the biosensor systems will be uploaded to the appropriate database and housed separately, according to the company's data management practices and standard operating procedures.

12.4. Case Report Form Completion

Data within the eCRF will be monitored according to the study-specific Monitoring Plan. The completed eCRF for each patient must be signed and dated by the Investigator to signify that the eCRF has been certified to be complete and accurate.

12.5. Database Management, Data Clarification, and Quality Assurance

A designated Contract Research Organization (CRO) will be responsible for data management. Quality control procedures will be conducted prior to database lock according to the CRO's standard operating procedures. When the database has been declared to be complete and accurate, it will be locked.

12.6. Inspection of Records

All data required for completion of the eCRF for this study should be captured in source notes or electronic records. All source documents should be correctly labeled, filed, and associated with a single, verifiable patient, maintained by the Investigator, and made available for inspection by authorized persons.

12.7. Retention of Records

For investigational studies, Investigators must retain study records for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it

is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and FDA is notified.

13. PUBLICATION POLICY

The results of this study may be published in a medical publication, journal, or another public dissemination, or may be presented at a medical conference or used for teaching purposes. This study and its results may be submitted for inclusion in health authority study registry websites as required by local health authority regulations.

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