A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, SIX-MONTH STUDY TO EVALUATE THE EFFICACY, SAFETY AND TOLERABILITY OF SARIZOTAN IN PATIENTS WITH RETT SYNDROME WITH RESPIRATORY SYMPTOMS

SARIZOTAN

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1 SYNOPSIS

Title of Study: A Randomized, Double-Blind, Placebo-Controlled, Six-Month Study to Evaluate the Efficacy, Safety and Tolerability of Sarizotan in Patients with Rett Syndrome with Respiratory Symptoms

Protocol No.: Sarizotan/001/II/2015

Phase of Development: II/III

Objectives:
The objectives of the study are as follows:
Primary Efficacy Objective:

- To evaluate the effect of sarizotan (high dose), compared to placebo, on reducing the number of apnea episodes, during awake time, in patients with RTT with respiratory abnormalities.

Key Secondary Efficacy Objective:

- To evaluate the effect of sarizotan (high dose), compared to placebo, on the Caregiver-rated Impression of Change (CIC) from baseline;

Secondary Objectives:

- Safety: To evaluate the safety and tolerability of sarizotan in patients with Rett syndrome (RTT) with respiratory abnormalities.
- Efficacy: To evaluate the efficacy of sarizotan, compared to placebo, on the following:
    - Severity of patient symptoms, based on evaluation of Caregiver Top 3 Concerns (Visual Analogue Scale [VAS]);
    - Global change from baseline, assessed by the Clinical Global Impression of Change (CGI-C);
    - Motor behavior, assessed by the Motor-Behavioral Assessment Scale;
    - Other respiratory symptoms of Rett syndrome, assessed during awake time, including:
        - Percent time spent with breathing dysrhythmia (% time apnea + % time hyperventilation) per hour;
        - Number of hyperventilation episodes (≥10 seconds each) per hour;
        - Oxygen saturation (number of episodes of oxygen desaturation below 90% per hour).
        - *Respiratory Distress Index* - The sum of the following parameters calculated per hour of wakefulness: 1) number of breath-holding episodes, 2) number of episodes of hyperventilation [1 and 2 are as defined in inclusion criteria; each would have to be ≥10 seconds in duration], and 3) number of drops in oxygen values to < 90%.
    - Overall assessment of symptoms of RTT using the Rett syndrome Clinical Severity Scale (RCSS).
    - To determine the efficacy of sarizotan in patients who attain a minimum plasma exposure of 400 ng/ml on Day 15 (expected efficacious concentration based on the knockout mouse model);
- To determine the pharmacokinetic (PK) profile of sarizotan at the doses tested and compare with the PK profile in adults.

Study Design and Methods:
Initial 24-Week Double-blind Treatment Period
This is a prospective, 24-week, randomized, double-blind, placebo-controlled study designed to evaluate the safety, tolerability, and efficacy of multiple oral doses of sarizotan in patients with RTT with respiratory abnormalities. At least 129 patients will be randomized (approximately 43/group) to receive either low dose sarizotan (2 mg or 5 mg *bid*), high dose sarizotan (5 mg *bid* or 10 mg *bid*) or placebo *bid*. Patients 4 to <13 years of age will be randomized

equally (1:1:1) to sarizotan 2 mg *bid* (low dose group), sarizotan 5 mg *bid* (high dose group), or placebo. Patients ≥13 years of age and weighing ≥25 kg will be randomized equally (1:1:1) to sarizotan 5 mg *bid* (low dose group), sarizotan 10 mg *bid* (high dose group), or placebo *[Note: patients ≥13 years of age and weighing <25 kg will be eligible for the study, but will be included with the younger patients and randomized equally (1:1:1) to sarizotan 2 mg bid (low dose), sarizotan 5 mg bid (high dose) or placebo]*.

All patients 4 to <13 years of age, as well as those ≥13 years of age and weighing <25 kg, randomized to sarizotan 2 or 5 mg *bid* will start at a single dose of 2 mg on the morning of Day 1. Patients assigned to the 2 mg *bid* dose will remain on 2 mg once daily on Days 2-7, and will have their dose increased to 2 mg *bid* on Days 8-14. Patients assigned to the 5 mg *bid* dose will have their daily dose of sarizotan increased to 4 mg (2 mg *bid*) on Days 2-7, 7 mg (5 mg AM, 2 mg PM) on Day 8, and 10 mg (5 mg *bid*) on Days 9-14. Patients ≥13 years of age and weighing ≥25 kg randomized to sarizotan 5 or 10 mg *bid* will start at a single dose of 5 mg on the morning of Day 1, with an increase to 5 mg *bid* on Day 2. Patients randomized to 5 mg *bid* will continue on this dose for the rest of the study, while patients randomized to 10 mg *bid* will receive a single dose of 10 mg on Day 8 and have their dose increased to 10 mg *bid* on Day 9, and continue on this dose. If patients do not tolerate the proposed titration and target dose, the investigator will provide only one of the two daily doses (e.g. 5 mg/day) for a few days prior to reinitiating the intended *bid* dose (e.g. 5 mg *bid*). If the dose is still not tolerated, a reduction to a lower rescue dose will be permitted. Dose titration should be completed by Day 14, after which patients will continue on their maximum tolerated dose (MTD) through the final dose, unless intolerance develops and a dose reduction is warranted.

Each patient's parent, legal guardian, or representative, must provide written informed consent prior to the patient's participation in the trial. The patient should provide assent, if capable of doing so. Each patient will enter a 4-week period during which the screening evaluations will be performed. Patients who meet the respiratory criteria for enrolment in the study in one of the first 3 weeks may have their screening period shortened. The screening assessments, which must be completed and results available prior to baseline, will consist of the following: a confirmation of the diagnosis of Rett syndrome, based on clinical criteria (a proven mutation of the methyl CpG-binding protein 2 (MECP2) gene is not required), a review of the patient's medical history and current use of medications, vital signs, 12-lead electrocardiogram (ECG), laboratory tests (hematology, blood chemistry, urinalysis, virology, serum pregnancy test for post-pubertal females, and thyroid function tests), physical and neurological examination. The patient's respiratory abnormalities will be documented, based on parental feedback for the presence of one of the following: periodic apnea during wakefulness; intermittent hyperventilation; breath holding spells; air swallowing; forced expulsion of air or saliva.

Cardio-respiratory monitoring using a specialized ambulatory data acquisition system, the BioRadio™ system, will be performed at home during the first week of the screening period to ensure proficiency of the caregiver/patient in using the system, and to evaluate the quality of the data and resolve any problems the caregiver may be experiencing in using the device (this will be determined by the remote data monitoring service provider, Vivonoetics). Data from the BioRadio system will be used to confirm that the patient has at least 10 episodes of breathing dysrhythmia per hour over a 24-hour period. Home monitoring should be attempted for 6 hours each day, during the time the patient is awake, on at least 3 days during the first, second and third week of screening. The patient must meet all criteria related to breathing abnormalities in at least one of the three screening period assessments, as specified in the Inclusion Criteria, to be eligible for enrollment. If the patient meets the criteria, as determined by Vivonoetics, in any week of the Screening period, home monitoring in the subsequent week(s) of Screening will not be required. Adverse events and use of concomitant medication will be recorded from the time of signing of the consent form.

At the Baseline visit (Day 0), the inclusion/exclusion criteria (respiratory inclusion criteria will be assessed based on home respiratory monitoring performed during screening) will be reviewed to ensure that the patient is suitable for enrolment. Patients meeting all entry criteria at baseline will undergo a series of evaluations on the day prior to dosing (Day 0). Baseline assessments of respiratory function will be performed in the clinic using a stretch-sensitive resistance plethysmograph (SOMNOtouch™ device), Tanner Staging will be performed, and a blood sample will be taken for measurement of serum prolactin, and plasma ACTH and cortisol. The routine laboratory tests and physical and neurological examinations do not need to be repeated if performed less than 28 days before baseline, unless abnormalities are noted that require follow-up; however, a serum pregnancy test must be performed for post-pubertal females. An ophthalmologist would be required to perform a routine ophthalmological examination at Baseline,

including Optical Coherence Tomography (OCT; only if feasible, for the patient). In addition, baseline assessments for all of the efficacy variables [respiratory measures, heart rate, Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and Rett syndrome Clinical Severity Scale (RCSS)], as well as the suicidality assessment, will be performed on Day 0. Baseline vital signs and ECG evaluations will be performed in triplicate (10 min apart) on Day 0 or at least one hour prior to dosing on Day 1.

On Day 1 the patient will be randomized to treatment, with the dose assigned based on age, and for patients ≥13 years of age, body weight categories, as described above, and will receive the initial oral dose of study medication (Dose Level 1: 2 or 5 mg sarizotan or matching placebo) in the morning. In the initial 12-hour period after the first dose, safety evaluations (vital signs, 12-lead ECG) will be performed at 1 and 4 hr post-dose. Blood samples for the pharmacokinetic (PK) analysis will be collected prior to dosing (blank sample) and at approximately 1 and 4 hr post-dose, following completion of the vital signs and ECG assessments. The patient will receive the evening dose (Dose Level 1: placebo for all patients) in the hospital (minimally 6 hours after the morning dose) and will be housed overnight, either in the hospital or with their parent/caregiver in a separate facility. The morning dose of Dose Level 2 (2 or 5 mg sarizotan or placebo) will be administered on Day 2 in the clinic. Safety evaluations [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis, and measurement of plasma ACTH and cortisol), physical/neurological examination, assessment of adverse events] will be repeated, and if no significant side effects are noted within the 4-hr period following this dose, the patient will be released from the hospital and given a supply of study medication for the remainder of the first week of dosing at Dose Level 2 (Days 2 [PM] to 7) and for the second week of dosing at Dose Level 3 [2 mg sarizotan *bid,* 5 mg sarizotan *bid*, 7 mg sarizotan/day (5 mg in AM + 2 mg in PM), 10 mg sarizotan once daily (in AM, with placebo in PM) or placebo *bid*; on Day 8] and Dose Level 4 [2 mg sarizotan *bid*, 5 mg sarizotan *bid*, 10 mg sarizotan *bid*, or placebo *bid*; on Days 9 to 14].

The caregiver will be contacted by telephone by the Investigator/site staff on Day 7 to inquire about the tolerability of Dose Level 2 of the study medication and any adverse events the patient might have experienced. If there are no tolerability issues, the patient/caregiver will be instructed to increase the dose to Dose Level 3 on Day 8 and Dose Level 4 on Day 9. During the time the patient is at home, monitoring of their cardio-respiratory function will be attempted for 6 hours each day, during the time the patient is awake, on any 3 days in the week preceding the next scheduled office visit on Day 14, using the BioRadio. Data recorded using this system will be downloaded from the device at home using the Internet/WiFi or a dedicated laptop provided by the Sponsor, if Internet access is not available.

The patient will be required to return for a scheduled visit on Day 14 and safety [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis), physical/neurological examination] and selected efficacy (respiratory measures, CGI-C, CIC) evaluations, will be performed. The tolerability of the current dose of study medication will be evaluated, and the patient will be housed overnight, if necessary. On Day 15, if there are no safety or tolerability issues that would require a dose reduction, the patient will be given the morning dose of Dose Level 4 (2 mg sarizotan, 5 mg sarizotan, 10 mg sarizotan or placebo, *bid*). Prior to administering the dose, a PK blood sample for assessing steady state trough levels of sarizotan at Dose Level 4 will be taken, and PK blood samples will be collected at 1 and 4 hr post-dose following the dose. Post-dose safety assessments (vital signs, ECG) will be performed, and if there are no tolerability issues, the patient will be released from the hospital after the last PK sample, and will be provided with a supply of Dose Level 4 (or a lower dose if Dose Level 4 was not tolerated) for dosing on Days 15 [PM] to 56 (Week 8). Home respiratory monitoring will be attempted for 6 hours each day on any 3 days in the week prior to each of the scheduled office visits at Weeks 8, 16 and 24.

The patient will be required to return for scheduled visits on Weeks 8 and 16 and safety (vital signs, routine laboratory tests, ECG, physical and neurological examinations) and efficacy evaluations will be performed. The suicidality assessment will be performed at Week 8. In addition, serum prolactin, and plasma ACTH and cortisol measurements, and an ophthalmological examination will be done at Week 16. At each of these visits, the patient will be dispensed an 8-week supply of their current dose level of study medication for dosing during the period until the next scheduled visit, provided there are no safety or tolerability issues that would require a dose reduction.

The patient will return for the final scheduled visit at Week 24, or if they discontinue from the study prematurely. The caregiver should be instructed not to administer the morning dose of the study medication on the day of the Week

24 visit to the clinic, as patients who continue in the open-label extension treatment period will receive their first dose of study medication on this day. All final safety [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis, serum pregnancy test for post-pubertal females, and measurement of serum prolactin, and plasma ACTH and cortisol), Tanner Staging, and physical, neurological and ophthalmological examinations (including OCT, if feasible for the patient), and the suicidality assessment] and efficacy assessments [respiratory measures, heart rate, CGI-C, CIC, Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and RCSS] will be performed at this visit. The results from the Week 24 assessment of the primary efficacy measure of respiratory function (data from home respiratory monitoring on 3 days in the week preceding the visit), as well as all other secondary efficacy measures assessed during the visit, will be considered the endpoint value for statistical analysis purposes.

At Week 24, all patients who have completed the final evaluations at Week 24 (Day 168), have no safety or tolerability issues that would preclude continuing on the study medication, have been compliant with the trial requirements, and have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator, will be eligible to enter the initial 24-week, open-label, extension treatment period of the study. Patients who do not wish to continue treatment will have their study medication discontinued and will be asked to return for a safety follow-up visit 2 weeks after their final dose, during which vital signs and an assessment of adverse events will be performed. Any Serious Adverse Events (SAEs) occurring within 30 days after the final dose will be reported.

168-Week Open-label Extension Treatment Period
All patients who have completed the final evaluations at Week 24 (Day 168), have no safety or tolerability issues that would preclude continuing on the study medication, have been compliant with the trial requirements, and have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator, will have the option of continuing open-label treatment with sarizotan for 24 weeks. Each patient's parent, legal guardian, or representative, must provide written informed consent prior to the patient's participation in this initial extension period. The patient should provide assent, if capable of doing so. At the end of the initial 24 weeks of open-label treatment, the Investigator will make a global assessment of the patient. If in the Investigator's opinion, the patient is benefitting from treatment with sarizotan and is not experiencing any significant adverse events, they will be eligible to continue open-label treatment. Informed consent must be obtained at Week 48, for the patient to continue open-label treatment for an additional 48 weeks (through Week 96), again at Week 96 for the patient to continue open-label treatment for another 48 weeks (through Week 144), and again at Week 144 for the patient to continue open-label treatment for another 48 weeks (through Week 192).

At Week 24 (Day 168), all patients ≥13 years of age and weighing ≥25 kg, including those originally randomized to placebo and those receiving 5 mg once daily, will receive treatment with sarizotan, and will start at a dose of 5 mg *bid*. Patients 4 to <13 years of age and patients ≥13 years of age but weighing <25 kg, including those who were randomized to placebo, will receive sarizotan at a starting dose of 2 mg *bid*. If patients do not tolerate this starting dose, the Investigator will instruct the caregiver to administer only the morning dose (i.e. 2 or 5 mg once daily) for a few days prior to reinitiating the intended *bid* dose (i.e. 2 or 5 mg *bid*). If the 2 or 5 mg *bid* dose is still not tolerated, a reduction to 2 or 5 mg once daily will be permitted.

At Week 28 (Day 196), patients ≥13 years of age and weighing ≥25 kg will have their dose increased to 10 mg *bid*, if the starting dose was well tolerated, or may remain on a lower dose. Patients 4 to <13 years of age and weighing 10 to <18 kg will remain on a dose of 2 mg *bid*, while those weighing ≥18 kg, as well as patients ≥13 years of age but weighing <25 kg, will have their dose increased to 5 mg *bid*, provided there are no tolerability issues.

If the patient does not tolerate the target dose (2, 5 or 10 mg bid, based on age and weight criteria), the Investigator will instruct the caregiver to administer only the morning dose (i.e. 2, 5 or 10 mg/day) for a few days prior to reinitiating the intended *bid* dose (i.e. 2, 5 or 10 mg *bid*). If the target dose of 2, 5 or 10 mg *bid* dose is still not tolerated, a reduction to 2 mg once daily, 2 mg *bid* or 5 mg *bid*, respectively will be permitted. Patients will continue on their maximum tolerated dose (MTD) through the final dose, unless intolerance develops, and a dose reduction is warranted.

The final evaluations performed at Week 24 in the double-blind period, including assessments of respiratory function performed in the clinic using a stretch-sensitive resistance plethysmograph (SOMNOtouch), vital signs (body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic blood pressure), laboratory tests (hematology, biochemistry, urinalysis, serum prolactin, plasma ACTH and cortisol, serum pregnancy test for post-pubertal females), ECG, physical and neurological examinations, Tanner Staging, routine ophthalmological examination [including Optical Coherence Tomography (OCT), if feasible] and final assessments for other efficacy variables [Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and Rett syndrome Clinical Severity Scale (RCSS)] will serve as the baseline assessments for the open-label extension period. Once all of these Week 24 assessments have been completed, and the consent form for the extension period has been signed, patients meeting all entry criteria will start on the low dose (5 mg *bid*) of sarizotan.

The first dose of the extension period (2 or 5 mg sarizotan) should be administered in the clinic (if the morning dose was given, wait at least 6 hours after the prior dose before administration) on the same day (Day 168); however, if necessary, it can be postponed until the following morning. Patients will be observed for the occurrence of any adverse events in the initial 4hour period after the first dose. Safety evaluations (vital signs, 12-lead ECG, physical and neurological examinations) will be performed at 1 and 4 hr- post-dose. If no significant side effects are noted within the 4-hr period following the first dose, the patient will be released from the hospital and given a supply of study medication for the remainder of the first four weeks of dosing at 2 or 5 mg *bid*.

The patient/caregiver will receive a telephone contact from the Investigator/site staff on Day~~s~~ 175 (Week 25) to inquire about the tolerability of the current dose of the study medication. If there are no tolerability issues, the patient/caregiver will be instructed to continue on the current dose. If intolerance develops, a reduction to 2 or 5 mg once daily can be instituted, or if necessary, the patient can be asked to return to the clinic for an unscheduled visit for a full assessment.

The patient will be required to return for a scheduled visit on Day 196 (Week 28). The caregiver should be instructed not to administer the morning dose of the study medication on the day of the Week 28 visit to the clinic, as the first dose of the next dose level will be administered in the clinic. At this visit, safety [vital signs, 12-lead ECG, physical/neurological examination] and selected efficacy (in-clinic respiratory measures, CIC, CGI-C) evaluations, will be performed. The tolerability of the current dose of study medication will be evaluated. If there are no safety or tolerability issues that would require the dose to be maintained or reduced, the patient will have their dose maintained at 2 mg *bid*, or increased to 5 or 10 mg *bid*, and will be given their first dose in the clinic (if the morning dose was given, wait at least 6 hours after the prior dose before administration). Safety evaluations (vital signs, 12-lead ECG, physical and neurological examinations) will be performed prior to dosing and at 1 and 4 hr post-dose. If no significant side effects are noted within the 4-hr period following the dose, the patient will be released from the hospital and given a supply of study medication for the next 4 weeks of dosing at 2 mg *bid*, 5 mg *bid* or 10 mg *bid*.

The caregiver will be contacted by telephone by the Investigator/site staff on Day 203 (Week 29) to inquire about the tolerability of the study medication and any adverse events the patient might have experienced. If there are no tolerability issues, the patient/caregiver will be instructed to continue on the current dose. If intolerance develops, a reduction in dose can be instituted, or if necessary, the patient can be asked to return to the clinic for an unscheduled visit for a full assessment. Similar telephone contacts will be made at Weeks 54, 66, 78, 90, 102, 114, 126, 138, 150, 162, 174 and 186 during the additional open-label extension treatment period, during which adverse events, concomitant medication usage and overall compliance with the study medication will be assessed.

Patients will be required to return for scheduled visits at Weeks 32, 40, and 48 in the initial 24-week open-label treatment period, and Weeks 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 if they continue open-label treatment. At each of these visits, safety assessments, comprising vital signs, laboratory tests (hematology, blood chemistry, urinalysis), ECG, and physical and neurological examinations, as well as selected efficacy assessments (in-clinic respiratory measures, CIC, CGI-C), will be performed. In addition, at Weeks 48, 72, 96, 120, 144, 168 and 192 the following assessments will be performed: serum prolactin, plasma ACTH and cortisol, a serum pregnancy test (for post-pubertal females), Tanner Staging, an ophthalmological examination (including OCT, if feasible for the patient), and the suicidality assessment, as well as additional efficacy assessments (Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and RCSS). At each of these visits, the patient will be dispensed an 8-week or

12-week supply of their current dose of study medication, provided there are no safety or tolerability issues that would require a dose reduction, for dosing during the period until the next scheduled visit. The Week 48 assessments will be considered the final evaluation for patients discontinuing after 24 weeks of open-label treatment.

All patients continuing open-label treatment after Week 48 will return for the final evaluations at Week 96, if they complete 72 weeks of open-label treatment and do not continue further treatment, or at the time of discontinuation, if they discontinue from the study prematurely prior to Week 96. Patients continuing open-label treatment after Week 96, will return for the final evaluations at Week 144, or at the time of discontinuation, if they discontinue from the study prematurely prior to Week 144. Patients continuing open-label treatment after Week 144, will return for the final evaluations at Week 192, or at the time of discontinuation, if they discontinue from the study prematurely prior to Week 192. All final safety [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis, serum prolactin, plasma ACTH and cortisol, and serum pregnancy test for post-pubertal females), Tanner Staging, and physical, neurological and ophthalmological (including OCT, if feasible for the patient) examinations, and the suicidality assessment] and efficacy [in-clinic respiratory measures, CIC, CGI-C, Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and RCSS] assessments will be performed at this visit. The results from the final (Week 48, 96, 144, or 192, or at early discontinuation) assessment of the respiratory function (data from in-clinic respiratory monitoring using the SOMNOtouch), as well as all other efficacy measures assessed during the visit, will be considered the endpoint values for the open-label extension period for statistical analysis purposes.

Patients who discontinue treatment prematurely or at the end of the initial open-label extension treatment period (Week 48), as well as those who discontinue treatment after completing Part 1 (through Week 96), Part 2 (through Week 144) or Part 3 (through Week 192) of the additional extension treatment period, will be asked to return for a safety follow-up visit 2 weeks after their final dose. Vital signs and an assessment of adverse events will be performed at this visit. Any SAEs occurring within 30 days after the final dose will be reported.

Planned Study Duration:
The study will last up to 196 weeks, including a 4-week screening period, followed by a 24-week randomized, double-blind treatment period, and a 168-week open-label extension period. A safety follow-up visit will be performed 2 weeks after the final dose of study medication.

Study Population:
A minimum of 129 patients with RTT will be randomized to either sarizotan 2 mg *bid*, sarizotan 5 mg *bid*, sarizotan 10 mg *bid* or placebo *bid*, based on age and weight criteria.

Inclusion Criteria:

The patient must meet all of the following inclusion criteria to be eligible for enrolment into the study:

Demographics

1. Female or male ≥ 4 years of age.
2. Body weight ≥ 10 kg, and within the expected range for an RTT patient, based on age and height.

Diagnostic

3. Diagnosis of Rett syndrome based on consensus clinical criteria (Neul et al, 2010; Appendix 4). *A test for MECP2 mutations (Xq28) will be performed at screening if results from an accredited laboratory are not available; selection for the trial is not contingent on the results of the MECP2 test. Patients with known MECP2 duplications will not be eligible.*
4. One or more of the following breathing dysfunctions: periodic apnea during wakefulness; intermittent hyperventilation; breath holding spells; air swallowing; forced expulsion of air or saliva.
5. Patient meets all of the following criteria related to breathing abnormalities:
    a. Parent report of 10 episodes or more of breathing abnormality per day during wakefulness in the week prior to the screening visit;

b. Time per hour spent on normal breathing is less than 90% of the total time per hour of wakefulness (i.e., ≥10% of the time should be abnormal breathing);
c. Has at least 10 episodes of breathing dysrhythmia, defined by episodes ≥10 seconds of breath holding (apnea), per hour, during cardiorespiratory monitoring (performed with home/ambulatory monitoring system during screening period).

6. Stable medication regimen for 4 weeks prior to beginning the study (if receiving services - physical, occupational, or speech therapy - subjects must be on a stable regimen of these services for 3 months prior to beginning the study). Female patients of childbearing potential are to use adequate contraception as recommended by their Health Care Provider (see Section 13.1.8).

Procedural

7. Parent/legal guardian/representative has provided written consent prior to the patient participating in the study. Where feasible, consent or assent for patients less than 18 years of age, has also been provided by the patient.
8. Ability to take study medication provided either as capsules or combined with food/drink.
9. Patient is cooperative, willing to complete all aspects of the study, and capable of doing so with assistance of a caregiver.
10. Caregiver is able to understand the instructions and fully participate.

Exclusion Criteria:

The presence of any of the following will exclude a patient from study enrollment:

1. Meets any of the diagnostic exclusion criteria for Rett syndrome, Typical (Neul et al, 2010; Appendix 4);
2. Patient is participating in a clinical trial with another investigational drug or has taken an investigational drug within one month or 5 half-lives (whichever is longer) prior to screening;
3. Hypersensitivity to sarizotan or other 5-HT1a agonists, [*UK only*: or to the capsule material gelatin or microcrystalline cellulose used in the placebo capsules];
4. Current clinically significant (as determined by Investigator)
   a. cardiovascular, respiratory (e.g. severe asthma), gastrointestinal, renal, hepatic, hematologic or other medical disorders, in addition to those directly related to the patient's Rett syndrome *[for US, Italy, India and Australia]*;
   b. cardiovascular, respiratory (e.g. severe asthma), or gastrointestinal disease, renal impairment (as indicated by creatinine >2X ULN), hepatic impairment (as indicated by total bilirubin >2X ULN), history of moderate or severe hepatic insufficiency or moderate or severe liver cirrhosis, or hematologic or other medical disorders, in addition to those directly related to the patient's Rett syndrome [*for UK only*];
5. QTcF interval on the ECG is greater than 450 msec.
6. Surgery planned during the study (except for insertion of gastrostomy tube);
7. Severe diabetes mellitus or fatty acid oxidation disorder.
8. Ophthalmologic history including any of the following conditions: albino patients, family history of hereditary retinal disease, retinitis pigmentosa, any active retinopathy or severe diabetic retinopathy.
9. Females who are pregnant, breastfeeding, or of childbearing potential and not using adequate contraception (see Section 13.1.8), as recommended by their Health Care Provider.
10. [*UK only*: Subjects who have an inborn error of metabolism.]
11. Evidence of clinically significant malnutrition.

Study Medication Description:
Sarizotan is formulated as capsules of 2-mg, 5-mg and 10-mg dosage strength.

Study Medication – Dose and Mode of Administration:

The study medication will be administered as capsules of 2-mg, 5-mg or 10-mg dosage strength of sarizotan or matching placebo capsules. The capsules should be given orally; however, if necessary, they can be dissolved in an appropriate liquid and administered as a solution either orally or through an enteral feeding tube, e.g. gastrostomy tube (G-tube). Patients will receive 1 capsule *bid*, with combinations of active and placebo (double-blind period only) capsules being used to achieve the desired daily dose of sarizotan. Study medication with each different dosage (2-mg, 5-mg or 10-mg sarizotan, or placebo) will be provided in kits for dispensing to the patient for twice daily dosing over the period between scheduled visits. In the double-blind period, the study medication (sarizotan 2 mg, 5 mg or 10 mg, or placebo) administered to each patient will be determined according to a computer-generated randomization scheme. Drug supplies, randomization of patients to treatment (double-blind period only), and dispensing of appropriate medication kits (double-blind and open-label periods) will be managed by an Interactive Voice or Web Response System (IWRS).

Comparator – Dose and Mode of Administration:
In the initial 24-week double-blind period, patients randomized to the placebo group will be administered an equal number of matching placebo capsules (1 capsule *bid*) at each dose level.

Methodology:
Safety Outcomes – Secondary Objective
Safety will be assessed by the following:

- Adverse events (AEs)
- Vital signs (systolic/diastolic blood pressure, pulse, body weight, body temperature, respiratory rate)
- Laboratory evaluations (blood chemistry, hematology, urinalysis, serum prolactin, and plasma ACTH and cortisol)
- Electrocardiogram (ECG) – 12-lead standard
- Physical examination
- Neurological examination
- Routine ophthalmology examination (including OCT, if feasible for the patient)
- Tanner Staging
- Suicidality assessment.

Primary Efficacy Outcome
The primary efficacy outcome in the double-blind period will be the percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour, during awake time. This will be calculated from the data obtained during home respiratory monitoring by means of an ambulatory data acquisition system (BioRadio™) designed to record specific respiratory and cardiac parameters. Assessment of the respiratory outcomes will be performed at home on any 3 days in each of the first 3 weeks of the screening period, and in the week prior to each of the visits at Weeks 2, 8, 16, and 24. In parallel, cardio-respiratory monitoring will be performed at the hospital/clinic at Baseline and at Weeks 16 and 24, using a stretch-sensitive resistance plethysmograph (SOMNOtouch).

Home respiratory monitoring will be performed during the screening period (three 3-day periods during the first 3 weeks) and for 3 days in the week prior to each scheduled clinic visit at Weeks 2, 8, 16, and 24. Monitoring will be performed when the patient is awake and should be attempted for 6 hours on each of the 3 days. Data from all of the quality recording time (minimally 3 hours) in the Screening period, and from the week prior to each of the visits at Weeks 2, 8, 16, and 24, will be averaged and used in calculating the value for the primary efficacy measure. If the patient meets the criterion for number of apnea episodes, as determined by Vivonoetics, in any week of the Screening period, home monitoring in the subsequent week(s) would not be required, and the screening data would be used to calculate the baseline value for the analyses.

During the open-label extension phase of the study patients will return for visits every 4, 8 or 12 weeks, and respiratory assessments will be performed in the clinic at Weeks 28, 32, 40, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 using the SOMNOtouch. Respiratory measures derived from the SOMNOtouch data will be similar to those from the BioRadio™, including apnea episodes. Efficacy data from the extension period from patients randomized to sarizotan at Baseline will be analyzed to determine if there is any attenuation of the benefit noted during the first six months. In addition, a comparison will be made between patients who received sarizotan throughout both periods of the study and those who were randomized to placebo in the double-blind period and switched to sarizotan in the open-label extension period.

The following system will be used for home monitoring of cardio-respiratory function:

- BioRadio™ wireless physiological monitor (http://glneurotech.com/bioradio/physiological-signal-monitoring/wireless-respiration/).

Key Secondary Efficacy Outcome

The following global assessment performed by the caregiver will be the key secondary efficacy outcome:

- **Caregiver-rated Impression of Change (CIC):** 7-point scale requiring the caregiver to rate how much the patient's illness has improved or worsened relative to the baseline state.

Other Secondary Efficacy Outcomes

The Secondary efficacy outcomes will include the following:

- **Caregiver Top 3 Concerns:** Visual Analogue Scale-based evaluation of three priority concerns identified by caregivers related to the patient's RTT syndrome which they would like to see change as a result of treatment. The severity of these concerns is rated by the caregiver at baseline and is evaluated again at subsequent follow-up visits.
- **Clinical Global Impression of Change (CGI-C):** 7-point scale requiring the clinician to rate how much the patient's illness has improved or worsened relative to the baseline state.
- **Motor-Behavioral Assessment Scale:** comprised of three sub-scales:
    I. Behavioral/Social Assessment – 16 items
    II. Orofacial/Respiratory Assessment – 7 items
    III. Motor Assessment/Physical Signs – 14 items.
- **Respiratory Measures**:
    - Percent time spent with breathing dysrhythmia (% time apnea + % time hyperventilation) per hour;
    - Number of hyperventilation episodes (≥10 seconds each);
    - Oxygen saturation (# of episodes of oxygen desaturation below 90% per hour).
    - *Respiratory Distress Index* - The sum of the following parameters calculated per hour of wakefulness: 1) number of breath-holding episodes, 2) number of episodes of hyperventilation [1 and 2 are as defined in inclusion criteria; each would have to be ≥10 seconds in duration], and 3) number of drops in oxygen values to < 90%.
- **Rett Syndrome Clinical Severity Scale (RCSS):** frequency and manageability of seizures, respiratory irregularities, scoliosis, ability to walk (gait apraxia), hand use, speech and sleep; yielding total and feature-specific scores.
- Efficacy in patients with a sarizotan plasma concentration of >400 ng/mL on Day 15.

Pharmacokinetics – Secondary Objective

Blood samples for measurement of plasma levels of sarizotan and its major metabolites and determination of PK parameters will be collected just prior to the first dose of Dose Level 1 (blank sample) and at approximately 1 and 4 hr post-dose (±15 min) on Day 1. On Day 15, a blood sample will be taken prior to dosing on Day 15 (trough level for Dose Level 4) and at 1 and 4 hr post-dose (Dose Level 4).

Details of the population PK analysis and determination of dose-proportionality for sarizotan will be provided in a separate document.

Safety Monitoring Board
Safety data from all patients will be examined periodically by an Independent Safety Monitoring Board (ISMB). The ISMB may request modifications to the study design or request that the study be terminated, should any significant safety concerns become evident.

Statistical Methods:

Sample Size:
A minimum of 129 patients with RTT will be included in this study, with approximately equal numbers of patients randomly assigned to each of the three treatment groups:

- low dose sarizotan [sarizotan 2 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg) or 5 mg *bid* (age ≥13 years, weight ≥25 kg)],
- high dose sarizotan [sarizotan 5 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg), or 10 mg *bid* (age ≥13 years, weight ≥25 kg)],
- or placebo *bid*.

The sample size estimate was based on the following assumptions:

- 3 treatment groups (2 active + placebo)
- Primary endpoint: Percent reduction in the number of apnea episodes (≥10 sec each) per hour
- H_0: Percent reduction in apnea episodes in placebo and active groups is the same
- H_A: There is a difference in the % reduction in apnea episodes between high dose sarizotan and placebo.

The assumptions for the estimated standard deviation (SD) are based on limited data from a study by Khwaja et al. (2014), in RTT patients. There were only 5 patients with data reported for apnea index (for apnea episodes ≥ 10 seconds). Pilot observational data collected using the BioRadio device in 26 patients with RTT indicated that the number of episodes of apnea may approach 60 per hour. Based on the range of 10-60 episodes per hour, ranges of 10-30, 10-45 and 10-60 apnea episodes per hour indicate SDs of 5.0, 8.75 and 12.5, respectively.

Sample sizes were computed to determine the number of patients required to detect a minimum difference in mean percent reduction of 20% (high dose sarizotan 30%, placebo 10%) and 10% (high dose sarizotan 20%, placebo 10%), based on differences between high dose sarizotan and placebo.

Sample sizes of 23 or 34 in each treatment group will have 90% power to detect a difference in mean percent reduction of -20% or -10%, respectively, assuming a common SD of 20 (for the 20% reduction) and 12.5 (for the 10% reduction), using a two-group t-test with a 0.050 two-sided significance level. Assuming an attrition rate of 25% (7 or 9 patients) per group, a total of 30 or 43 patients would need to be enrolled in each group to get 23 or 34 patients completing the study. As this is the first large-scale study evaluating the effect of sarizotan on respiratory symptoms in patients with RTT, a conservative estimate of 10% reduction in apnea episodes was used to estimate the sample size required, i.e. approximately 43 patients per group.

The sample size may be revised based on a prospective assessment of variability in the frequency of apnea episodes, of 10 seconds or greater duration, from data collected from the first 20 patients who complete screening.

Subject Characteristics:
The background and demographic characteristics (age, race, ethnicity, weight, height, education, past and current medical conditions, etc.), disease characteristics, and prior/concomitant medication use for randomized patients will be summarized by treatment group. Continuous variables will be summarized by minimum, maximum, mean, median, and standard deviation, and discrete variables will be summarized using frequencies and percentages.

Safety Analysis:
The safety population will consist of all subjects who took at least one dose of study medication. All AEs will be summarized by body system and preferred term. The incidence (%) of SAEs, AEs that are newly occurring or worsened after administration of study medication (i.e. treatment-emergent AEs [TEAEs]), and AEs leading to discontinuation (ADOs) will also be summarized; severity of each AE and relatedness to study medication will be

assessed and presented. Changes from baseline in vital signs, ECG and laboratory values, and findings on physical, neurological and routine ophthalmology examinations will be summarized, with abnormal and clinically notable values/findings being identified. In the initial 24-week, double-blind, placebo-controlled period, a comparison will be made between the sarizotan and placebo groups in the proportion of patients experiencing SAEs, ADOs, TEAEs and any clinically significant abnormalities in other safety parameters. Where feasible, mean changes from baseline will be examined using a paired t-test. Safety data for the open-label extension period will be analyzed separately and will use the Week 24 evaluations from the double-blind period as the baseline assessments.

Efficacy Analysis:
Primary Efficacy Outcome. The primary efficacy analysis for the initial, 24-week, double-blind, treatment period will be performed on the Intent-to-Treat (ITT) population, consisting of all patients who were randomized to treatment. The percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour, during awake time, will be compared between treatment groups using a Mixed Model Repeated Measures (MMRM) analysis. The MMRM model will include percent change from baseline as response, the fixed, categorical effects of treatment group, visit, treatment group-by-visit interaction, and the continuous terms age and baseline value as covariate. A statistically significant difference between high dose sarizotan (5 or 10 mg *bid*) and placebo will be considered evidence of efficacy in reducing apnea.

Key Secondary Efficacy Outcome. The mean rating of the caregiver-rated CIC at Week 24/endpoint will be compared between treatment groups using the Van Elteren test (a stratified Cochran Mantel Haenszel test with modified ridit scores). The following age strata will be considered in the analysis: <13 years and ≥ 13 years. In addition to the above analysis, comparisons between treatments for the proportion of patients rated improved at Week 24 will be done using logistic regression.

Other Secondary Efficacy Outcomes.
The analysis of the Caregiver Top 3 Concerns will be performed first, comparing the change from baseline in the total score on the VAS for each of the 3 symptoms identified as most concerning at baseline using an ANCOVA-LOCF analysis. The model will include treatment group and the baseline value along with age as covariates. Change will be estimated using the least-squares mean derived from the ANCOVA. Comparisons between treatment groups at Week 24 will be made using the difference in least-squares mean, 95% confidence interval (CI) and p-values from the ANCOVA model.

Descriptive statistics will be presented for the other secondary efficacy outcomes. The proportion of patients rated improved, as well as the mean score, on the Investigator-rated CGI-C at each of the scheduled visits will be presented. Actual values and the change from baseline in other secondary efficacy variables (Motor Behavioral Assessment Scale and subscales, other respiratory parameters, and RCSS) at all scheduled visits will be presented. An additional analysis of efficacy will be performed for patients with a sarizotan plasma concentration of >400 ng/mL.

Multiple comparisons/ multiplicity.
The study level error rate will be controlled by following a sequential multiple testing procedure, where testing will begin with the primary efficacy endpoint. The effects of the high dose sarizotan versus placebo will be analyzed first for all parameters in a pre-specified sequence.

The sequence of testing will be as presented below:

- Percentage change from baseline in the number of apnea episodes,
- Mean rating of CIC,
- Mean change from baseline in the Top Three Concerns Total Score.

Initial 24-week Double-Blind Treatment Period. The primary efficacy assessment for the study will be performed using data from the initial 24-week double-blind, placebo-controlled treatment period. A statistical "Win" will be claimed if the following results are obtained in the primary and key secondary efficacy analyses:

- Statistically significant difference between high dose sarizotan (5 or 10 mg *bid*) and placebo in the percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour, during awake time;
- Directional change on the CIC in favor of high dose sarizotan (5 or 10 mg *bid*) compared to placebo.

168-week Open-label Extension. Efficacy data from the extension period obtained from patients randomized to sarizotan at Baseline will be analyzed to determine if there is any attenuation of the benefit noted during the first six months. The response in placebo patients switched to sarizotan in the extension period will be compared with that in patients who were treated with sarizotan throughout the study.

2 SIGNATURE PAGE

Study Title:
A Randomized, Double-Blind, Placebo-Controlled, Six-Month Study to Evaluate the Efficacy, Safety and Tolerability of Sarizotan in Patients with Rett Syndrome with Respiratory Symptoms.

Study Code:
Sarizotan/001/II/2015

Principal Investigator

Name	Title
Signature	Date

Sponsor Representative
Ravi Anand, M.D.
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The Sponsor has transferred all responsibilities for the conduct of the trial to the CRO.

3 TABLE OF CONTENTS

4 ABBREVIATIONS AND DEFINITIONS OF TERMS

ACTH	=	adrenocorticotrophic hormone
ADO	=	adverse dropout
ADR	=	adverse drug reaction
AE	=	adverse event
AED(s)	=	antiepileptic drug(s)
AIC	=	Active-In-Capsule
ALT	=	alanine-aminotransferase
ANCOVA	=	analysis of covariance
ANOVA	=	analysis of variance
AST	=	aspartic-aminotransferase
AUC	=	area under the plasma drug concentration vs. time curve
bid	=	twice daily
BUN	=	blood urea nitrogen
CGI-C	=	Clinical Global Impression – Change from baseline
CIC	=	Caregiver-rated Impression of Change from baseline
CI	=	confidence intervals
C_{max}	=	maximum post-dose plasma drug concentration
CNS	=	Central Nervous System
CRF	=	case report form
CTCAE	=	Common Terminology Criteria for Adverse Events
CYP	=	Cytochrome P450
DBP	=	diastolic blood pressure
EC	=	Ethics Committee
ECG	=	Electrocardiogram
ESR	=	Erythrocyte sedimentation rate
GCP	=	Good Clinical Practice
GMP	=	Good Manufacturing Practice
γGT	=	gamma-glutamyl transpeptidase
GLP	=	Good Laboratory Practice
HDL	=	high density lipoprotein
HBV	=	Hepatitis-B Virus
HCV	=	Hepatitis-C Virus
hERG	=	human *Ether-a-go-go* Related Gene
HIV	=	human immunodeficiency virus
HR	=	heart rate
HPA	=	hypothalamic – pituitary – adrenal

ICH	=	International Conference on Harmonisation
IMP	=	Investigational Medicinal Product
i.p.	=	Intraperitoneal
IRB	=	Institutional Review Board
ITT	=	Intent to Treat
IWRS	=	Interactive Web Response System
LDH	=	lactate dehydrogenase
LC-MS/MS	=	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
LDL	=	low density lipoprotein
LOCF	=	Last Observation Carried Forward
MECP2	=	methyl-CpG-binding protein 2 (human gene)
MeCP2	=	methyl-CpG-binding protein 2 (human protein)
Mecp2	=	methyl-CpG-binding protein 2 (mouse gene)
Mecp2	=	methyl-CpG-binding protein 2 (mouse protein)
MTD	=	Maximum tolerated dose
NOAEL	=	No observed adverse effect level
OCT	=	Optical Coherence Tomography
OTC	=	Over-The-Counter
PK	=	Pharmacokinetics
PRL	=	Prolactin
RBC	=	red blood cells
RCSS	=	Rett syndrome Clinical Severity Scale
RTT	=	Rett syndrome
RDO	=	Retrieved dropout
SAE	=	Serious Adverse Event
SBP	=	systolic blood pressure
SD	=	standard deviation
SMB	=	Safety Monitoring Board
SOP	=	standard operating procedure
T_3	=	Triiodothyronine
T_4	=	Thyroxine
TEAE	=	Treatment-emergent adverse event
TSH	=	Thyroid Stimulating Hormone
VLDL	=	very low density lipoprotein
WBC	=	white blood cells

5 TITLE OF STUDY

A Randomized, Double-Blind, Placebo-Controlled, Six-Month Study to Evaluate the Efficacy, Safety and Tolerability of Sarizotan in Patients with Rett Syndrome with Respiratory Symptoms.

6 PROTOCOL NUMBER

This study is being conducted under protocol no.: Sarizotan/001/II/2015.

7 INTRODUCTION

7.1 Background Information

7.1.1 Pharmacology

Sarizotan hydrochloride (also referred to as Sarizotan and EMD 128130), binds with high affinity to h5-HT_{1A} and 5-HT7 serotonin receptors, and to D2-like dopamine receptors, including hD2S, hD2L, hD3, hD4.2, hD4.4 and hD4.7 subtypes.

In functional *in vitro* tests, sarizotan hydrochloride exhibited the profile of a full agonist at serotonin 5-HT1A and dopamine D4.4 receptors and of an antagonist at dopamine D2 receptors (with weak intrinsic D2 agonistic activity at high doses). Although the role of its D4.4 agonistic properties, as well as its high affinity for D3 receptors, remains unclear, sarizotan hydrochloride displays a highly favorable profile, since theoretically possible dopamine D2 receptor mediated motor impairments are counteracted by its 5-HT_{1A} agonism. The role of the high affinity of sarizotan hydrochloride for 5-HT7 receptors is unclear. EMD 148 107 and EMD 329 989, two minor metabolites of sarizotan hydrochloride, show pharmacological activity at the D2 and D4 receptors, respectively; however, there is no relevant contribution of these metabolites to the activity of sarizotan. EMD 329 989 also binds to the 5-HT1A receptors.

Functional *in vivo* studies on the turnover of biogenic amines in rats indicate both serotoninergic agonistic activity and dopaminergic antagonistic activity of sarizotan in the brain; the dopaminergic agonist activity is more than 10 times weaker.

Antidyskinetic and neuroleptic activity

In vivo behavioral studies indicate that sarizotan acts as a **5-HT_{1A} agonist.** Sarizotan hydrochloride inhibited, and at higher doses totally suppressed, ultrasonic vocalization induced by foot shock, with an ID_{50} of 1.4 mg/kg po, suggestive of a serotoninergic activity at the 5-HT1A receptor. Anti-dyskinetic effects, seen both in L-dopa treated parkinsonian rats and monkeys have also been attributed to its 5-HT_{1A} agonistic component, as anti-dyskinetic effects were abolished by the selective 5-HT_{1A} antagonist WAY 100635. In 6-OHDA lesioned rats, sarizotan decreased L-dopa induced dyskinesia /dystonia at a dose of 3 mg/kg sc, inhibited the L-dopa induced abnormal involuntary movements at doses of 1-5 mg/kg ip, and at the single oral dose of 2.5 mg/kg normalized the shortening of turning behavior duration induced by chronic (22 days) treatment with L-dopa. In parkinsonian MPTP –treated monkeys, sarizotan dose-dependently (0.2 – 2 mg/kg po) reduced the L-dopa- induced dyskinesia (Figure 1).

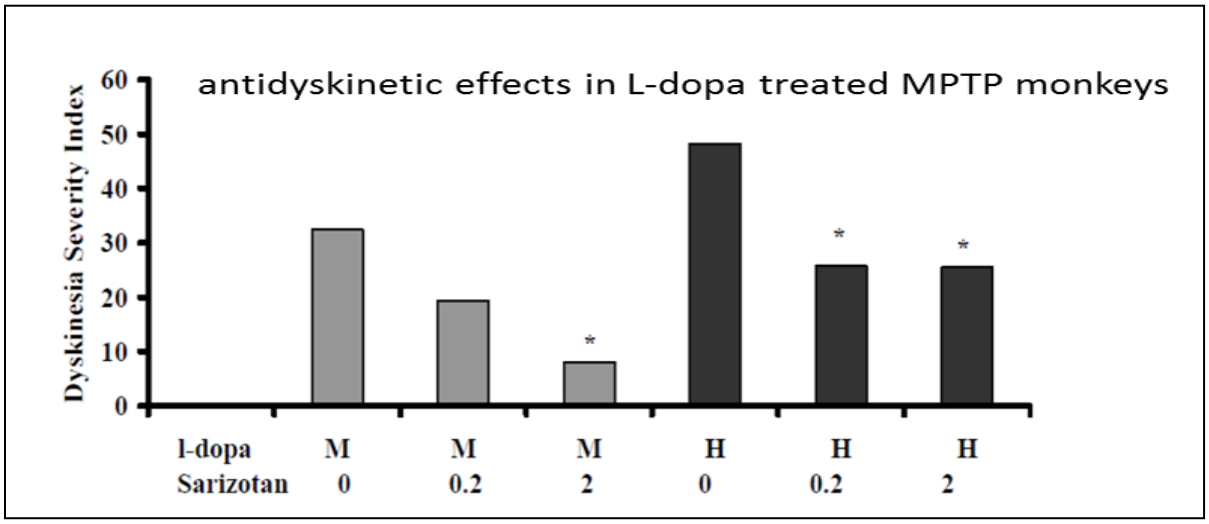


Figure 1: effects of sarizotan in L-dopa treated MPTP-lesioned monkeys. Dose-dependent reduction of total dyskinesia (expressed as Dyskinesia Severity Index) by sarizotan 0.2 - 2 mg/kg po. '0' refers to the placebo-treated control of sarizotan. M and H refer to the medium and high dose of L-dopa. * $p<0.05$ compared to placebo.

Sarizotan showed lower potency in models that are suggestive of **dopamine D2 antagonistic properties:** doses of 16-20 mg/kg were needed to inhibit apomorphine-induced stereotyped behaviors, and avoidance reaction in rodents.

Sarizotan does not induce catalepsy even at high doses (single doses of 100 and 1000 mg/kg p.o in mice and rats; sub-chronic 100 mg/kg po in rats); at lower doses it counteracts haloperidol-induced catalepsy (ID50 = 10 mg/kg/po, Figure 2) and chronic haloperidol-induced repetitive jaw movements (model of tardive dyskinesia; ID50 between 1.5 and 9 mg/kg po, Figure 3). The lack of catalepsy and the suppression of the D2 antagonist induced EPS is due to 5-HT_{1A} agonistic effects of sarizotan, as these effects were reversed by the 5-HT_{1A} antagonist WAY 100635.

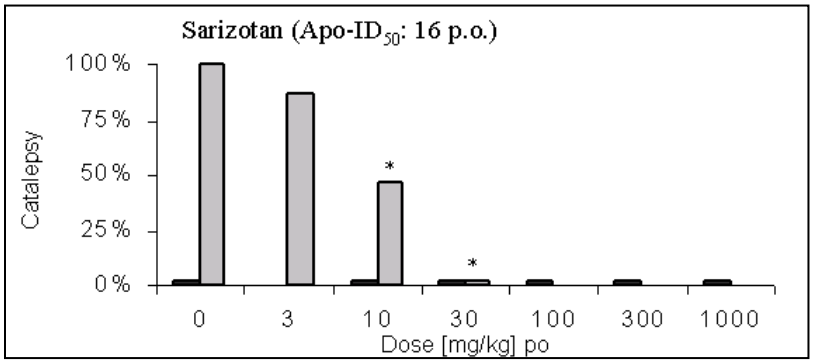


Figure 2: Sarizotan does not induce catalepsy in rats up to 1000 mg/kg (black bars); sarizotan reverses haloperidol-induced catalepsy with ID50 of 10 mg/kg po (gray bars) * $p < 0.05$ versus haloperidol alone. In the title is reported the oral ID50 (16 mg/kg) for the inhibition of apomorphine-induced stereotyped behavior indicating D2 antagonism.

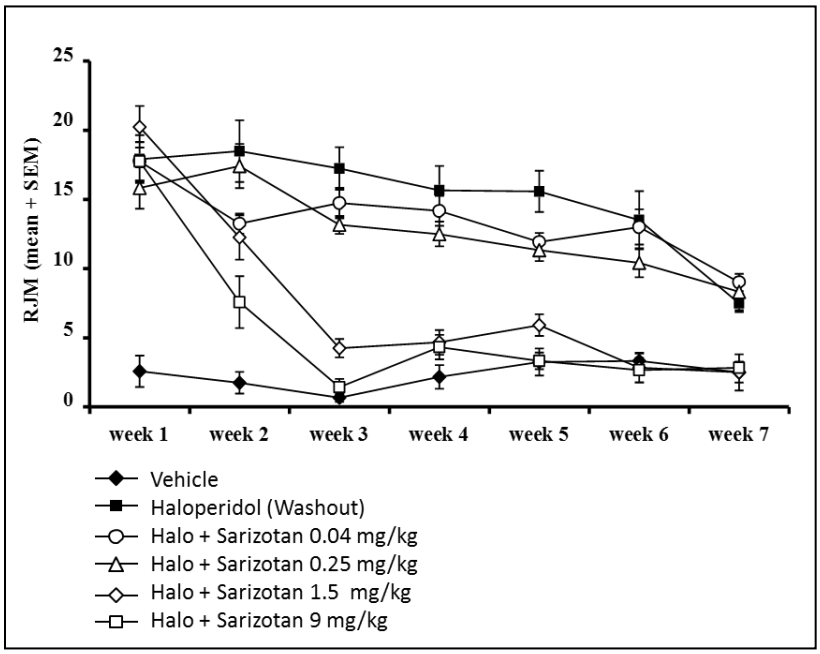


Figure 3: sarizotan reduces repetitive jaw movements (RJM) produced by chronic treatment with haloperidol. Haloperidol (1 mg/kg/day) was administered for 6 months. After 3 weeks of haloperidol washout, sarizotan hydrochloride was administered at the given doses (mg/kg/day) for 2 months. (n=12 rats per group).

Respiratory dysfunction

The new proposed indication for sarizotan is "breathing dysfunction in Rett syndrome (RTT) patients".

Dysregulation of breathing is very common in RTT, and is particularly severe. It is characterized by alternating periods of hyperventilation and apneas, breath holds terminated by Valsalva's maneuvers, forced and deep breathing, as well as apneustic breathing, and it commonly reveals abnormal cardiorespiratory coupling. It has been reported that complications of cardiorespiratory dysfunctions may result in sudden and unexpected deaths that account for 26% of all deaths in RTT (Julu et al, 1997; Kerr et al 1997; Julu et al, 2008; Byard et al, 2006). Neurophysiological studies have shown that breathing defects and abnormal cardiac responses reflect the immaturity of the brainstem (Julu et al 2001). Deficits in various neurotransmitter systems in the brainstem have been observed in patients and animal models of RTT (Katz et al., 2009; Weng et al., 2011). Activation of 5-HT_{1A} receptors in specific areas of the brainstem involved in the neuronal control of respiration plays a key role in modulation of the respiratory rhythm, including the response to CO_2 concentration (Abdala, May 2014). Studies in in Mecp2 mutant mice (validated preclinical model of RTT) have indicated that breathing irregularities are mostly characterized by overactive brainstem expiratory neurons, possibly due to a lack of synaptic inhibitory control (Abdala 2010). Agonism at the 5-HT_{1A} receptor using the reference standard 8-OH-DPAT has been shown to provide inhibitory control of expiratory neurons within the lower brainstem, resulting in potential benefit in reducing the breathing irregularities and apneas (Lalley et al, 1994, Abdala et al, 2010; Abdala et al May 2014).

There are no treatments available that ameliorate breathing irregularities in RTT patients. Effective treatment of respiratory abnormalities contributing to the RTT morbidity and mortality would address a significant unmet medical need. The potential benefits of sarizotan on respiratory symptoms in patients with RTT were evaluated by examining its effects in Mecp2 deficient RTT mice models (Abdala et al, June 2014).

The effect of acute administration of sarizotan was evaluated in heterozygous female mice strains with different *Mecp2* deletions (Mecp2 Jae/ + and Mecp2 Bird/+, respectively) and in homozygous null male mice (Mecp2 Jae/y). These transgenic mice models present a significantly higher basal incidence of apnea (number of apneas per hour) and breathing irregularities compared to their corresponding wild-type (WT) strains.

A sarizotan dose of 5.0 mg/kg significantly reduced the incidence of apnea in Mecp2 deficient heterozygous females (n=10) from 143 ± 31 to 20 ± 8/hr (~ 86% reduction, p=0.001). Sarizotan also resulted in a significant decrease in the irregularity variance from 0.34 ± 0.07 to 0.06 ± 0.01 ($p < 0.0001$), approximating the irregularity variance measured in the WT. Frequency was also significantly increased, although not to the WT level.

Sarizotan (10.0 mg/kg) was equally effective in improving respiration in Mecp2 null male mice. The incidence of apnea fell from 200 ± 42 to 30 ± 16/hr (~ 85% reduction, p=0.003). Irregularity significantly declined from 0.55 ± 0.21 to 0.11 ± 0.05 ($p = 0.048$).

The effect of sarizotan was also evaluated in two separate long-term studies (7 and 14 days administration) in two heterozygous female mice strains (Mecp2 Bird/+ and Mecp2R168X/+; the latter represents a common nonsense mutation seen in RTT patients). Sarizotan was administered by dissolving it in drinking water (0.0625 mg/ml, vehicle 1.25% DMSO + 0.1% saccharin). The dose of sarizotan ingested by drinking averaged ~ 14 mg/kg per day.

The results in Mecp2 Bird/+ mice, indicated a significant reduction of 63.3% ($p = 0.009$) and 66.1% ($p = < 0.001$) in the incidence of apnea on days 5 and 7 respectively, and a significant reduction of the irregularity score towards the WT level. The second study performed in Mecp2R168X/+ mice indicated a significant reduction of apnea of approximately 75% that was maintained between days 7-14 compared to the control ($p = 0.008–0.022$). Irregularity score was significantly improved as compared to that of animals receiving vehicle (on day 7: $p = 0.006$; on day 10: $p = 0.049$; on day 14: $p = 0.05$).

7.1.2 Toxicology

Completed toxicology studies

Sarizotan was initially developed for other indications (Parkinson's disease and schizophrenia), and the preclinical safety program is considered to be almost complete and includes numerous studies, i.e. pharmacology studies including evaluating the effects of sarizotan in RTT mice, studies relevant to the earlier indications and a full safety pharmacology package. There is a comprehensive DMPK package with toxicokinetic studies. Toxicology studies consist of single and repeat dose oral toxicity studies, including chronic studies in rats and dogs; a battery of *in vitro* and *in vivo* mutagenicity tests, and carcinogenicity bioassays in rats and mice; a full range of reproductive toxicity studies in rats and rabbits; juvenile toxicity studies in rats; local tolerance (eye, skin) in rabbits, sensitization in guinea pigs, and an *in vitro* phototoxicity study; *in vitro* (adrenal steroid biosynthesis) and *in vivo* (monkey, dog) mechanistic studies; and combination repeated-dose studies with L-dopa/carbidopa in rats and dogs.

During the course of the large preclinical safety program the key findings identified include:

CNS signs (mainly reduced spontaneous activity & unsteady gait) were the predominant feature in pharmacology and toxicity studies. In the toxicity studies, rats showed prone position, salivation and burrowing in the litter, dogs showed reduced activity, unsteady gait and salivation and monkeys showed reduced activity, tremor, salivation and hunched position. In the proposed clinical trial, RTT patients will be monitored for clinical signs.

CV effects: Sarizotan and its active metabolites EMD 148 107 and EMD 329 989 inhibited *in vitro* hERG channel activity at low concentrations (IC50s of 1.95, 0.2 and 0.35 µM, respectively). Sarizotan was not associated with any increase in cardiac action potential duration (APD) or prolongation of the QT interval in functional studies, but was associated with APD shortening at high concentrations in cardiac *in vitro* preparations (10µM in rabbit isolated Purkinje fibers, 30 µM in Guinea pig heart papillary muscles, and at 6 µM in sheep isolated cardiac Purkinje fibers). No APD shortening is expected in humans at the free plasma concentration of 0.35 µM that would be reached at a 10 mg single sarizotan dose in a subject with a 25-kg body weight. The two active metabolites EMD 148 107 and EMD 329 989 increased the APD duration in isolated rabbit Purkinje fibers at concentrations of 1µM. However, no APD prolongation is expected in humans, as the plasma concentrations of these two metabolites are < 10% of those of the parent compound sarizotan. No relevant cardiovascular adverse effects were noted in telemetric studies in conscious normotensive rats (oral administration) and in anaesthetized open-chest dogs (intra-duodenal administration). There were no adverse ECG findings on the repeated-dose dog studies (oral 4-, 26-, 52-weeks duration, i.v. 2-weeks duration). In clinical trials, no meaningful increase in QTc was observed at doses of 20 - 40 mg *bid*. Based on these data, no relevant (> 10 msec) increase in QTc is expected in RTT patients weighing 25 kg or more, who will be receiving a maximum dose of 10 mg bid in the current study. Patients with a QTcF at baseline which exceeds 450 msec will be excluded from the study. In addition, ECGs will be performed at regular intervals in all patients throughout the trial.

Adrenal cortical changes consisting principally of hypertrophy/hyperplasia in the rat (≥ 125 mg/kg/day) and dog (≥ 25 mg/kg/day) and atrophy in the monkey (≥ 10 mg/kg/day) and ***mammary*** changes of hyperplasia and atrophy in rats at ≥5 mg/kg/day and hyperplasia/neoplasia in the mouse carcinogenicity study.

The mammary hyperplasia/neoplasia in rodents, as well as the adrenal hypertrophy/hyperplasia/atrophy in various animal species, were a consequence of the pharmacological action of sarizotan i.e. 5-HT1A receptor activation which induces secretion of ACTH and PRL. In addition, *in vitro* data indicate that sarizotan can cause a relatively selective inhibition of the 11ß-hydroxylase isoenzyme in the biogenesis pathway of corticosterone/cortisol and aldosterone. This conclusion is supported by the results of a 4-week explanatory study in dogs. It is therefore possible that this mechanism contributed to the elevated ACTH concentrations via the adrenal-pituitary axis feedback system. The induction of PRL-related mammary proliferative lesions is rodent specific. PRL is luteotrophic in rodents, but not in humans; the synergistic effect of PRL, progesterone (from corpora lutea) and estrogen leads to mammary proliferation in rodents.

Clinical data showed no clear dose- or time-dependent effect on ACTH concentrations in Phase I clinical studies. In Phase II studies, high sarizotan doses (≥40 mg/day) elicited abnormal responses in cortisol following an ACTH test. However, at lower doses (≤20 mg/day), no abnormal tests were observed. Sarizotan did not impair the cortisol response to ACTH stimulation as assessed in the Phase III Open-Label PADDY-O study. No dose-dependent effects on PRL secretion or the mammary gland were observed in Phase I and II clinical studies.

Testicular atrophy was present in monkeys at ≥ 10 mg/kg/day. As the RTT syndrome almost exclusively affects females, testicular atrophy in monkeys is of low concern. Testicular effects were not recorded in clinical trials.

An increased incidence of ***retinal atrophy*** was recorded in the albino rat carcinogenicity study at ≥25 mg/kg/day. Retinal atrophy was seen in all groups including the controls. However, when compared with the controls, the incidence and severity of this change was higher in both sexes at ≥25 mg/kg/day. Retinal atrophy was not found in the mouse carcinogenicity (105/107-weeks), dog (52-weeks), monkey (4-weeks) or rat (26-weeks) studies.

The histopathological description of the atrophy is similar to light -induced atrophy in albino rats, i.e. bilaterally increased incidence of atrophy of the outer nuclear layer of the retina. As sarizotan reduced the spontaneous activity at ≥5 mg/kg and frequently prevented the rats from seeking the provided means to hide from light, it is possible that the atrophy is not a direct compound-related effect. This form of retinal atrophy is not unique to sarizotan. In a literature review of marketed compounds, retinal degeneration was found in toxicity studies primarily with CNS-active drugs and in rodents but not in non-rodent species (dogs, monkeys). The published data from these compounds show that the features of the atrophy are similar to that of sarizotan, particularly the rat specificity, dose and light dependency. The vast majority of these compounds have not been associated with retinal changes in humans, suggesting a general lack of predictability for this type of toxicological finding.

In the clinic, sarizotan in the dose range of 1 to 200 mg/day was administered as single and/or repeated doses to more than 1500 subjects in Phase II-III studies. More than 1000 patients received treatment with sarizotan in Phase III trials, with over 300 patients completing 2 years of treatment without any evidence of an increase in the incidence of ocular adverse events compared with placebo.

In the current RTT study, ophthalmological monitoring has been included at baseline and at Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192, or at early discontinuation.

Completed juvenile toxicology studies

The preclinical data discussed in Section 7.1.1, and in the section above, together with adequate clinical data in the adult population without adverse events of concern, are considered sufficient for the conduct of the proposed 6-month initial trial that will include adolescent RTT patients (≥13 years of age). Juvenile toxicology studies in rats 7-21 days of ages are described in detail in Section 5.3.7 of the Sarizotan Investigator's Brochure, v.3, 14 September 2018, and summarized below. The main juvenile toxicity study (YG47PW) commenced in 21-day old rat pups, which correspond to a 2-year old child (Buelke-Sam, 2003). The results of this study, therefore, support the treatment of RTT patients as young as 4 years of age and up to 13 years of age.

Two sarizotan *dose-range-finding studies* conducted in juvenile rats, an initial study (ONP0106) in which treatment started in 21-day old pups (corresponding to a 2-year old child), and a 2nd study (ONP0113) in 7-day old pups (corresponding to a newly born child), indicated the presence of effects on body weight, and relatively severe CNS signs in the 7-day old pups, thus limiting the *main study* (YG47PW) to be performed in 21-day old pups, at doses of 0, 12.5, 25 and 50 mg/kg/day. In this study, at the 50 mg/kg dose, a treatment-related death was observed, as well as CNS signs (underactive behavior, flat posture, piloerection, and splayed limbs); similar signs, but of a lower severity, were encountered in all dose groups (including 12.5 and 25 mg/kg), which resolved during the day. In addition, reduced ulnar growth (50 mg/kg), abnormalities in weight gain (50 mg/kg males, all groups females), oestrus irregularity (all groups), delay in reproductive maturity (50 mg/kg), abnormalities in motor activity (50 mg/kg) and during the functional operational battery (FOB) (all groups), and impairments in learning and memory (all groups) were noted. All of the above changes showed recovery, apart from the increase in motor activity. Despite treatment-related histopathological changes (large corpora lutea, vaginal dioestrus, mammary gland hypertrophy/ hyperplasia, and adrenal gland zona glomerulosa hypertrophy) during treatment, there was no difference between sarizotan and placebo-treated animals during recovery. There was no evidence of an effect of any of the doses on mating behavior, pregnancy, conception and fertility indices, or the number of live embryos or litter size.

The Functional Observational Battery (FOB) revealed an increase in vocalisation, particularly in females, in all treated groups during the in-hand observations, and an increase in the incidence of flattened posture, abnormal gait, tremors, urination and defecation, and a decrease in the levels of arousal and rearing during the 2-minute arena observation. All arena counts, except high urination and fecal counts, showed full recovery.

The Morris water maze assessment revealed a clear and generally dose-dependent effect on cognition (learning and memory) in all treated groups. In Weeks 3-4 of the recovery period, there was clear evidence of long-term memory retention and no deterioration of long-term memory over time. The overall percentage improvement in mean trial times over the four days of testing during recovery was unaffected by previous treatment with sarizotan.

The occurrence of the treatment-related death and the lack of recovery for motor activity at the 50 mg/kg/day dose indicate that the next lower dose of 25 mg/kg/day should be considered as the No-Observed-Adverse-Effect-Level (NOAEL). Safety margins based on the NOAEL and compared to the predicted exposures in RTT patients are in the range of x1 to x5 (Section 5.3.8 of the Sarizotan Investigator's Brochure, v.3, 14 September 2018).

Many of the sarizotan-related changes in the juvenile toxicity study had been noted in earlier toxicity studies in rats, and were attributable to activation of 5-HT1A receptors, with subsequent perturbation of the pituitary-adrenal-gonadal hormone axis. Data from previous trials in human subjects did not detect any effects on growth, motor activity or memory. There was no effect of sarizotan at doses of 20 mg or lower on prolactin, growth hormone, progesterone and testosterone concentrations, or on the cortisol response to ACTH. In this study, RTT patients will undergo regular neurological and clinical examinations, as well as measurements of serum prolactin, and plasma ACTH and cortisol.

Evidence of good tolerability, without any signal of safety risk, in over 1800 human subjects treated at doses of 0.2-200 mg/day, including >200 subjects treated for 2 years or longer, together with the safety precautions in the trial, including oversight by an Independent Safety Monitoring Board, support dosing with sarizotan in children from 4 years of age in the ongoing clinical trial.

7.1.3 Pharmacokinetics and Metabolism

Twenty-three Phase I studies were performed to investigate the safety, tolerability, pharmacodynamic and pharmacokinetic effects of sarizotan after single and multiple administrations. A total of 549 subjects were treated with sarizotan in these studies.

Single doses of 20 mg sarizotan hydrochloride and multiple doses up to 50 mg BID have been given in studies in healthy volunteers, including 12 elderly subjects; these doses were generally well tolerated. The most frequent adverse events following administration of sarizotan, affected the nervous system (central (CNS) or peripheral nervous system). The most frequent events within this class were dizziness, headache, and somnolence. Other adverse events were predominantly categorized as either general disorders (fatigue), or those affecting the gastro-intestinal tract.

Pharmacokinetics (PK) of sarizotan are linear in the dose range investigated (0.5-25 mg). Cmax occurred within 1 hour after administration, indicating a rapid absorption. Absorption is almost complete, with an absolute bioavailability of ~90%. Sarizotan hydrochloride concentrations declined poly-exponentially with a terminal elimination half-life of 5-7 hours.

Sarizotan is extensively metabolized, less than 5 % of unchanged drug was found in excreta (urine, feces). Major routes of metabolism are hydroxylation at various positions in the chromane ring system and N-dealkylation. Plasma metabolite concentrations are considerably lower than those of the parent drug.

Age does not affect the pharmacokinetics of sarizotan in a clinically relevant manner. Sarizotan does not change the PK profile of L-dopa or digoxin. The plasma levels of sarizotan (Cmax) are decreased by approximately 40% when taken with food, compared to when taken fasted; however, the AUC is unaffected.

Sarizotan had no impact on the PK profiles and the metabolic ratios of five probe drugs for the CYP P450 enzymes 2D6, 1A2, 2C19, 2C9 and 3A4. Therefore, no clinically relevant drug-drug interactions between sarizotan and potential co-medications being cleared by these P450s are expected. Erythromycin, a CYP3A4 inhibitor, did not influence the PK properties of sarizotan to a clinically relevant extent. Fluvoxamine, a CYP1A2 inhibitor, had a moderate effect on the PK of sarizotan and its metabolites. The effect of St. John's Wort, a CYP3A4 inducer, on the PK of sarizotan and its metabolites was moderate.

Total exposure of sarizotan was reduced in subjects with severe renal impairment, as compared to normal subjects, in accordance with a shorter terminal half-life. In subjects with hepatic impairment, exposure to sarizotan was increased to 140% and 190% in moderately and severely impaired subjects, respectively, as compared to normal subjects.

In summary, the PK profile of the drug indicates that dosing in clinical studies should be based on a twice -daily dosing regimen.

All pre-clinical studies performed to date, including general and specific pharmacology studies on the mechanism of action, pharmacokinetics and toxicology, are described in the Investigator's Brochure [Sarizotan Investigator's Brochure (IB), version 3 for Rett Syndrome, dated 14 September 2018].

7.1.4 Clinical Studies

Exposure

The safety and tolerability of sarizotan in the dose range of 0.2 to 200 mg/day has been adequately established in other programs with over 1800 subjects having been exposed to at least a single dose of sarizotan, with over 1000 patients having received treatment for 6 months, and over 400 for 1 year, >200 for 2 years, >150 for 3 years, and >50 for 4 years, during long-term, open-label treatment, without evidence of any significant treatment-related adverse outcomes.

Sarizotan was administered to 433 subjects in the dose range of 0.2 to 200 mg/day in phase I studies. The Phase II and III Clinical program explored the safety and efficacy of sarizotan in patients with schizophrenia (N=112) at doses ranging from 40 to 180 mg/day (Study EMD 128130-008). Four other studies in Parkinson's disease (PD) patients (three double-blind, placebo-controlled studies, and one open-label study) recruited 1447 patients, of which 862 received sarizotan (dose range 1 to 20 mg/day). In addition, 456 patients who received placebo in the randomized phase of the studies received sarizotan in the long-term open label extension. Over 300 patients were treated with doses of 1 to 5 mg BID, with approximately 100 patients receiving doses of 10 mg/day for over 1 year.

Therapeutic Studies

The phase II and III Clinical program completed with sarizotan explored the safety and efficacy of sarizotan in two indications: schizophrenia (one study at doses ranging from 40 to 180 mg/day) and L-dopa induced dyskinesias at doses ranging from 1 to 20 mg/day in PD (4 double-blind, placebo-controlled studies and 3 long-term open-label studies). No clinical studies have been performed to date with sarizotan in patients with Rett syndrome, and no clinical studies are currently ongoing for any indication. Details of all clinical studies performed to date with sarizotan are described in the Investigator's Brochure [Sarizotan Investigator's Brochure (IB), version 3 for Rett Syndrome, dated 14 September 2018].

Studies in schizophrenia

A 6-week, double-blind, Phase II study was performed in 231 patients with chronic or sub-chronic schizophrenia to test the antipsychotic efficacy of different dose ranges of sarizotan (i.e. 40-80 mg/day and 100-180 mg/day, overall 112 patients) compared to haloperidol (4-16 mg/day, 57 patients) and placebo (62 patients). Patient enrolment was terminated prematurely as approximately 70% of the 231 enrolled patients (83 out of 112 on sarizotan - 74.1%; 44 out of 62 on placebo -70.9%; 40 out of 57 on haloperidol - 70.2%) discontinued from the study prematurely.

Abnormal (blunted) cortisol responses to ACTH challenge were observed in both sarizotan treatment groups (17.8% of patients in the sarizotan groups), compared with the placebo and haloperidol groups (1.6% in the placebo group and 5.3% in the haloperidol group).

Studies in Parkinson's disease (L-dopa induced dyskinesia)

An open-label study in 64 PD patients with treatment-related dyskinesia (SPLENDID) with a dose range of sarizotan of 2 -10 mg *bid* indicated significant improvement of dyskinesia compared to baseline. Subsequently, three double-blind, placebo-controlled studies were performed in PD patients with L-dopa induced dyskinesia.

Neither the two 12-week studies (SPIRID, 100 patients/group [4 treatment arms, sarizotan 1 mg *bid*, 2 mg *bid*, 5 mg *bid*, and placebo] and PADDY-1, 253 patients/group [2 treatment arms, sarizotan 1 mg *bid* and placebo]), nor the 24-week study (PADDY-2, 235 patients on sarizotan 1 mg *bid* and 246 patients on placebo) demonstrated any significant difference compared to placebo on the variables 'ON time without dyskinesia', OFF time, and the proportion of "Responders", based on change from baseline to endpoint of at least 25% on the UPDRS Section IV, Items 32 + 33.

Overview of Tolerability and Efficacy

Sarizotan was well tolerated in the schizophrenia study in the dose range up to 180 mg/day; however, it was associated with a blunted cortisol response after ACTH stimulation in some patients, at doses of 80 mg/day and higher. No such effect was noted at doses of 2-20 mg/day that were evaluated in patients with PD. In patients with PD, there was no difference in the incidence of these findings between sarizotan and placebo. No tolerability or safety findings were identified in these studies at doses up to and including 10 mg BID. Neither the two 12-week studies, nor the 24-week study, in PD patients demonstrated any significant difference compared to placebo for any efficacy variable related to PD or dyskinesia.

No tolerability or safety findings were identified in any of these studies that would preclude further evaluation of sarizotan.

7.2 Chemistry and Pharmaceutical Properties of Sarizotan

The nomenclature, structure and chemical properties of sarizotan and the composition of the available dosage forms (5.0 and 10.0 mg capsules) are provided below.

International Nonproprietary Name (INN)

Sarizotan hydrochloride

United States Adopted Name (USAN) (RR-123)

Sarizotan hydrochloride

Chemical Name

(-)-3-[[[(R)-2-Chromanylmethyl]amino]methyl]-5-(p-fluorophenyl)pyridine hydrochloride

(R)-2-[5-(4-Fluorophenyl)-3-pyridylmethylaminomethyl]-chroman hydrochloride, or

3-[(2R)-3,4-Dihydro-2H-1-benzopyran-2- pyridylmethylamino-methyl]-5-(4-fluorophenyl)-pyridine hydrochloride, or

(R)-1-(chroman-2-yl)-N-((5-(4-fluorophenyl)pyridin-3-yl)methyl)methanamine hydrochloride

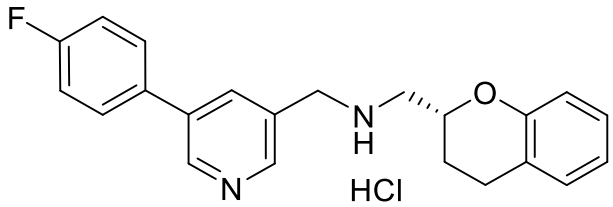
Company Name or Laboratory Code

EMD 128130

Chemical Abstract Service (CAS) Registry Number (RN)

195068-07-6

Structure



Molecular formula: $C_{22}H_{21}FN_2O.HCl$

Molecular weight: 384.88 g/mol

Description:

Sarizotan hydrochloride is an off-white, fine crystalline powder with a melting point at 242 °C (combined with decomposition)

Hygroscopicity

The substance is not hygroscopic.

General Description of Sarizotan Drug Product

The dosage form used in this study is an orally administered immediate release capsule containing only drug substance; commonly referred to as Active-In-Capsule (AIC). Sarizotan AIC for the clinical study will be provided in dose strengths of 2 mg, 5 mg and 10 mg. The capsule shells are made of animal gelatin, just under a standard size 4, opaque, Swedish Orange color and purchased from Capsugel ™. There are no other excipients in the capsule. Placebo capsules, manufactured to contain a small amount of microcrystalline cellulose, match the sarizotan AIC investigational product.

Route of Administration and Dosing Regimen

Sarizotan is manufactured using an AIC formulation for oral administration. The maximum daily dose is 20 mg (10 mg *bid*). The intended dosing regimen is twice daily.

Sarizotan hydrochloride AIC may be administered as an oral solid dosage that can be swallowed or as a drinkable oral liquid dosage. The oral capsule is administered in the standard manner with sips of water or juice before and after swallowing to help the pill go down. The administration of sarizotan as an oral liquid, for patients unable to swallow a capsule, requires dissolving the sarizotan hydrochloride AIC in apple juice, cranberry juice, orange juice, tea (with or without milk) or water. Sarizotan may also be administered via an enteral feeding tube by dissolving the capsules in water. The procedures for the preparation and administration of these solutions are described in Section 10.2 and Appendix 6.

7.3 Study Rationale

Rationale for use of Sarizotan in treating respiratory abnormalities in RTT patients

Sarizotan is a full agonist at 5-HT1A receptors and an antagonist/partial agonist at the dopamine-D2 and related dopamine receptors. It is effective in animal models predictive of anti-dyskinetic activity. Sarizotan was initially evaluated for the treatment of schizophrenia and L-dopa induced dyskinesia in patients with Parkinson's disease (PD); efficacy could not be demonstrated in either indication. Sarizotan at doses of 1 – 20 mg was well tolerated, even when administered for extended periods of time.

Serotonin is an important neuromodulator in the central control of the respiratory processes. Dysregulation of the serotonin pathways in the brainstem respiratory network has been described in RTT. There is currently no approved treatment for managing the breathing irregularities observed in RTT. Therefore, effective treatment of respiratory abnormalities contributing to the RTT morbidity and mortality would address a significant unmet medical need. Mecp2-deficient mice (a preclinical model of RTT), show respiratory alterations that could benefit from increasing serotonergic activity at the nuclei involved in control of respiratory activity. It is postulated that sarizotan, a full 5-HT1A receptor agonist, would modulate respiratory neurons and provide beneficial effects in reducing breathing irregularities in RTT. Sarizotan is effective in reducing apnea incidence and to regularize the abnormal respiratory pattern in Mecp2-deficient mice of different strains. Benefits are observed after single and repeated dosing. These findings suggest that sarizotan could represent a new pharmacotherapeutic approach to treat respiratory dysregulation in RTT patients.

Rationale for study design

The primary objectives of the current study are to evaluate the safety and tolerability of sarizotan given as an oral dose (2 to 10 mg *bid*) in patients with Rett syndrome (RTT) with respiratory abnormalities and to evaluate the effect of sarizotan, compared to placebo, on reducing the number of apnea episodes in these patients.

A randomized, double-blind study design will be used for the initial 24-week treatment period of this study to reduce potential bias in the assignment of patients to treatment and in the assessment of efficacy and safety parameters. A total of 129 patients will be included in this study, with approximately equal numbers of patients randomly assigned (1:1:1) to treatment with low dose sarizotan [2 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg) or 5 mg *bid* (age ≥13 years, weight ≥25 kg)], high dose sarizotan [5 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg), or 10 mg *bid* (age ≥13 years, weight ≥25 kg)], or placebo *bid*. The approximately equal randomization to each group allows for a drug-placebo comparison for safety and efficacy for each dose. Samples sizes were computed based on percent reductions of 20% and 10% between the sarizotan high dose and placebo groups, assuming an attrition rate of 25%, and a SD of 20 or 12.5, respectively. The sample sizes of 30 or 43 per group provides 90% power to detect a difference of -20% (-30% for sarizotan high dose vs. -10% for placebo) or -10% (-20% for sarizotan high dose vs. -10% for placebo) in the percent reduction in apnea episodes per hour (apnea index).

There are currently no approved treatments for respiratory abnormalities in RTT patients; therefore, a placebo control, rather than an active control, will be used in this study. The use of a device to record respiration as well as the structured method of observation may lead to learning effects. The placebo control has been included so that a background rate of spontaneous occurrence of adverse events, as well as changes in other safety and efficacy parameters, can be established in placebo-treated patients, against which the active treatment will be compared, without introducing a bias from knowledge of the treatment by the caregivers or investigators.

The initial treatment period of the study will be 6 months, which is a standard period of observation for chronic neurological diseases. All patients who have completed the final evaluations at Week 24 (Day 168), have no safety or tolerability issues that would preclude continuing on the study medication, have been compliant with the trial requirements, and have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator, will be eligible for continuing open-label treatment with sarizotan for up to 24 weeks, to allow for collection of long-term safety and efficacy data.

Each patient will return to the hospital on the day prior to the administration of the first dose to allow for performance of safety and efficacy evaluations, and will remain in the facility until 4 hours after the morning dose on Day 2 to facilitate safety monitoring and collection of samples for PK analysis, and to ensure standardized conditions for all patients. Subsequent dose increases will be performed during outpatient treatment, with monitoring of tolerability through scheduled telephone contacts.

The study incorporates a dose titration design in which the target dose and dose titration will be dependent on the age and, in the case of patients ≥13 years of age, weight of the patient. All patients will start on the lowest dose (Dose Level 1: 2 or 5 mg sarizotan or placebo), and will have their doses increased to Dose Level 2 (2 mg sarizotan once daily, 2 mg sarizotan *bid*, 5 mg sarizotan *bid*, or placebo *bid*) on Day 2, then to Dose Level 3 (2 mg sarizotan *bid*, 5 mg AM + 2 mg PM sarizotan, 5 mg sarizotan *bid*, 10 mg sarizotan once daily, or placebo *bid*) on Day 8; and finally to Dose Level 4 (2 mg sarizotan *bid*, 5 mg sarizotan *bid*, 10 mg sarizotan *bid*, or placebo *bid*) on Day 9, dependent on tolerability. The dose may be maintained at the current level or reduced if needed if tolerability issues develop. Patients will be titrated to their maximum tolerated dose (MTD) and would be expected to complete the study on this dose; however, dose reductions are permitted at any time if intolerance develops.

Patients will return to the hospital for a visit on Day 14 to assess their tolerability of their current dose, and will be housed overnight with their parent/caregiver in a separate facility (hotel/hostel/etc.) and return to the hospital on the following morning, so that blood samples for PK analysis at the highest dose (Dose Level 4) can be collected prior to and 1 and 4 hours after the morning dose on Day 15. If the dose is well tolerated, the patient will be dispensed medication for the next 6-week period at the current dose level. The data collected from the device (BioRadio™) for monitoring cardio-respiratory function at home will be reviewed on an ongoing basis throughout the study, and if this review of the cardiorespiratory data indicates a problem with the use of the device, additional training will be provided to the caregiver at the next visit. [*See discussion below regarding rationale for selection of the primary efficacy variable and the procedure for its measurement*].

Subsequent visits will take place at Weeks 8 and 16, at which time safety and efficacy assessments will be performed and medication will be dispensed to the patient of the next dosing period. Final evaluations will be performed at Week 24 (or at early discontinuation) and will be used for assessing the safety and efficacy of sarizotan, compared to placebo. For patients who discontinue prematurely or complete 24 weeks of treatment, but do not continue in the open-label extension period, safety follow-up assessments (vital signs and adverse events) will be performed 14 days after the final dose of study medication.

The 168-week extension treatment period of the study uses an open-label design, such that the Investigator, site staff, caregiver/patient and Sponsor will know what treatment the patient is receiving. To ease the burden on the patient/caregiver, the number of assessments has been reduced in this open-label extension period; in particular in-home cardio-respiratory monitoring using the BioRadio will not be performed. All patients treated in the initial double-blind period, including those randomized to placebo, will have an opportunity to receive open-label treatment with sarizotan for up to 24 weeks in the initial open-label period. Only those patients who are judged by the Investigator to be benefitting from sarizotan treatment, and not experiencing significant adverse events, will be eligible to continue open-label treatment for an additional 144 weeks. The open-label design allows simplification of the packaging, dispensing and dosing of the

study medication. All patients enrolled in the extension period, including those originally randomized to placebo, will receive treatment with sarizotan, and will start at a dose of 2 mg *bid* (patients 4 to <13 years of age and weighing 10 to <18 kg, and patients ≥13 years of age but weighing <25 kg) or 5 mg *bid* (patients ≥13 years of age and weighing ≥25 kg). At Week 28, patients will return to the clinic for safety and efficacy assessments, and will have their dose maintained at 2 mg *bid* (patients 4 to <13 years of age and weighing 10 to <18 kg), increased to 5 mg *bid* (patients 4 to <13 years of age and weighing ≥18 kg, and patients ≥13 years of age and weighing <25 kg) or increased to 10 mg *bid* (patients ≥13 years of age and weighing ≥25 kg), if the starting dose was well tolerated. Since patients may be receiving sarizotan for the first time or receiving a different dose than in the initial double-blind period, the Week 24 evaluations will be used as the baseline assessments for evaluating changes in the safety and efficacy parameters measured in the extension period.

Sarizotan has not been administered previously to pediatric patients, or patients with RTT; therefore, safety data from all patients will be evaluated periodically by a Safety Monitoring Board (SMB) throughout the study.

Rationale for pharmacokinetic sampling times

No PK data has been collected previously for sarizotan in pediatric patients or patients with RTT; therefore, blood samples will be collected in this study for measurement of sarizotan. However, due to the small size and fragile nature of RTT patients, PK sampling in this study will be limited to samples taken pre-dose and 1 and 4 hours post-dose for the initial dose (Dose Level 1) on Day 1, and the morning dose at Dose Level 4 on Day 15. In adults, sarizotan had a rapid T_{max} of ~1 hr and a relatively short terminal elimination half-life of 5-7 hr (see Section 7.1.3). Therefore, if metabolism in these RTT patients is similar to adults, these sampling times should capture the post-dose peak concentration and the initial tail of the curve.

Rationale for choice of patient population

To be eligible for the study patients must have a diagnosis of RTT based on consensus clinical criteria (Neul et al, 2010; Appendix 4); a test for MECP2 mutations (Xq28) will be performed, but is not required for the diagnosis. The selected population for this first study will include patients 4 years of age or older, and weighing at least 10 kg (Tarquinio et al, 2102). As shown in a recent study (Mackay et al, 2017), autonomic breathing abnormalities, including breath-holding and hyperventilation, have been observed in a substantial proportion of RTT patients below the age of 6 years. Dosing of patients as young as 4 years of age is supported by the results of a recently completed juvenile toxicity study performed in 21-day old rats (see Section 7.1.2).

Both male and female patients will be eligible; however, male patients rarely survive past 2 years of age, and most male patients with loss of functional mutations in MECP2 do not meet clinical criteria for RTT. Since sarizotan is being developed for the treatment of respiratory abnormalities, patients must have a significant level of breathing dysfunctions, e.g. periodic apnea during wakefulness, and must meet the following criteria: at least 10 episodes of breathing abnormality per hour, time spent in normal breathing is <90% of total time per hour of wakefulness, and at least 10 episodes of breath holding (apnea) ≥10 seconds in duration per hour during home cardiorespiratory monitoring. Ensuring that the patients have a substantial level of respiratory symptoms, in particular apnea, at baseline will facilitate measurement of a potential treatment effect for the primary efficacy endpoint, i.e. percent reduction in number of apnea episodes per hour, during awake time.

Excluding patients with significant or severe concomitant medical illnesses and ensuring that patients are on stable doses of any concomitant medication prior to baseline will prevent bias in determining the tolerability and safety of sarizotan. Since adverse ocular effects of sarizotan have been observed in studies in albino rats, patients will be excluded if they have an ophthalmologic history including any of the

following conditions: albino patients, family history of hereditary retinal disease, retinitis pigmentosa, any active retinopathy or severe diabetic retinopathy, will be excluded.

Rationale for dose selection

Currently, there are no PK or efficacy data in RTT patients. Sarizotan has been studied in 23 Phase 1 study in healthy adult volunteers that evaluated PK and safety of sarizotan in a wide range of deses from 0.5. A population PK analysis was performed on PK data collected from 230 healthy adult volunteers (with 5528 PK samples) who received doses of sarizotan ranging between 0.5-100 mg *bid*. The final population PK model was validated and shown to adequately represent the PK of sarizotan (Appendix 3 of the Sarizotan Investigator's Brochure, v.3, 14 September 2018). The final population model was used to allometrically (based on weight) extrapolate the adult healthy volunteer PK data to RTT patients who are known to be mostly below the age of 18 and have lower weight for a given age compared to healthy subjects. The predictions from the final population PK model shows:

- pediatric and adolescent RTT patients following, 10 mg *bid* of sarizotan are expected to have a median AUCss ranging between 6352 and 9907 ng*hr/mL and a median $Cmax_{,SS}$ ranging between 516 and 817 ng/mL for body weights in the ranges 45-55 kg and 15-20 kg, respectively.
- pediatric and adolescent RTT patients following 5 mg *bid* of sarizotan are expected to have a median AUC_{SS} ranging between 3176 and 4953 ng*hr/mL, and a median $Cmax_{,SS}$ ranging between 258 and 409 ng/mL for body weights in the ranges 45-55 kg and 15-20 kg, respectively.
- pediatric and adolescent RTT patients following 2 mg *bid* of sarizotan are expected to have a median AUC_{SS} ranging between 1270 and 1981 ng*hr/mL, and a median $Cmax_{,SS}$ ranging between 103 and 164 ng/mL for body weights in the ranges 45-55 kg and 15-20 kg, respectively.

Selection of the doses for the current study was based on targeting the predicted AUC for RTT patients (presented above) being above the efficacious AUC values associated with efficacy in improving respiratory abnormalities observed in a mice RTT model (i.e., an AUC of 2033 ng.h/ml), while staying below the AUC values associated with NOAEL in rats (i.e., 10020 ng.h/ml). These target exposures are approximately met at doses between 2-10 mg *bid* dose in RTT patients, with patients with a body weight of 10 kg or higher. The exposure in patients weighing 10 to <18 kg who are randomized to a dose of 5 mg bid would be expected to be lower than the NOAEL in rats.

Rationale for efficacy measures and method of measurement for respiratory parameters

Primary efficacy outcome

The primary efficacy endpoint in this study is the percent reduction in the number of apnea episodes (≥ 10 seconds in duration) observed per hour, during awake time. Apnea episodes of at least 10 seconds in duration are considered clinically meaningful (Khwaja et al, 2014). The selection of apnea as a primary endpoint was based on its prevalence at all stages of RTT, its impact on the patient and caregiver, its contribution to long-term adverse outcomes, the ability to measure it objectively over a long period of time under conditions that are not stressful for the patient, and the benefit that may accrue from its attenuation.

The apnea endpoint is considered clinically relevant and can be measured objectively over a longer continuous period of time than a point estimate. The use of an automated home monitoring device (BioRadio™) to measure cardiorespiratory parameters (episodes of apnea, hyperventilation, disordered breathing, respiratory volumes, oxygen saturation) provides the additional advantage of lack of measurement bias, higher sensitivity, lower variability, and more robust data. The BioRadio will be used only in the initial 24-week double-blind period of the study.

For the primary efficacy outcome, apnea episodes will be defined as those with duration considered to be of clinical significance (i.e., ≥ 10 seconds). The Sponsor will also determine the effect on apnea episodes

that are associated with a biological change that would make it clinically meaningful, e.g. oxygen saturation lower than 90%.

Secondary efficacy outcomes

Key secondary efficacy outcome. The Sponsor, based on a request from regulatory authorities to include a measure that assesses the clinical meaningfulness of any drug effect, proposed that a caregiver-rated global assessment of change in the patient's condition should be used as a key secondary measure. This caregiver-rated measure will take into account activities, behavior, mood and functioning. Therefore, the Caregiver-rated Impression of Change (CIC), a 7-point Likert-type scale requiring the caregiver to rate how much the patient's illness has improved or worsened relative to the baseline state, has been included as the key secondary efficacy outcome. This rating will be performed in consultation with the study Investigator, but should be based largely on the caregiver's evaluation of the patient during the reporting period.

Other standard measurements of respiratory function, evaluated during awake time, will also be assessed as secondary efficacy outcomes: percent time spent with breathing dysrhythmia (% time apnea + % time hyperventilation) per hour; number of hyperventilation episodes (≥10 seconds each) per hour; oxygen saturation (# of episodes of oxygen desaturation below 90% per hour); Respiratory Distress Index [sum of the number of breath-holding episodes (>10 sec), number of episodes of hyperventilation (>10 sec), and number of drops in oxygen values to < 90%]; and incidence of breathing dysrhythmia episodes, including apneustic breathing style and forceful breathing style.

In addition, several scales, specifically assessing the symptoms of Rett syndrome have been included as secondary efficacy measures. These include the Rett syndrome Clinical Severity Scale (RCSS), which assesses multiples symptoms of the disease, a global assessment performed by the Investigator (CGI-C), an assessment of motor and behavioral symptoms (Motor-Behavioral Assessment Scale), and the Caregiver Top 3 Concerns, a visual analogue scale evaluating change in the symptoms the caregiver finds most troubling.

Rationale for safety measures

Adverse events (AEs) will be assessed throughout the study, and other standard safety assessments will be performed in the hospital following the first 3 doses, and at all scheduled visit, including vital signs, laboratory tests (hematology, biochemistry, urinalysis), ECGs (12-lead standard), and physical and neurological examinations. In addition, the cardiorespiratory function will be assessed during the screening period, at baseline and at Weeks 8, 16 and 24 in the initial double-blind treatment period, and at Weeks 28, 32, 40, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 (or at early discontinuation) in the open-label extension treatment period. As requested by the FDA, an assessment of treatment-emergent suicidal ideation and behavior will be performed at baseline and periodically throughout the study (Weeks 8, 24, 48, 72, 96, 120, 144, 168 and 192) using a structured suicidality assessment query. This simple questionnaire was compiled by the Sponsor, based on other instruments commonly used to assess suicidality, to help the caregiver answer questions on potential symptoms of suicidality in the patient. A follow-up safety assessment (vital signs and AEs) will be performed 14 days after the last dose of study medication for all patients to evaluate any adverse events emerging after withdrawal of the study drug. Testing for hepatitis B and C and HIV will be performed at Screening, and any patients testing positive will be excluded from the trial.

In a prior study evaluating two dose ranges of sarizotan (40-80 mg/day and 100-180 mg/day) in patients with schizophrenia, abnormal (blunted) cortisol responses to ACTH challenge were observed in both sarizotan treatment groups (17.8% of patients), compared with the placebo and haloperidol groups (1.6% and 5.3%, respectively). These effects are not anticipated at the lower doses (2, 5 and 10 mg, *bid*) to be administered in the current study; however, plasma ACTH and cortisol levels will be measured at Baseline and at Day 2 and Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192 during the treatment period of the trial.

In addition, serum prolactin will be measured at Baseline and at Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192 to monitor the effect of sarizotan on this hormone.

Adverse ocular effects (e.g. retinal atrophy) have been observed with sarizotan in carcinogenicity studies in albino rats at doses of ≥25 mg/kg/day. However, retinal atrophy was not found in mouse carcinogenicity (105/107-weeks), dog (52-weeks), monkey (4-weeks) or rat (26-weeks) studies. No adverse ocular effects have been observed or reported in any of the clinical studies performed with sarizotan to date at doses up to 180 mg/day. However, as a precaution, routine ophthalmological examinations (including OCT, if feasible for the patient) will be performed by an ophthalmologist at Baseline and at Weeks 16 and 24 in the initial treatment period and at Weeks 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) in the extension treatment period.

Finally, since pediatric patients will be enrolled in this study, the potential effects of sarizotan on their sexual maturity will be evaluated at Baseline and at Weeks 24, 48, 72, 96, 120, 144, 168 and 192 using the Tanner scale (Marshall and Tanner, 1969; Marshall and Tanner, 1970; see Appendix 7).

8 STUDY OBJECTIVES

The objectives of the study are as follows:

Primary Efficacy Objective:

- To evaluate the effect of sarizotan (high dose), compared to placebo, on reducing the number of apnea episodes, during awake time, in patients with RTT with respiratory abnormalities.

Key Secondary Efficacy Objective:

- To evaluate the effect of sarizotan (high dose), compared to placebo, on the Caregiver-rated Impression of Change (CIC) from baseline;

Secondary Objectives:

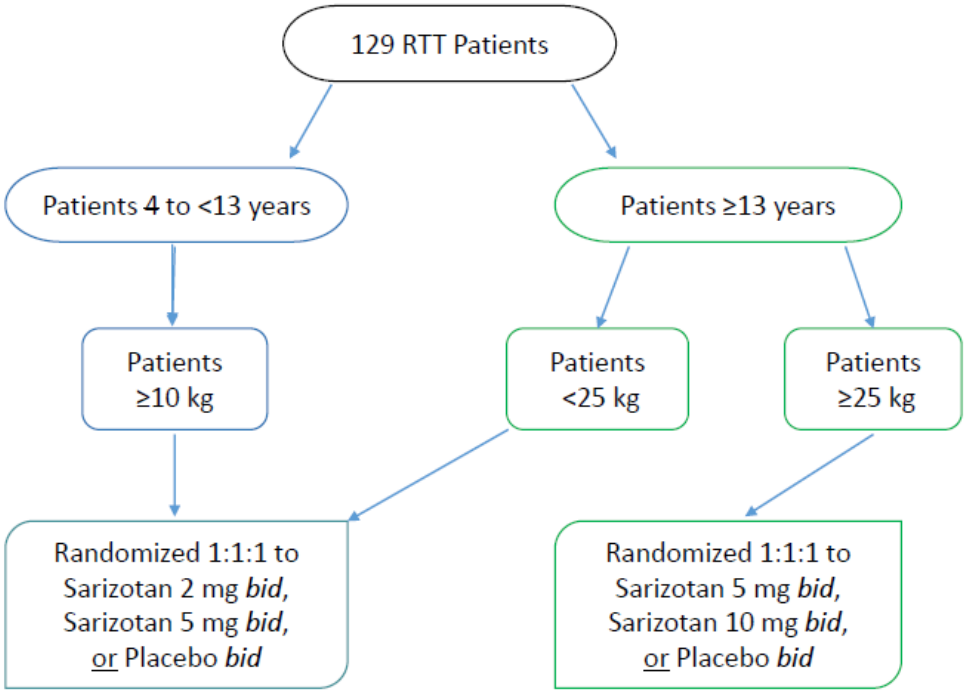
- Safety: To evaluate the safety and tolerability of sarizotan in patients with Rett syndrome (RTT) with respiratory abnormalities.
- Efficacy: To evaluate the efficacy of the dose range of sarizotan, compared to placebo, on the following:
    - Severity of patient symptoms, based on evaluation of Caregiver Top 3 Concerns (Visual Analogue Scale [VAS]);
    - Global change from baseline, assessed by the Clinical Global Impression of Change (CGI-C);
    - Motor behavior, assessed by the Motor-Behavioral Assessment Scale;
    - Other respiratory symptoms of Rett syndrome, assessed during awake time, including:
        - Percent time spent with breathing dysrhythmia (% time apnea + % time hyperventilation) per hour;
        - Number of hyperventilation episodes (≥10 seconds each) per hour;
        - Oxygen saturation (number of episodes of oxygen desaturation below 90% per hour).
        - *Respiratory Distress Index* - The sum of the following parameters calculated per hour of wakefulness: 1) number of breath-holding episodes, 2) number of episodes of hyperventilation [1 and 2 are as defined in inclusion criteria; each would have to be ≥10 seconds in duration], and 3) number of drops in oxygen values to < 90%.
    - Overall assessment of symptoms of RTT using the Rett syndrome Clinical Severity Scale (RCSS).
    - To determine the efficacy of sarizotan in patients who attain a minimum plasma exposure of 400 ng/ml on Day 15 (expected efficacious concentration based on the knockout mouse model);
- To determine the pharmacokinetic profile of sarizotan at the doses tested and compare with the pharmacokinetic profile in adults.

9 INVESTIGATIONAL PLAN

9.1 Study Design

This is a prospective, 24-week, randomized, double-blind, placebo-controlled study designed to evaluate the safety, tolerability, and efficacy of multiple oral doses of sarizotan in patients with RTT with respiratory abnormalities. At least 129 patients will be randomized (approximately 43/group) to receive either low dose sarizotan (2 mg *bid* or 5 mg *bid*), high dose sarizotan (5 mg *bid* or 10 mg *bid*) or placebo *bid.* Patients 4 to <13 years of age will be randomized equally (1:1:1) to sarizotan 2 mg *bid* (low dose group), sarizotan 5 mg *bid* (high dose group), or placebo. Patients ≥13 years of age and weighing ≥25 kg will be randomized equally (1:1:1) to sarizotan 5 mg *bid* (low dose group), sarizotan 10 mg *bid* (high dose group), or placebo *[Note: patients ≥13 years of age and weighing <25 kg will be eligible for the study, but will be included with the younger patients and randomized equally (1:1:1) to sarizotan 2 mg bid (low dose), sarizotan 5 mg bid (high dose) or placebo]*. The paradigm for randomizing patients based on age and weight criteria is summarized schematically in Figure 4.

Figure 4. Randomization of patients to treatment according to age and body weight criteria



All patients 4 to <13 years of age, as well as those ≥13 years of age and weighing <25 kg, randomized to sarizotan 2 or 5 mg *bid* will start at a single dose of 2 mg on the morning of Day 1. Patients assigned to the 2 mg *bid* dose will remain on 2 mg once daily on Days 2-7, and will have their dose increased to 2 mg *bid* on Days 8-14. Patients assigned to the 5 mg *bid* dose will have their daily dose of sarizotan increased to 4 mg (2 mg *bid*) on Days 2-7, 7 mg (5 mg AM, 2 mg PM) on Day 8, and 10 mg (5 mg *bid*) on Days 9-14. Patients ≥13 years of age and weighing ≥25 kg randomized to sarizotan 5 or 10 mg *bid* will start at a single dose of 5 mg on the morning of Day 1, with an increase to 5 mg *bid* on Day 2. Patients randomized to 5 mg *bid* will continue on this dose for the rest of the study, while patients randomized to 10 mg *bid* will receive a single dose of 10 mg on Day 8 and have their dose increased to 10 mg bid on Day 9, and continue

on this dose. If patients do not tolerate the proposed titration and target dose, the investigator will provide only one of the two daily doses (e.g. 5 mg/day) for a few days prior to reinitiating the intended *bid* dose (e.g. 5 mg *bid*). If the dose is still not tolerated, a reduction to a lower rescue dose will be permitted. Dose titration should be completed by Day 14, after which patients will continue on their maximum tolerated dose (MTD) through the final dose, unless intolerance develops, and a dose reduction is warranted. Patients will return for scheduled visits at Weeks 8, 16 and 24, at which time efficacy and safety assessments will be performed.

All patients, including those randomized to placebo, who have completed the final evaluations at Week 24 (Day 168), have no safety or tolerability issues that would preclude continuing on the study medication, have been compliant with the trial requirements, and have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator, will have the option of continuing open-label treatment with sarizotan for an additional 24 weeks. Patients who are judged to be benefitting from treatment with sarizotan, and are not experiencing significant adverse events, will be eligible for an additional 144 weeks of open-label treatment.

The final safety and efficacy evaluations performed at Week 24 will serve as the baseline assessments for this open-label extension period. All patients meeting the inclusion/exclusion criteria, including those previously randomized to placebo, will receive treatment with sarizotan, and will start at a dose of 2 mg *bid* (patients <13 year of age and patients ≥13 years of age weighing <25 kg) or 5 mg *bid* (patients ≥13 years of age weighing ≥25 kg). Patients will have their dose maintained at 2 mg bid (patients <13 year of age weighing 10 to <18 kg), increased to 5 mg *bid* (patients <13 year of age weighing ≥18 kg and patients ≥13 years of age weighing <25 kg), or increased to 10 mg *bid* (patients ≥13 years of age weighing ≥25 kg) at Week 28, if there are no tolerability issues, and will continue on this dose. Patients will return for scheduled visits at Weeks 32, 40 and 48, in the initial open-label period, and Weeks 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192, if they continue open-label treatment, at which time efficacy and safety assessments will be performed. A Safety Follow-up visit will be performed two weeks after the last dose of study medication.

An overview of the study design for the initial 24-week double-blind treatment period, the initial 24-week open-label extension treatment period, and Parts 1, 2 and 3 of the additional 144-week extension treatment period is provided in Table 1, and Tables 2A, 2B, 2C, and 2D, respectively.

Table 1. Summary of Study Design: Initial 24-Week, Double-blind, Treatment Period

Period	Screening	Baseline	Randomized Treatment Period				Final Evaluation	Safety follow-up
Study Day(s)/ Week	-28 to -1	Days 0 and 1 (pre-dose)	Days 1 and 2	Days 14 and 15	Week 8	Week 16	Week 24 (or early d/c)	2 weeks after last dose
Duration	28 days	1 day	2 days	2 days	1 day	1 day	1 day	1 day
Treatment/ Procedures	Informed consent; Screening evaluations; home respiratory monitoring (on 3 days in each of the first 3 weeks); I/E criteria assessed	Hospital visit; Baseline safety and efficacy assessments; confirm patient still meets I/E criteria; randomization	First 3 doses in hospital setting; dose titration, PK sampling and safety assessments; return home on Day 2	Hospital visit; dose titration complete (telephone contact on Day 7); home respiratory monitoring on 3 days in the week prior to visit; PK sampling, and safety and efficacy assessments	Home respiratory monitoring on 3 days in the week prior to visit; all safety and efficacy assessments	Home respiratory monitoring on 3 days in the week prior to visit; all safety and efficacy assessments	Home respiratory monitoring on 3 days in the week prior to visit; all safety and efficacy assessments	Vital signs and adverse events for patients not continuing treatment in the open-label extension period

Table 2A. Summary of Study Design: Open-Label Extension Treatment Period (Weeks 24 – 48)

Period	Baseline (extension)	Initial Open-Label Treatment Period						Safety follow-up
Study Week	Week 24	Week 25	Week 28	Week 29	Week 32	Week 40	Week 48 (or early d/c)	2 weeks after last dose*
Duration	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day
Treatment/Procedures	Informed consent; Week 24 safety and efficacy assessments from initial period will serve as baseline for extension; confirm patient is eligible to continue treatment; all patients start re-titration with sarizotan 5 mg *bid* on same day #	Telephone contact on Day 175 to confirm tolerability of dose; assess AEs and conc. medication	Selected safety and efficacy assessments; dose increase to 10 mg *bid*, if no tolerability issues	Telephone contact on Day 203 to confirm tolerability of dose; assess AEs and conc. medication	Selected safety and efficacy assessments	Selected safety and efficacy assessments	All safety and efficacy assessments; this will be the final evaluation for patients not continuing open-label treatment	Vital signs and adverse events for all patients

[#] Additional procedures and administration of the first dose of study medication in the open-label extension period should be performed on the same day as the Week 24 final assessments from the double-blind period, if possible.

*To be performed for patients who discontinue prematurely and those not continuing open-label treatment.

Table 2B. Summary of Study Design: Open-Label Extension Treatment Period (Weeks 49 - 96)

Period	**Additional Open-Label Treatment Period (Part 1)***							**Final (#) Evaluation**	**Safety follow-up**
Study Week	Week 54	Week 60	Week 66	Week 72	Week 78	Week 84	Week 90	Week 96 (or early d/c)	2 weeks after last dose
Duration	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day
Treatment/ Procedures	Telephone contact to assess AEs, conc. medication and compliance with study medication	Selected safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	All safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	Selected safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	All safety and efficacy assessments	Vital signs and adverse events for all patients

*Only those patients who have completed 24 weeks of open-label treatment, and at the Week 48 visit are judged by the Investigator to be benefitting from treatment, are not experiencing significant adverse events, and for whom informed consent has been provided, will be eligible to continue open-label treatment in this period of the study.

The Week 96 evaluation will be the final assessment for patients in Part 1 who are not continuing in Part 2 of the additional open-label extension treatment period (Weeks 97-144).

Table 2C. Summary of Study Design: Open-Label Extension Treatment Period (Weeks 97 - 144)

Period	**Additional Open-Label Treatment Period (Part 2)***							**Final** (#) **Evaluation**	**Safety follow-up**
Study Week	Week 102	Week 108	Week 114	Week 120	Week 126	Week 132	Week 138	Week 144 (or early d/c)	2 weeks after last dose
Duration	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day
Treatment/ Procedures	Telephone contact to assess AEs, conc. medication and compliance with study medication	Selected safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	All safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	Selected safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	All safety and efficacy assessments	Vital signs and adverse events for all patients

*Only those patients who have completed 72 weeks of open-label treatment, and at the Week 96 visit are judged by the Investigator to be benefitting from treatment, are not experiencing significant adverse events, and for whom informed consent has been provided, will be eligible to continue open-label treatment in this period of the study.

The Week 144 evaluation will be the final assessment for patients in Part 2 who are not continuing in Part 3 of the additional open-label extension treatment period (Weeks 145-192).

Table 2D. Summary of Study Design: Open-Label Extension Treatment Period (Weeks 145 - 192)

Period	**Additional Open-Label Treatment Period (Part 3)***							**Final Evaluation**	**Safety follow-up**
Study Week	Week 150	Week 156	Week 162	Week 168	Week 174	Week 180	Week 186	Week 192 (or early d/c)	2 weeks after last dose
Duration	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day	1 day
Treatment/ Procedures	Telephone contact to assess AEs, conc. medication and compliance with study medication	Selected safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	All safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	Selected safety and efficacy assessments	Telephone contact to assess AEs, conc. medication and compliance with study medication	All safety and efficacy assessments	Vital signs and adverse events for all patients

*Only those patients who have completed 120 weeks of open-label treatment, and at the Week 144 visit are judged by the Investigator to be benefitting from treatment, are not experiencing significant adverse events, and for whom informed consent has been provided, will be eligible to continue open-label treatment in this period of the study.

9.2 Study Population

9.2.1 Inclusion Criteria

The patient must meet all of the following inclusion criteria to be eligible for enrolment into the study:

Demographics

1. Female or male ≥ 4 years of age.
2. Body weight ≥ 10 kg, and within the expected range for an RTT patient, based on age and height.

Diagnostic

3. Diagnosis of Rett syndrome based on consensus clinical criteria (Neul et al, 2010; Appendix 4). *A test for MECP2 mutations (Xq28) will be performed if results from an accredited laboratory are not available; selection for the trial is not contingent on the results of the MECP2 test. Patients with known MECP2 duplications will not be eligible*.
4. One or more of the following breathing dysfunctions: periodic apnea during wakefulness; intermittent hyperventilation; breath holding spells; air swallowing; forced expulsion of air or saliva.
5. Patient meets all of the following criteria related to breathing abnormalities:
    a. Parent report of 10 episodes or more of breathing abnormality per day during wakefulness in the week prior to the screening visit;
    b. Time per hour spent on normal breathing is less than 90% of the total time per hour of wakefulness (i.e., ≥10% of the time should be abnormal breathing);

c. Has at least 10 episodes of breathing dysrhythmia, defined by episodes ≥10 seconds of breath holding (apnea), per hour, during cardiorespiratory monitoring (performed with home/ambulatory monitoring system during screening period).

6. Stable medication regimen for 4 weeks prior to beginning the study (if receiving services - physical, occupational, or speech therapy - subjects must be on a stable regimen of these services for 3 months prior to beginning the study). Female patients of childbearing potential are to use adequate contraception as recommended by their Health Care Provider (see Section 13.1.8).

Procedural

7. Parent/legal guardian/representative has provided written consent prior to the patient participating in the study. Where feasible, consent or assent for patients less than 18 years of age, has also been provided by the patient.
8. Ability to take study medication provided either as capsules or combined with food/drink.
9. Patient is cooperative, willing to complete all aspects of the study, and capable of doing so with assistance of a caregiver.
10. Caregiver is able to understand the instructions and fully participate.

9.2.2 Exclusion Criteria

The presence of any of the following will exclude a patient from study enrollment:

1. Meets any of the diagnostic exclusion criteria for Rett syndrome, Typical (Neul et al, 2010);
2. Patient is participating in a clinical trial with another investigational drug or has taken an investigational drug within one month or 5 half-lives (whichever is longer) prior to screening;
3. Hypersensitivity to sarizotan or other 5-HT1a agonists, [*UK only*: or to the capsule material gelatin or microcrystalline cellulose used in the placebo capsules];
4. Current clinically significant (as determined by Investigator)
   a. cardiovascular, respiratory (e.g. severe asthma), gastrointestinal, renal, hepatic, hematologic or other medical disorders, in addition to those directly related to the patient's Rett syndrome [*for US, Italy, India and Australia*];
   b. cardiovascular, respiratory (e.g. severe asthma), or gastrointestinal disease, renal impairment (as indicated by creatinine >2X ULN), hepatic impairment (as indicated by total bilirubin >2X ULN), history of moderate or severe hepatic insufficiency or moderate or severe liver cirrhosis, or hematologic or other medical disorders, in addition to those directly related to the patient's Rett syndrome [*for UK only*];
5. QTcF interval on the ECG is greater than 450 msec.
6. Surgery planned during the study (except for insertion of gastrostomy tube);
7. Severe diabetes mellitus or fatty acid oxidation disorder.
8. Ophthalmologic history including any of the following conditions: albino patients, family history of hereditary retinal disease, retinitis pigmentosa, any active retinopathy or severe diabetic retinopathy.
9. Females who are pregnant, breastfeeding, or of childbearing potential and not using adequate contraception (see Section 13.1.8), as recommended by their Health Care Provider.
10. [*UK only*: Subjects who have an inborn error of metabolism.]

11. Evidence of clinically significant malnutrition.

9.2.3 Documentation of Randomization

Only subjects who meet all of the inclusion criteria and none of the exclusion criteria for randomization are eligible for randomization into the treatment phase. In addition to violations of inclusion and exclusion criteria, reasons for randomization failure include a major protocol deviation, lost to follow-up, voluntary withdrawal, and study termination; the primary reason must be noted on the applicable CRF. Randomization will be on Day 1 at the start of dosing.

9.2.4 Premature Discontinuation

Subjects who discontinue from the study prematurely must have their reason for discontinuation entered in the CRF. Subjects who discontinue from the study after having received at least one dose of study medication will not be replaced. All subjects who discontinue prematurely from the initial 24-week double-blind treatment period will be asked to return for a final assessment at which time all Week 24 assessments are to be performed. Similarly, all subjects who discontinue prematurely from the open-label extension treatment period will be asked to return for a final assessment at which time all Week 48 (initial 24-week open-label period), Week 96 (Part 1 of the additional open-label period), Week 144 (Part 2 of the additional open-label period), or Week 192 (Part 3 of the additional open-label period) assessments are to be performed.

9.2.5 Record of Screening Failures

Investigators must account for all subjects who failed screening and have signed informed consent forms. The primary reason for screen failure will be recorded on the screen failure CRF using the following categories:

- Did not meet entry criteria
- Major protocol deviation
- Pretreatment Event/Adverse Event
- Lost to follow-up
- Voluntary withdrawal (specify reason)
- Study termination
- Other (specify reason)

Subject numbers assigned to subjects who fail screening will not be reused.

9.2.6 Record of Study Participants

The investigator will be required to maintain a confidential record of all study participants, including all subjects who were screened for the study, but were not actually randomized to treatment. The confidential record must include sufficient information so that it would be possible to contact the study subject.

9.2.7 Enrolment in Extension Treatment Period

All patients who have completed the final evaluations at Week 24 (Day 168) will be eligible for continuing open-label treatment with sarizotan in the initial 24-week open-label extension treatment period, if they meet the following criteria:

- have no safety or tolerability issues that would preclude continuing on the study medication,
- have been compliant with the trial requirements, and

- have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator.

At the end of the initial 24 weeks of open-label treatment, the Investigator will make a global assessment of the patient. If in the Investigator's opinion, the patient is benefitting from treatment with sarizotan and is not experiencing any significant adverse events, they will be eligible to continue open-label treatment. Informed consent must be obtained at Week 48, for the patient to continue open-label treatment for an additional 48 weeks in Part 1 of the additional open-label treatment period (through Week 96). A similar assessment of the patient will be performed at Week 96 for patients being considered for continuing treatment in Part 2 of the additional open-label treatment period. Informed consent must be obtained for the patient to continue open-label treatment for another 48 weeks (through Week 144). For patients completing Part 2, a similar assessment of the patient will be performed at Week 144 for patients being considered for continuing treatment in Part 3 of the additional open-label treatment period. Informed consent must be obtained for the patient to continue open-label treatment for another 48 weeks (through Week192).

10 STUDY MEDICATION

10.1 Description, Labeling and Packaging

10.1.1 Description of the Supplies

The supplies for the study will consist of sarizotan capsules (2-mg, 5-mg and 10-mg dosage strengths) provided to each site in separate bottles with a child-proof screw cap. Bottles with matching placebo will also be provided for the initial double-blind treatment period. Bottles will be packaged as separate 2-bottle kits that will be blinded for use during the double-blind dosing period, and will have the dose of sarizotan indicated on the labels of the box and bottles for the open-label dosing period. Medication kits will be packaged for 1-day, 6-day and 2-week periods. Four kits will be provided for the initial 2-week double-blind titration period: Dose Level 1 (1-day kit); Dose Level 2 (6-day kit), Dose Level 3 (1-day kit) and Dose Level 4 (6-day kit). Multiple 2-week kits will be provided to cover the 4-week (2 kits), 6-week (3 kits) and 8-week (4 kits) intervals between scheduled clinic visits.

10.1.2 Labelling

The following information will appear on the study medication bottles:

- Protocol No. Sarizotan-001-II-2015
- Space for entering the Subject No.
- Bottle A (for morning dose) or Bottle B (for evening dose)
- Unique medication kit number
- Dose strength (5 mg or 10 mg sarizotan) – *open-label period only*
- Project Code and Batch Number
- Quantity of capsules
- Date of manufacture
- Stored at 15 - 25°C
- Caution: New Drug – Limited by Federal (United States) Law to Investigational Use Only
- Newron Pharmaceuticals
- Space for entering name of Investigator
- Any other country-specific requirements.

In addition, labels for designating the Study Days for which a kit is to be used will be provided to the site pharmacist for attaching to the kits designated for the initial titration period. For the initial dose titration period these labels would be as follows: Day 1, Days 2-7, Day 8, and Days 9-14.

10.1.3 Packaging

The Investigational Medicinal Product (IMP) will be packaged and labeled in accordance with all applicable regulatory requirements and GMP Guidelines. Combinations of capsules containing the active drug (2 mg, 5 mg or 10 mg sarizotan) or matching placebo capsules will be used for the initial double-blind period to achieve the planned target doses of 2, 5 or 10 mg, or placebo, which will be given twice daily. The packaging of the medication for each treatment group by study day and dose level is presented in Table 3 for the initial treatment period. Extra capsules will be provided in each bottle for each period of dosing in case of lost or damaged medication, or a delay in the patient returning for a scheduled visit. The medication for each dosing interval (1-day, 6-day or 2-week) will be packaged as 2-bottle kits, with expiry date on the carton label. Separate bottles will be provided for morning (AM) and evening (PM) dosing. Two-bottle kits

for dosing after the titration period will contain enough medication for 2 weeks of dosing plus 2 extra capsules (16 capsules total). Multiple kits will be supplied to cover the period of dosing prior to the next scheduled visit.

Table 3. Drug Packaging According to the Planned Dose Titration – Initial Double-Blind Treatment Period

Group	Target Dose	Suggested Dosing Time	Study Days				
			1 #	2-7 #	8 #	9-14 #	15-168*
			Dose Level 1	*Dose Level 2*	*Dose Level 3*	*Dose Level 4*	*Dose Level 4 or MTD*
1 [Low Dose]	2 mg *bid* Sarizotan	8:00 AM	1 x 2 mg Sar	6 x 2 mg Sar	1 x 2 mg Sar	6 x 2 mg Sar	7 x 2 mg Sar
		8:00 PM	1 x Pbo	6 x Pbo	1 x 2 mg Sar	6 x 2 mg Sar	7 x 2 mg Sar
	5 mg *bid* Sarizotan	8:00 AM	1 x 5 mg Sar	6 x 5 mg Sar	1 x 5 mg Sar	6 x 5 mg Sar	7 x 5 mg Sar
		8:00 PM	1 x Pbo	6 x 5 mg Sar	1 x 5 mg Sar	6 x 5 mg Sar	7 x 5 mg Sar
2 [High Dose]	5 mg *bid* Sarizotan	8:00 AM	1 x 2 mg Sar	6 x 2 mg Sar	1 x 5 mg Sar	6 x 5 mg Sar	7 x 5 mg Sar
		8:00 PM	1 x Pbo	6 x 2 mg Sar	1 x 2 mg Sar	6 x 5 mg Sar	7 x 5 mg Sar
	10 mg *bid* Sarizotan	8:00 AM	1 x 5 mg Sar	6 x 5 mg Sar	1 x 10 mg Sar	6 x 10 mg Sar	7 x 10 mg Sar
		8:00 PM	1 x Pbo	6 x 5 mg Sar	1 x Pbo	6 x 10 mg Sar	7 x 10 mg Sar
3	Placebo	8:00 AM	1 x Pbo	6 x Pbo	1 x Pbo	6 x Pbo	7 x Pbo
		8:00 PM	1 x Pbo	6 x Pbo	1 x Pbo	6 x Pbo	7 x Pbo

Each bottle for the 14-day titration period will contain one extra capsule in case of loss or damage.

*Number of capsules supplied for each week of dosing; patient will continue at maximum tolerated dose (MTD). Each of the 2-week kits dispensed during this maintenance period will have 2 extra capsules in each bottle in case of loss or damage.

Abbreviations: Pbo = Placebo; Sar = Sarizotan

Packaging for the 168-week open-label extension treatment period will consist of 2-bottle kits containing enough medication (2-mg, 5-mg or 10-mg sarizotan capsules) for 2 weeks of dosing plus 2 extra capsules (16 capsules total). The dose strength will be pre-printed on the labels on the boxes for the 2-bottle kits, as well as on the individual bottles. Multiple kits will be supplied to cover the period of dosing prior to the next scheduled visit. Study medication dispensing for the open-label period by visit is presented in Tables 4A and 4B.

Table 4A. Study Medication Dispensing by Visit and Age/Body Weight Category: Initial Open-Label Extension Period (Weeks 24 - 40)

Study Week (Day)	Age / Body Wt. Category	Dose Type	Sarizotan Daily Dose	Dosing Period	No. of Two-Bottle Kits (Bottles) Dispensed [$]	Total No. of Capsules Dispensed
Week 24 (Day 168)	<13 yr / ≥10 kg, **or** ≥13 yr / <25 kg	Starting	2 mg *bid*	4 weeks	2 (4)	64
	≥13 yr / ≥25 kg	Starting	5 mg *bid*	4 weeks	2 (4)	64
Week 28 (Day 196)	<13 yr / ≥10 to <18 kg	Target	2 mg *bid*	4 weeks	2 (4)	64
		Drop-back (*od* dosing) #	2 mg *od*	4 weeks	1 (2)	32
	<13 yr / ≥18 kg, **or** ≥13 yr / <25 kg	Target	5 mg *bid*	4 weeks	2 (4)	64
		Drop-back *	2 mg *bid*	4 weeks	2 (4)	64
		Drop-back (*od* dosing) #	2 mg *od*	4 weeks	1 (2)	32
	≥13 yr / ≥25 kg	Target	10 mg *bid*	4 weeks	2 (4)	64
		Drop-back *	5 mg *bid*	4 weeks	2 (4)	64
		Drop-back (*od* dosing) #	5 mg *od*	4 weeks	1 (2)	32
Weeks 32 & 40 (Days 224 & 280)	<13 yr / ≥10 to <18 kg	Target	2 mg *bid*	8 weeks	4 (8)	128
		Drop-back (*od* dosing) #	2 mg *od*	8 weeks	2 (4)	64
	<13 yr / ≥18 kg, **or** ≥13 yr / <25 kg	Target	5 mg *bid*	8 weeks	4 (8)	128
		Drop-back *	2 mg *bid*	8 weeks	4 (8)	128
		Drop-back (*od* dosing) #	2 mg *od*	8 weeks	2 (4)	64
	≥13 yr / ≥25 kg	Target	10 mg *bid*	8 weeks	4 (8)	128
		Drop-back *	5 mg *bid*	8 weeks	4 (8)	128
		Drop-back (*od* dosing) #	5 mg *od*	8 weeks	2 (4)	64

Patients unable to tolerate the starting dose (2 or 5 mg *bid*), may drop back to a once daily dose (2 or 5 mg *od*), and later reattempt the starting dose; or they may continue on this lower dose throughout the study.

* Patients having minor tolerability issues at the starting dose (2 or 5 mg *bid*), as well as patients who are unable to tolerate the target dose (5 or 10 mg *bid*), may remain on, or drop back to, the starting dose of 2 or 5 mg *bid,* respectively, and continue on this dose throughout the study.

[$] Each bottle will contain 16 capsules, enough for 2 capsules/day for 7 days + 2 extra capsules in case of loss or damage, or a delay in returning for a scheduled visit.

Table 4B. Study Medication Dispensing by Visit and Age/Body Weight Category: Additional Open-Label Extension Period (Weeks 48 – 180)

Study Week (Day)	Age / Body Wt. Category	Dose Type	Sarizotan Daily Dose	Dosing Period	No. of Two-Bottle Kits (Bottles) Dispensed [$]	Total No. of Capsules Dispensed
Weeks 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, and 180 (Days 336, 420, 504, 588, 672, 756, 840, 924, 1008, 1092, 1176 and 1260)	<13 yr / ≥10 to <18 kg	Target	2 mg *bid*	12 weeks	6 (12)	192
		Drop-back (*od* dosing) #	2 mg *od*	12 weeks	3 (6)	96
	<13 yr / ≥18 kg, **or** ≥13 yr / <25 kg	Target	5 mg *bid*	12 weeks	6 (12)	192
		Drop-back *	2 mg *bid*	12 weeks	6 (12)	192
		Drop-back (*od* dosing) #	2 mg *od*	12 weeks	3 (6)	96
	≥13 yr / ≥25 kg	Target	10 mg *bid*	12 weeks	6 (12)	192
		Drop-back *	5 mg *bid*	12 weeks	6 (12)	192
		Drop-back (*od* dosing) #	5 mg *od*	12 weeks	3 (6)	96

Patients unable to tolerate the starting dose (2 or 5 mg *bid*), may drop back to a once daily dose (2 or 5 mg *od*), and later reattempt the starting dose; or they may continue on this lower dose throughout the study.

* Patients having minor tolerability issues at the starting dose (2 or 5 mg *bid*), as well as patients who are unable to tolerate the target dose (5 or 10 mg *bid*), may remain on, or drop back to, the starting dose of 2 or 5 mg *bid,* respectively, and continue on this dose throughout the study.

[$] Each bottle will contain 16 capsules, enough for 2 capsules/day for 7 days + 2 extra capsules in case of loss or damage, or a delay in returning for a scheduled visit.

Drug supplies and randomization of patients to treatment will be managed by an Interactive Voice or Web Response System (IWRS). At the start of each dosing interval, the Investigator will enter the patient number in the IWRS and indicate whether it is the first dose, or if the patient's dose will be increased, decreased or maintained at the current level. The IWRS will provide the Investigator with the appropriate unique number(s) corresponding to the blinded/unblinded medication kit(s) that is (are) to be dispensed to the patient for that dosing period.

10.2 Administration

The test drug (sarizotan) will be provided by the Sponsor in the form of capsules at dosage strengths of 2, 5 and 10 mg. The placebo for use in the double-blind period will be provided in matching capsules. All study drugs, together with relevant documentation, will be supplied to the investigational site.

The first 2 doses of study medication (Dose Level 1) will be administered to the patient in the clinic on Day 1 in the presence of a licensed physician. On Day 2, if there are no tolerability issues, the patient will receive Dose Level 2 in the clinic and will be observed for at least 4 hours post-dose. If there are no tolerability issues, the patient will then receive a supply of study medication for the next 12 days and return home. Dose increases to Dose Levels 3 and 4 will be performed on Days 8 and 9 when the patient is residing at home. The patient/caregiver will be contacted by telephone on Day 7 to assess the tolerability of the current dose before the dose titration is continued. The date, time, and number of capsules administered in the hospital setting will be recorded on the CRFs. The doses of sarizotan or matching placebo to be administered to the patient for each dose level are summarized by treatment group in Table 5.

Table 5. Dose Titration by Treatment Group in Initial Double-Blind Treatment Period

		Sarizotan Doses								Matching Placebo
		Low Dose				High Dose				
		2 mg *bid* [a,c]		5 mg *bid* [b]		5 mg *bid* [a,c]		10 mg *bid* [b]		
Dose Level	Study Day(s)	AM Dose	PM Dose	AM Dose	PM Dose	AM Dose	PM Dose	AM Dose	PM Dose	
1	1	2 mg	Placebo	5 mg	Placebo	2 mg	Placebo	5 mg	Placebo	1 capsule *bid*
2	2-7	2 mg	Placebo	5 mg	5 mg	2 mg	2 mg	5 mg	5 mg	1 capsule *bid*
3	8	2 mg	2 mg	5 mg	5 mg	5 mg	2 mg	10 mg	Placebo	1 capsule *bid*
4	9-14	2 mg	2 mg	5 mg	5 mg	5 mg	5 mg	10 mg	10 mg	1 capsule *bid*
4	15-168	2 mg	2 mg	5 mg	5 mg	5 mg	5 mg	10 mg	10 mg	1 capsule *bid*

[a] Patients 4 to <13 years of age, (randomized 1:1:1 to sarizotan 2 mg *bid*, sarizotan 5 mg bid or placebo *bid*)
[b] Patients ≥13 years of age, weighing ≥25 kg (randomized 1:1:1 to sarizotan 5 mg *bid*, sarizotan 10 mg *bid*, or placebo *bid*)
[c] Patients ≥13 years of age, weighing <25 kg (randomized 1:1:1 to sarizotan 2 mg *bid*, sarizotan 5 mg *bid* or placebo *bid*)

In the initial double-blind period, the study medication will be administered as capsules of 2, 5 and 10 mg dosage strengths of sarizotan, or an equal number of matching placebo capsules. Doses will be administered as 1 capsule *bid* given at approximately 8:00 AM and 8:00 PM, with an interval of approximately 12 hours (minimally 6 hours) between doses.

If intolerance develops, the Investigator may reduce a patient's dose to the next lower level at any time. If patients do not tolerate the proposed titration and target dose, the investigator will instruct the caregiver to administer only one of the two daily doses (e.g. 2 or 5 mg once daily) for a few days prior to reinitiating the intended *bid* dose (e.g. 2 or 5 mg *bid*). If the dose is still not tolerated, a reduction to a lower rescue dose will be permitted. Patients unable to tolerate Dose Level 1, even after a dose interruption, should be discontinued from the study. Dose titration should be completed by Day 14, after which patients will continue on their maximum tolerated dose (MTD) through the final dose in the double-blind period, unless intolerance develops and a dose reduction is warranted.

In the open-label extension treatment period, all patients, including those originally randomized to placebo, will receive treatment with sarizotan, and will start at a dose of 2 mg *bid* (patients 4 to <13 years of age and weighing 10 to <18 kg, and patients ≥13 years of age but weighing <25 kg) or 5 mg *bid* (patients ≥13 years of age and weighing ≥25 kg). The first dose (2 or 5 mg) will be administered in the clinic (if the morning dose was given, wait at least 6 hours after the prior dose before administration), in the presence of a licensed physician, on the same day as the Week 24 evaluations from the initial period, if possible. The date, time, and number of capsules administered in the hospital setting on the first day of open-label dosing will be recorded on the CRF. If no tolerability issues are noted in the 4-hr post-dose follow-up period, the patient will then receive a supply of study medication (sarizotan 2-mg or 5-mg capsules) for the 4-week period until the next scheduled visit and return home.

The caregiver will be contacted by telephone by the Investigator/site staff on Day 175 (Week 25) to inquire about the tolerability of the study medication and any adverse events the patient might have experienced. If patients do not tolerate this starting dose, the Investigator will instruct the caregiver to administer only the morning dose (i.e. 2 or 5 mg/day) for a few days prior to reinitiating the intended *bid* dose (i.e. 2 or 5 mg *bid*). If the 2 or 5 mg *bid* dose is still not tolerated, a reduction to 2 or 5 mg once daily will be permitted.

At Week 28, patients will return to the clinic for safety and efficacy assessments, and will have their dose maintained at 2 mg *bid* (patients 4 to <13 years of age and weighing 10 to <18 kg), increased to 5 mg *bid* (patients 4 to <13 years of age and weighing ≥18 kg, and patients ≥13 years of age and weighing <25 kg) or increased to 10 mg *bid* (patients ≥13 years of age and weighing ≥25 kg), if the starting dose was well tolerated. The first 2-mg, 5-mg or 10-mg dose will be administered in the clinic (if the morning dose was given, wait at least 6 hours after the prior dose before administration). The date, time, and number of capsules administered in the hospital setting will be recorded on the CRF. If no tolerability issues are noted in the 4-hr post-dose follow-up period, the patient will then receive a supply of study medication (sarizotan 2-mg, 5-mg or 10-mg capsules) for the 4-week period until the next scheduled visit and return home.

The caregiver will be contacted by telephone by the Investigator/site staff on Day 203 (Week 29) to inquire about the tolerability of the study medication. If the patient does not tolerate this dose, the Investigator will instruct the caregiver to administer only the morning dose (i.e. 2, 5 or 10 mg/day) for a few days prior to reinitiating the intended *bid* dose (i.e. 2, 5 or 10 mg *bid*). If the target dose of 2, 5 or 10 mg *bid* is still not tolerated, a reduction to 2 mg once daily, 2 mg bid, or 5 mg *bid*, respectively, will be permitted. Patients will continue on their maximum tolerated dose (MTD) through the final dose, unless intolerance develops and a dose reduction is warranted. Patients completing the initial 24 weeks of open-label treatment and entering the additional open-label treatment period will continue on the same dose of sarizotan.

If intolerance develops at any time during the extension treatment period, the Investigator may reduce a patient's dose to the next lower level. Alternatively, the Investigator may instruct the caregiver to administer

only one of the two daily doses (e.g. 2 mg, 5 mg or 10 mg, once daily) for a few days prior to reinitiating the intended *bid* dose (e.g. 2 mg *bid*, 5 mg *bid* or 10 mg *bid*). If the dose is still not tolerated, a reduction to a lower dose will be permitted. Patients unable to tolerate a dose of 2 mg or 5 mg once daily (dependent on age and weight) must be discontinued from the study.

Flexibility will be allowed in dosing during the open-label extension period to accommodate the needs of individual patients. Investigators will use medical judgement to improve tolerability by adjusting dosing in cases of intolerance. If deemed necessary by the Investigator for minimizing intolerance and maintaining efficacy, different doses may be administered in the morning and evening, e.g., 10 mg in the morning and 5 mg in the evening for a patient not tolerating the assigned 10 mg *bid* dose. In all of these cases, the Investigator must contact the Medical Monitor to discuss the planned dosing. Appropriate medication kits will be dispensed and instructions provided to the caregiver by the Investigator to implement the revised dosing paradigm.

For patients unable to swallow a capsule, sarizotan may be administered as a drinkable solution. This requires dissolving the sarizotan hydrochloride AIC in apple juice, cranberry juice, orange juice, tea (with or without milk), or water. The details of these procedures are provided in Appendix 6. In the case of patients who have severe difficulty with swallowing and are currently receiving alimentation through a gastrostomy tube (G-tube), sarizotan- may be administered through the G-tube as capsules dissolved in water (see Appendix 6 for details).

10.3 Storage

In the clinic, supplies of the study medication must be stored at 15 - 25°C, in a secure locked area. During out-patient treatment, patients will be requested to store the medication at room (ambient) temperature. All unused study medication and medication bottles must be returned to Newron or its designee at the end of the trial or destroyed by the study site per their local SOP following completion of accountability by the CRO's monitor and authorization from Newron. The destruction of unused medication will be documented in accordance with ICH GCP.

10.4 Blinding and Randomization

Subject identifier

Each screened subject will receive a five-digit subject number, with the first two digits specifying the center number and the last 3 digits the subject at the center. The "ID" field will appear in the header at the top-center of each CRF page, with a space for entering the "Subject No."

The Investigator will assign the five-digit subject number to each patient at screening. The subject number should be assigned sequentially, beginning with **##-001**, in the order of the patients (or their parent/legal guardian/representative) signing the informed consent. This five-digit number should be entered in the "Subject No." space on every CRF for a given patient.

Should a patient discontinue from the study for any reason after having been assigned a subject number, the number will not be reassigned to another patient. Information regarding the demographics of the patient and reason for discontinuation will be recorded in an End of Screening Log.

In cases where the Investigator is uncertain of a patient's eligibility for the study (e.g., selection criteria, coexistent medical conditions or concomitant therapy) the Medical Monitor should be contacted to confirm the appropriateness of the inclusion of the patient.

Patient Randomization

A minimum of 129 patients will be randomized approximately equally to treatment with low dose or high dose sarizotan or placebo *bid* as follows:

- Age 4 to <13 years randomized 1:1:1 to sarizotan 2 mg *bid* [low dose sarizotan group], sarizotan 5 mg *bid* [high dose sarizotan group] or placebo *bid*;
- Age ≥13 years and weight <25 kg - randomized 1:1:1 to sarizotan 2 mg *bid* [low dose sarizotan group], sarizotan 5 mg *bid* [high dose sarizotan group] or placebo *bid;*
- Age ≥13 years, weight ≥25 kg – randomized 1:1:1 to sarizotan 5 mg *bid* [low dose sarizotan group], sarizotan 10 mg *bid* [high dose sarizotan group] or placebo *bid*.

A randomization number corresponding to the treatment assignment will be assigned to each randomized subject using the IVRS/IWRS, and must be registered in the subject's files for identification. Each patient randomized at the site should receive the next sequential randomization and treatment assignment.

Breaking the Blind

Should it be necessary to break the blind during the double-blind period for reasons of safety, the Investigator will be able to break the blind using the IWRS. The Investigator will be asked to inform the Sponsor and their representative of this as soon as possible and to provide a reason for the unblinding.

10.5 Accountability

In accordance with ICH and FDA requirements, the Investigator or Drug Dispensing Person (e.g., pharmacist) must at all times be able to account for all study drugs furnished to the institution. The pharmacist or the Investigator must sign the Drug Receipt Form, confirming that he/she has received the clinical supplies for the study and provide assurance that the investigational products will be handled and stored properly.

The investigational treatments must only be dispensed to subjects in accordance with the protocol. At the end of the study, it must be possible to reconcile delivery records with those of usage and returned stocks. Account must be given of any discrepancy. Any unused products must be returned to the Drug Broker. Certificates of returns must be signed with assurance from the pharmacist/investigator that all unused investigational drugs and all medication containers provided for the stated study have been returned.

Under no circumstances is the Investigator allowed to use the study medications other than as directed by the protocol.

10.6 Overdosage

To date more than 1800 subjects have been exposed to at least one dose of sarizotan in clinical studies in healthy volunteers and patients with schizophrenia or Parkinson's disease. The maximum single dose given in these studies was 200 mg. This dose, which is 20 times higher than the maximum individual dose to be administered in the current trial, was not associated with any significant adverse effects. Therefore, in case of an accidental overdosage, conservative management of symptoms and signs is advised. The procedure for reporting a symptomatic overdose is described in Section 13.1.6.

10.7 Occupational safety

Sarizotan is not expected to pose any significant occupational safety risks to investigational staff under normal conditions of use and administration. Caregivers and site staff should take appropriate precautions when heating juice or other liquids to 80°C for dissolving capsules for administration to patients who are unable to swallow the medication in a capsule form.

11 EVALUATIONS AND PROCEDURES

11.1 Written Informed Consent

Written informed consent must be obtained from the patient or his/her parent (if the patient is below the age of consent or unable to consent), or legal guardian/representative (if necessary), at screening prior to initiating any study procedures required by the protocol, according to the procedure described in Section 16.1. The patient, if able, may provide assent. The details of the study should be discussed with the patient and parent/caregiver or legal representative (if necessary) prior to obtaining informed consent, and the Informed Consent Form (ICF) must be signed and dated by the patient, parent/caregiver, or legal representative (if necessary), and by the Investigator or his/her designee. A copy of the signed ICF will be provided to the patient/ parent/ legal guardian/representative, and the original will be retained with the source documents. The initial consent will cover one year (48 weeks) of treatment; however, the patient/ parent/ legal guardian/representative will be asked to provide an additional consent/assent after completing the 6-month initial treatment period, before continuing in the initial 24-week, open-label extension treatment period. At the end of the initial 24 weeks of open-label treatment, the Investigator will make a global assessment of the patient. If in the Investigator's opinion, the patient is benefitting from treatment with sarizotan and is not experiencing any significant adverse events, they will be eligible to continue open-label treatment. The patient/ parent/ legal guardian/representative must provide informed consent/assent at Week 48 for the patient to continue open-label treatment for an additional 48 weeks in Part 1 of the additional open-label extension period (through Week 96), again at Week 96 for the patient to continue open-label treatment for another 48 weeks in Part 2 of the additional open-label extension period (through Week 144), and again at Week 144 for the patient to continue open-label treatment for another 48 weeks in Part 3 of the additional open-label extension period (through Week 192).

11.2 Study Conduct

Initial 24-Week Double-blind Treatment Period

Each patient's parent, legal guardian, or representative, must provide written informed consent prior to the patient's participation in the trial. The patient should provide assent, if capable of doing so. Each patient will enter a 4-week period during which the screening evaluations will be performed. Patients who meet the respiratory criteria for enrolment in the study in one of the first 3 weeks may have their screening period shortened. The screening assessments, which must be completed and results available prior to baseline, will consist of the following: a confirmation of the diagnosis of Rett syndrome, based on clinical criteria (a proven mutation of the methyl CpG-binding protein 2 (MECP2) gene is not required), a review of the patient's medical history and current use of medications, vital signs, 12-lead electrocardiogram (ECG), laboratory tests (hematology, blood chemistry, urinalysis, virology, serum pregnancy test for post-pubertal females, and thyroid function tests), and physical and neurological examinations. The patient's respiratory abnormalities will be, documented, based on parental feedback for the presence of one of the following: periodic apnea during wakefulness; intermittent hyperventilation; breath holding spells; air swallowing; forced expulsion of air or saliva.

Cardio-respiratory monitoring using a specialized ambulatory data acquisition system, the BioRadio™ system, will be performed at home during the first week of the screening period to ensure proficiency of the caregiver/patient in using the system, and to evaluate the quality of the data and resolve any problems (this will be determined by the remote data monitoring service provider, Vivonoetics). Data from the BioRadio system will be used to confirm that the patient has at least 10 episodes of breathing dysrhythmia per hour over a 24-hour period. Home monitoring should be attempted for 6 hours each day, during the time the patient is awake, on at least 3 days during the first, second and third week of screening. The patient

must meet all criteria related to breathing abnormalities in at least one of the three screening period assessments, as specified in the Inclusion Criteria, to be eligible for enrollment. If the patient meets the criteria, as determined by Vivonoetics, in any week of the Screening period, home monitoring in the subsequent week(s) of Screening would not be required. Adverse events and use of concomitant medication will be recorded from the time of signing of the consent form.

At the Baseline visit (Day 0), the inclusion/exclusion criteria, (respiratory inclusion criteria will be assessed based on home respiratory monitoring performed during screening) will be reviewed to ensure that the patient is suitable for enrolment. Patients meeting all entry criteria at baseline will undergo a series of evaluations on the day prior to dosing (Day 0). Baseline assessments of respiratory function will be performed in the clinic using a stretch-sensitive resistance plethysmograph (SOMNOtouch™ device), Tanner Staging will be performed, and a blood sample will be taken for measurement of serum prolactin, and plasma ACTH and cortisol. The routine laboratory tests and physical and neurological examinations do not need to be repeated if performed less than 28 days before baseline, unless abnormalities are noted that require follow-up. A routine ophthalmological examination (including visual acuity, lens, intraocular pressure and dilated examination of the fundus, and OCT, if feasible for the patient) will be performed. A serum pregnancy test must be performed for post-pubertal females. In addition, baseline assessments for all of the efficacy variables [respiratory measures, heart rate, Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and RCSS], as well as the suicidality assessment, will be performed on Day 0. Baseline vital signs and ECG evaluations will be performed in triplicate (10 min apart) on Day 0 or at least one hour prior to dosing on Day 1.

On Day 1 the patient will be randomized to treatment, with the dose assigned based on age and body weight categories, as described in Section 10.4, and will receive the initial oral dose of study medication (Dose Level 1: 2 or 5 mg sarizotan or matching placebo) in the morning. In the initial 12-hour period after the first dose, safety evaluations (vital signs, 12-lead ECG) will be performed at 1 and 4 hr post-dose. Blood samples for the pharmacokinetic (PK) analysis will be collected prior to dosing (blank sample) and at approximately 1 and 4 hr post-dose, following completion of the vital signs and ECG assessments. The patient will receive the evening dose (Dose Level 1: placebo for all patients) in the hospital (minimally 6 hours after the morning dose) and will be housed overnight, either in the hospital or with their parent/caregiver in a separate facility. The morning dose of Dose Level 2 (2 or 5 mg sarizotan or placebo, *bid*) will be administered on Day 2 in the clinic. Safety evaluations [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis, and measurement of plasma ACTH and cortisol), physical/neurological examination, assessment of adverse events] will be repeated, and if no significant side effects are noted within the 4-hr period following this dose, the patient will be released from the hospital given a supply of study medication for the remainder of the first week of dosing at Dose Level 2 (Days 2 [PM] to 7) and for the second week of dosing at Dose Level 3 [2 mg sarizotan *bid,* 5 mg sarizotan *bid*, 7 mg sarizotan/day (5 mg in AM + 2 mg in PM), 10 mg sarizotan once daily (in AM, with placebo in PM) or placebo *bid*; on Day 8] and Dose Level 4 [2 mg sarizotan *bid,* 5 mg sarizotan *bid*, 10 mg sarizotan *bid*, or placebo *bid*; on Days 9 to 14].

The caregiver will receive a telephone contact from the Investigator/site staff on Day 7 to inquire about the tolerability of Dose Level 2 of the study medication and any adverse events the patient might have experienced. If, in the Investigator's judgement, there are no tolerability issues that would preclude a dose increase, the patient/caregiver will be instructed to increase the dose to Dose Level 3 on Day 8 and Dose Level 4 on Day 9. During the time the patient is at home, monitoring of their cardio-respiratory function will be attempted for 6 hours each day, during the time the patient is awake, on any 3 days in the week preceding the next scheduled office visit on Day 14, using the BioRadio. Data recorded using this system will be downloaded from the device at home using the Internet/WiFi or a dedicated laptop provided by the Sponsor, if Internet access is not available.

The patient will be required to return for a scheduled visit on Day 14 and safety [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis), physical/neurological examination] and selected efficacy (respiratory measures, CGI-C, CIC) evaluations, will be performed. The tolerability of the current dose of study medication will be evaluated, and the patient will be housed overnight, if necessary. On Day 15, if there are no safety or tolerability issues that would require a dose reduction, the patient will be given the morning dose of Dose Level 4 (2 mg sarizotan, 5 mg sarizotan, 10 mg sarizotan or placebo, *bid*). Prior to administering the dose, a PK blood sample for assessing steady state trough levels of sarizotan at Dose Level 4 will be taken, and PK blood samples will be collected at 1 and 4 hr post-dose. Post-dose safety assessments (vital signs, ECG) will be performed, and if there are no tolerability issues, the patient will be released from the hospital after the last PK sample, and will be provided with a supply of Dose Level 4 (or a lower dose if Dose Level 4 was not tolerated) for dosing on Days 15 [PM] to 56 (Week 8). Home respiratory monitoring will be attempted for 6 hours each day on any 3 days in the week prior to each of the scheduled office visits at Weeks 8, 16 and 24.

The patient will be required to return for scheduled visits on Weeks 8 and 16, and safety (vital signs, routine laboratory tests, ECG, physical and neurological examinations) and efficacy evaluations will be performed. The suicidality assessment will be performed at Week 8. In addition, serum prolactin and plasma ACTH and cortisol measurements, and an ophthalmological examination will be done at Week 16. At each of these visits, the patient will be dispensed an 8-week supply of their current dose level of study medication for dosing during the period until the next scheduled visit, provided there are no safety or tolerability issues that would require a dose reduction.

The patient will return for the final scheduled visit at Week 24, or if they discontinue from the study prematurely. The caregiver should be instructed not to administer the morning dose of the study medication on the day of the Week 24 visit to the clinic, as patients who continue in the open-label extension treatment period will receive their first dose of study medication on this day. All final safety [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis, and measurement of serum prolactin, and plasma ACTH and cortisol), Tanner Staging, and physical, neurological and ophthalmological examinations (including OCT, if feasible for the patient), and the suicidality assessment] and efficacy assessments [respiratory measures, heart rate, CGI-C, CIC, Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and RCSS] will be performed at this visit. The results from the Week 24 assessment of the primary efficacy measure of respiratory function (data from home respiratory monitoring on 3 days in the week preceding the visit), as well as all other secondary efficacy measures assessed during the visit, will be considered the endpoint value for statistical analysis purposes.

All patients who have completed the final evaluations at Week 24 (Day 168), have no safety or tolerability issues that would preclude continuing on the study medication, have been compliant with the trial requirements, and have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator, will be eligible to enter the initial 24-week, open-label, extension treatment period of the study. Patients who do not wish to continue treatment will have their study medication discontinued and will be asked to return for a safety follow-up visit 2 weeks after their final dose, during which vital signs and an assessment of adverse events will be performed. Any Serious Adverse Events (SAEs) occurring within 30 days after the final dose will be reported.

168-Week Open-label Extension Treatment Period

All patients, including those randomized to placebo, who have completed the final evaluations at Week 24 (Day 168), have no safety or tolerability issues that would preclude continuing on the study medication, have been compliant with the trial requirements, and have not worsened to the extent that they are considered at an increased risk for morbidity or mortality, as judged by the Investigator, will have the option of continuing open-label treatment with sarizotan for 24 weeks. Each patient's parent, legal guardian, or representative, must provide written informed consent prior to the patient's participation in this initial

extension period. The patient should provide assent, if capable of doing so. At the end of the initial 24 weeks of open-label treatment, the Investigator will make a global assessment of the patient. If in the Investigator's opinion, the patient is benefitting from treatment with sarizotan and is not experiencing any significant adverse events, they will be eligible to continue open-label treatment. Informed consent must be obtained at Week 48, for the patient to continue open-label treatment for an additional 48 weeks (through Week 96), again at Week 96 for the patient to continue open-label treatment for another 48 weeks (through Week 144), and again at Week 144 for the patient to continue open-label treatment for another 48 weeks (through Week 192).

At Week 24 (Day 168), all patients ≥13 years of age and weighing ≥25 kg, including those originally randomized to placebo and those receiving 5 mg once daily, will receive treatment with sarizotan, and will start at a dose of 5 mg *bid*. Patients 4 to <13 years of age and patients ≥13 years of age but weighing <25 kg, including those who were randomized to placebo, will receive sarizotan at a starting dose of 2 mg *bid*. If patients do not tolerate this starting dose, the Investigator will instruct the caregiver to administer only the morning dose (i.e. **2 or** 5 mg once daily) for a few days prior to reinitiating the intended *bid* dose (i.e. 2 or 5 mg *bid*). If the 2 or 5 mg *bid* dose is still not tolerated, a reduction to 2 or 5 mg once daily will be permitted.

At Week 28 (Day 196), patients ≥13 years of age and weighing ≥25 kg will have their dose increased to 10 mg *bid*, if the starting dose was well tolerated, or may remain on a lower dose. Patients 4 to <13 years of age and weighing 10 to <18 kg will remain on a dose of 2 mg *bid*, while those weighing ≥18 kg, as well as patients ≥13 years of age but weighing <25 kg, will have their dose increased to 5 mg *bid*, provided there are no tolerability issues. If the patient does not tolerate the target dose (2, 5 or 10 mg bid, based on age and weight criteria, the Investigator will instruct the caregiver to administer only the morning dose (i.e. 2, 5 or 10 mg/day) for a few days prior to reinitiating the intended *bid* dose (i.e. 2, 5 or 10 mg *bid*). If the target dose of 2, 5 or 10 mg *bid* is still not tolerated, a reduction to 2 mg once daily, 2 mg *bid* or 5 mg *bid*, respectively, will be permitted. Patients will continue on their maximum tolerated dose (MTD) through the final dose, unless intolerance develops, and a dose reduction is warranted.

The final evaluations performed at Week 24 in the double-blind period, including assessments of respiratory function performed in the clinic using a stretch-sensitive resistance plethysmograph (SOMNOtouch), vital signs (body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic blood pressure), laboratory tests (hematology, biochemistry, urinalysis, serum prolactin, plasma ACTH and cortisol, serum pregnancy test for post-pubertal females), ECG, physical and neurological examinations, Tanner Staging, routine ophthalmological examination [including Optical Coherence Tomography (OCT), if feasible] and final assessments for other efficacy variables [Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and Rett syndrome Clinical Severity Scale (RCSS)] will serve as the baseline assessments for the open-label extension period. Once all of these Week 24 assessments have been completed, and the consent form for the extension period has been signed, patients meeting all entry criteria will start on the low dose (5 mg *bid*) of sarizotan.

The first dose of the extension period (2 or 5 mg sarizotan) should be administered in the clinic (if the morning dose was given, wait at least 6 hours after the prior dose before administration) on the same day (Day 168); however, if necessary, it can be postponed until the following morning. Patients will be observed for the occurrence of any adverse events in the initial 4hour period after the first dose. Safety evaluations (vital signs, 12-lead ECG, physical and neurological examinations) will be performed at 1 and 4 hr- post-dose. If no significant side effects are noted within the 4-hr period following the first dose, the patient will be released from the hospital and given a supply of study medication for the remainder of the first four weeks of dosing at 2 or 5 mg *bid*.

The patient/caregiver will receive a telephone contact from the Investigator/site staff on Day 175 (Week 25) to inquire about the tolerability of the current dose of the study medication. If there are no tolerability

issues, the patient/caregiver will be instructed to continue on the current dose. If intolerance develops, a reduction to 2 or 5 mg once daily can be instituted, or if necessary, the patient can be asked to return to the clinic for an unscheduled visit for a full assessment.

The patient will be required to return for a scheduled visit on Day 196 (Week 28). The caregiver should be instructed not to administer the morning dose of the study medication on the day of the Week 28 visit to the clinic, as the first dose of the next dose level will be administered in the clinic. At this visit, safety [vital signs, 12-lead ECG, physical/neurological examinations] and selected efficacy (in-clinic respiratory measures, CIC, CGI-C) evaluations, will be performed. The tolerability of the current dose of study medication will be evaluated. If there are no safety or tolerability issues that would require the dose to be maintained or reduced, the patient will have their dose maintained at 2 mg *bid*, or increased to 5 or 10 mg *bid*, and will be given their first dose in the clinic (if the morning dose was given, wait at least 6 hours after the prior dose before administration). Safety evaluations (vital signs, 12-lead ECG, physical and neurological examinations) will be performed prior to dosing and at 1 and 4 hr post-dose. If no significant side effects are noted within the 4-hr period following the dose, the patient will be released from the hospital and given a supply of study medication for the next 4 weeks of dosing at 2 mg *bid*, 5 mg *bid* or 10 mg *bid*.

The caregiver will be contacted by telephone by the Investigator/site staff on Day 203 (Week 29) to inquire about the tolerability of the study medication and any adverse events the patient might have experienced. If there are no tolerability issues, the patient/caregiver will be instructed to continue on the current dose. If intolerance develops, a reduction in dose can be instituted, or if necessary, the patient can be asked to return to the clinic for an unscheduled visit for a full assessment. Similar telephone contacts will be made at Weeks 54, 66, 78, 90, 102, 114, 126, 138, 150, 162, 174, and 186 in the additional open-label extension treatment period, during which adverse events, concomitant medication usage and overall compliance with the study medication will be assessed.

Patients will be required to return for scheduled visits at Weeks 32, 40, and 48 in the initial 24-week open-label treatment period, and Weeks 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 if they continue open-label treatment. At each of these visits, safety assessments comprising vital signs, laboratory tests (hematology, blood chemistry, urinalysis), ECG, and physical and neurological examinations, as well as selected efficacy assessments (in-clinic respiratory measures, CIC, CGI-C), will be performed. In addition, at Weeks 48, 72, 96, 120, 144 168 and 192, the following assessments will be performed: serum prolactin, plasma ACTH and cortisol, a serum pregnancy test (for post-pubertal females), Tanner Staging, an ophthalmological examination (including OCT, if feasible for the patient), and the suicidality assessment, as well as additional efficacy assessments (Motor-Behavioral Assessment Scale, Caregiver Top 3 Concerns, and RCSS). At each of these visits, the patient will be dispensed an 8-week or 12-week supply of their current dose of study medication, provided there are no safety or tolerability issues that would require a dose reduction, for dosing during the period until the next scheduled visit. The Week 48 assessments will be considered the final evaluation for patients discontinuing after 24 weeks of open-label treatment.

All patients continuing open-label treatment after Week 48 will return for the final evaluations at Week 96, if they complete 72 weeks of open-label treatment and do not continue further treatment, or at the time of discontinuation, if they discontinue from the study prematurely prior to Week 96. Patients continuing open-label treatment after Week 96, will return for the final evaluations at Week 144, or at the time of discontinuation, if they discontinue from the study prematurely prior to Week 144. Patients continuing open-label treatment after Week 144, will return for the final evaluations at Week 192, or at the time of discontinuation, if they discontinue from the study prematurely prior to Week 192. All final safety [vital signs, 12-lead ECG, laboratory tests (hematology, blood chemistry, urinalysis, serum prolactin, plasma ACTH and cortisol, and serum pregnancy test for post-pubertal females), Tanner Staging, and physical, neurological and ophthalmological (including OCT, if feasible for the patient) examinations, and the suicidality assessment] and efficacy [in-clinic respiratory measures, CIC, CGI-C, Motor-Behavioral

Assessment Scale, Caregiver Top 3 Concerns, and RCSS] assessments will be performed at this visit. The results from the final (Week 48, 96, 144 or 192, or at early discontinuation) assessment of the respiratory function (data from in-clinic respiratory monitoring using the SOMNOtouch), as well as all other secondary efficacy measures assessed during the visit, will be considered the endpoint values for the open-label extension period for statistical analysis purposes.

Patients who discontinue treatment prematurely or at the end of the initial open-label extension treatment period (Week 48), as well as those who discontinue treatment after completing Part 1 (through Week 96), Part 2 (through Week 144), or Part 3 (through Week 192) of the additional extension treatment period will be asked to return for a safety follow-up visit 2 weeks after their final dose. Vital signs and an assessment of adverse events will be performed at this visit. Any SAEs occurring within 30 days after the final dose will be reported.

Laboratory and PK Sample Collection and Processing

Blood sampling

Venous blood samples will be drawn for safety evaluations, comprising hematology, blood chemistry, virology (hepatitis B/C and HIV), thyroid function tests, serum prolactin and plasma ACTH and cortisol levels.

The maximum blood volume collected on any day for these tests will be 25 mL:

- 2 mL will be collected for hematology tests,
- 2.5 mL will be collected for blood chemistry tests,
- 4 mL will be collected for virology tests (Screening only),
- 2.5 mL will be collected for the thyroid tests (Screening only),
- 6.25 mL will be collected for plasma ACTH (3.75 mL) and cortisol (2.5 mL) levels (Baseline, Day 2, Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192),
- 1.25 mL will be collected for plasma prolactin levels (Baseline, Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192),
- 1.25 mL will be collected for a serum pregnancy test (post-pubertal females at Screening, Baseline, Weeks 24, 48, 72, 96, 120, 144, 168 and 192),
- 6 mL for MECP2 genetic mutation testing (optional; Screening only). This test will be performed only if the patient has not had the test performed previously, or does not have adequate documentation of the test results from an accredited laboratory. The results from this test do not need to be available prior to randomizing the patient to treatment.

Urine collection

The minimal quantity of urine needed for the urinalysis is 30 ml.

Pharmacokinetic sampling

In addition to the blood samples taken for laboratory tests, 7.5-ml blood samples will be collected into 10-ml Li-heparinized test tubes for measurement of plasma concentrations of sarizotan and its main metabolites and determination of PK parameters. Samples will be collected just prior to the first dose of Dose Level 1 (blank sample) and at approximately 1 and 4 hr post-dose (±15 min) on Day 1. On Day 15,

a blood sample will be taken prior to dosing on Day 15 (trough level for Dose Level 4) and at 1 and 4 hr post-dose (Dose Level 4). The exact time of sampling, relative to dosing must be noted in the eCRF.

For each sample time point, 7.5 mL of whole blood will be collected in standard Li-heparinized vacuum tubes, gently inverted several times and centrifuged within 15 minutes at 4°C. After centrifugation (15 minutes at approx. 1500g), the supernatant plasma (at least 2 x 1.5 mL) will be immediately pipetted off, transferred into two pre-labeled polypropylene cryo-vials and deep frozen at < -70°C as soon as possible. The tubes will be kept frozen at < -70°C pending shipment for analysis.

Processing of samples

All blood and urine samples for screening (virology/thyroid tests) and routine safety parameters (hematological/ biochemical/urinalysis), serum prolactin, plasma ACTH and cortisol evaluations, and MECP2 genetic testing (optional), will be sent to a Central Laboratory, following the instructions provided by the Central Laboratory.

All plasma samples for each cohort for the assay of systemic concentrations of sarizotan will be transferred to the central laboratory (Q2 Solutions) on dry ice in batches, where they will be stored at -70°C (urine and plasma PK) until analysis. Samples will only be destroyed or sent where indicated, upon approval/request by the Sponsor.

Determination of sarizotan in human plasma

The concentration of sarizotan in human plasma will be determined using a bioanalytical method based upon liquid chromatography – tandem mass spectrometry (LC-MS/MS), which has been validated in mouse and human plasma. Quantification of the parent drug and the three major metabolites EMD 148107, EMD 329989 and EMD 50929 will be performed. The LLOQ for sarizotan and the three metabolites was 0.5 – 1 ng/mL in mouse plasma and 0.1 ng/mL in human plasma.

11.3 Study Flow Chart

The schedules of evaluations to be performed during the initial 24-week double-blind treatment period, and the 24-week initial, and three 48-week additional (Part 1, Part 2 and Part 3), open-label extension treatment periods, are presented in Tables 6A, 6B, 6C, 6D, and 6E, respectively.

Table 6A. Schedule of Evaluations: Initial 24-Week Double-blind Treatment Period

Visit / Assessment	Screening	Baseline[A]	Day 1	Day 2	Day 7	Day 14	Day 15	Week 8	Week 16	Final (Week 24)[H]	Safety follow-up[J]
Study day(s)	**-28 to -1**	**0**	**1**	**2**	**3-7**	**8-14**	**15**	**16-56**	**57-112**	**113-168**	**169-182**
Informed consent (before any study procedure)	X										
Inclusion/Exclusion Criteria	X	X									
Demography/Background Information	X										
MECP2 gene mutation confirmation	X[P]										
Medical History and Current Medical Conditions	X										
Vital Signs	X	X[C]	X[C]	X		X	X	X	X	X	X
ECG (12-lead)	X	X[D]	X[D]	X		X	X	X	X	X	
Physical Examination	X	X[B]		X		X		X	X	X	
Neurological Examination	X	X[B]		X		X		X	X	X	
Ophthalmological Examination		X							X	X	
Laboratory Evaluation (Hematology, Biochemistry, Urinalysis)	X	X[B]				X		X	X	X	
Thyroid Function Tests (TSH, T3, T4)	X										
Virology (Hepatitis B/C, HIV)	X										
Plasma ACTH and cortisol		X		X					X	X	
Serum prolactin		X							X	X	
Serum pregnancy test [O]	X	X								X	
Tanner Staging		X								X	
Suicidality assessment		X						X		X	
Dosage administration and drug label record			X	X	X	X	X	X	X	X[K]	
Prior/Concomitant Medications and Significant Non-Drug Therapies	X	X	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	X[E]	X
Respiratory monitoring (in clinic) [M]		X							X	X	
Respiratory monitoring (at home) [N]	X					X		X	X	X	
CGI-C						X		X	X	X	
CIC						X		X	X	X	
RCSS		X								X	
Motor-Behavioral Assessment Scale		X						X	X	X	
Caregiver Top 3 Concerns		X						X	X	X	
Telephone contact [G]					X						
Pharmacokinetic blood sample			X[F]				X[F]				
Overnight stay (hotel/housing) required		X	X	X[L]		X	X[L]				
Study Completion										X[I]	X

[A] Patients meeting all entry criteria will report to the hospital/clinic on Day 0. Baseline evaluations may be started on Day 0 and continued on Day 1 pre-dose. Following completion of baseline evaluations, subjects will be randomized and dosed on Day 1.

[B] This evaluation does not need to be performed at baseline unless the screening assessment was done more than 28 days beforehand or there were abnormalities noted that require follow-up.

[C] Vital signs to be performed at baseline must be repeated 3 times, with an interval of at least 10 minutes between readings, on Day 0 or at least 1 hr prior to dosing on Day 1, and at 1 and 4 hr post-dose, just prior to taking blood samples for PK measurements.

[D] 12-lead ECG will be performed at baseline and repeated 3 times, with an interval of at least 10 minutes between readings, on Day 0 or at least 1 hr prior to dosing on Day 1, and at 1 and 4 hr post-dose, just prior to taking blood samples for PK measurements.

[E] Patients will be contacted 30 days after their final dose of study medication to follow up on the occurrence of any SAEs.

[F] A pre-dose PK sample will be taken, and samples will be collected at 1 and 4 hr post dose (window ± 15 min).

[G] Telephone contact with patient/caregiver will be done on Day 7 to assess tolerability of current dose of study medication, before the increase to Dose Level 3 and Dose Level 4 on Days 8 and 9, respectively; adverse events and concomitant medication use will be assessed.

[H] All Week 24 (Final) evaluations should be performed when a subject discontinues from the study prematurely.

[I] Patients completing 168 days (+/- 7 days) of treatment will be considered to have completed the initial 6-month period of the study.

[J] Safety follow-up assessments to be performed 14 days after the final dose of study medication, if the patient is not continuing in the open-label extension treatment period, or if the patient discontinues prematurely.

[K] Record dosing from last 8-week period. Dose administration will be done only for patients continuing in the open-label extension treatment period.

[L] Patient will be released from the hospital on Day 2 and Day 15, approximately 4 hr after the morning dose, if there are no tolerability issues.

[M] During outpatient treatment, patients will undergo cardio-respiratory monitoring using a specialized ambulatory data acquisition system (BioRadio). In addition, respiratory function will be assessed in the clinic using a similar device (SOMNOtouch).

[N] During outpatient treatment, patients will undergo cardio-respiratory monitoring using a specialized ambulatory data acquisition system (BioRadio). Respiratory monitoring will be attempted at home for 6 hours each day during the time awake at Screening (three 3-day periods during the first, second and third week), and on any 3 days in the week preceding each subsequent scheduled office visit. Data recorded using this system will be downloaded from the device at home using the Internet/WiFi or a dedicated laptop provided by the Sponsor, if Internet access is not available; data collected during the screening test period will be verified for accuracy by Vivonoetics.

[O] To be performed for all post-pubertal females.

[P] MECP2 genetic mutation testing will be performed only if the patient has not had the test performed previously, or does not have adequate documentation of the test results from an accredited laboratory.

Table 6B. Schedule of Evaluations: 24-Week Initial Open-label Extension Treatment Period (Weeks 24 - 48)

Visit / Assessment	Week 24[A]/ Baseline	Weeks 25	Week 28	Week 29	Week 32	Week 40	Week 48[B]	Safety follow-up[C]
Study day(s)	**168**	**175**	**169-196**	**203**	**197-224**	**225-280**	**281-336**	**337-350**
Informed consent (before any study procedure)	X						X[M]	
Inclusion/Exclusion Criteria	X						X[M]	
Vital Signs	X[I]		X[I]		X	X	X	X
ECG (12-lead)	X[I]		X[I]		X	X	X	
Physical Examination	X[I]		X[I]		X	X	X	
Neurological Examination	X[I]		X[I]		X	X	X	
Ophthalmological Examination							X	
Laboratory Evaluation (Hematology, Biochemistry, Urinalysis)					X	X	X	
Plasma ACTH/Cortisol							X	
Serum prolactin							X	
Serum pregnancy test [J]							X	
Tanner Staging							X	
Suicidality assessment							X	
Dosage administration and drug label record	X[L]	X	X[L]	X	X	X	X[D]	
Concomitant Medications and Significant Non-Drug Therapies	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X[E]	X
Respiratory monitoring (in clinic) [F]			X		X	X	X	
CGI-C [K]			X		X	X	X	
CIC [K]			X		X	X	X	
RCSS							X	
Motor-Behavioral Assessment Scale							X	
Caregiver Top 3 Concerns							X	
Telephone contact		X[G]		X[G]				
Study Completion							X[H]	X

Table 6C. Schedule of Evaluations: Additional Open-label Extension Treatment Period – Part 1 (Weeks 49 – 96)

Visit / Assessment	Week 54	Week 60	Week 66	Week 72	Week 78	Week 84	Week 90	Final (Week 96) [N]	Safety follow-up[C]
Study day(s)	**337-378**	**379-420**	**421-462**	**463-504**	**505-546**	**547-588**	**589-630**	**631-672**	**673-686**
Informed consent (before any study procedure)								X[M]	
Inclusion/Exclusion Criteria								X[M]	
Vital Signs		X		X		X		X	X
ECG (12-lead)		X		X		X		X	
Physical Examination		X		X		X		X	
Neurological Examination		X		X		X		X	
Ophthalmological Examination				X				X	
Laboratory Evaluation (Hematology, Biochemistry, Urinalysis)		X		X		X		X	
Plasma ACTH/Cortisol				X				X	
Serum prolactin				X				X	
Serum pregnancy test [J]				X				X	
Tanner Staging				X				X	
Suicidality assessment				X				X	
Dosage administration and drug label record	X	X	X	X	X	X	X	X[D]	
Concomitant Medications and Significant Non-Drug Therapies	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X[E]	X
Respiratory monitoring (in clinic) [F]		X		X		X		X	
CGI-C [K]		X		X		X		X	
CIC [K]		X		X		X		X	
RCSS				X				X	
Motor-Behavioral Assessment Scale				X				X	
Caregiver Top 3 Concerns				X				X	
Telephone contact	X[G]		X[G]		X[G]		X[G]		
Study Completion								X[O]	X

Table 6D. Schedule of Evaluations: Additional Open-label Extension Treatment Period – Part 2 (Weeks 97 – 144)

Visit / Assessment	Week 102	Week 108	Week 114	Week 120	Week 126	Week 132	Week 138	Final (Week 144) [P]	Safety follow-up[C]
Study day(s)	**673-714**	**715-756**	**757-798**	**799-840**	**841-882**	**883-924**	**925-966**	**967-1008**	**1009-1022**
Informed consent (before any study procedure)								X[M]	
Inclusion/Exclusion Criteria								X[M]	
Vital Signs		X		X		X		X	X
ECG (12-lead)		X		X		X		X	
Physical Examination		X		X		X		X	
Neurological Examination		X		X		X		X	
Ophthalmological Examination				X				X	
Laboratory Evaluation (Hematology, Biochemistry, Urinalysis)		X		X		X		X	
Plasma ACTH/Cortisol				X				X	
Serum prolactin				X				X	
Serum pregnancy test [J]				X				X	
Tanner Staging				X				X	
Suicidality assessment				X				X	
Dosage administration and drug label record	X	X	X	X	X	X	X	X[D]	
Concomitant Medications and Significant Non-Drug Therapies	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X[E]	X
Respiratory monitoring (in clinic) [F]		X		X		X		X	
CGI-C [K]		X		X		X		X	
CIC [K]		X		X		X		X	
RCSS				X				X	
Motor-Behavioral Assessment Scale				X				X	
Caregiver Top 3 Concerns				X				X	
Telephone contact	X[G]		X[G]		X[G]		X[G]		
Study Completion								X[Q]	X

Table 6E. Schedule of Evaluations: Additional Open-label Extension Treatment Period – Part 3 (Weeks 145 – 192)

Visit / Assessment	Week 150	Week 156	Week 162	Week 168	Week 174	Week 180	Week 186	Final (Week 192) [R]	Safety follow-up[C]
Study day(s)	**1009-1050**	**1051-1092**	**1093-1134**	**1135-1176**	**1177-1218**	**1219-1260**	**1261-1302**	**1303-1344**	**1345-1358**
Informed consent (before any study procedure)									
Inclusion/Exclusion Criteria									
Vital Signs		X		X		X		X	X
ECG (12-lead)		X		X		X		X	
Physical Examination		X		X		X		X	
Neurological Examination		X		X		X		X	
Ophthalmological Examination				X				X	
Laboratory Evaluation (Hematology, Biochemistry, Urinalysis)		X		X		X		X	
Plasma ACTH/Cortisol				X				X	
Serum prolactin				X				X	
Serum pregnancy test [J]				X				X	
Tanner Staging				X				X	
Suicidality assessment				X				X	
Dosage administration and drug label record	X	X	X	X	X	X	X	X[D]	
Concomitant Medications and Significant Non-Drug Therapies	X	X	X	X	X	X	X	X	
Adverse Events	X	X	X	X	X	X	X	X[E]	X
Respiratory monitoring (in clinic) [F]		X		X		X		X	
CGI-C [K]		X		X		X		X	
CIC [K]		X		X		X		X	
RCSS				X				X	
Motor-Behavioral Assessment Scale				X				X	
Caregiver Top 3 Concerns				X				X	
Telephone contact	X[G]		X[G]		X[G]		X[G]		
Study Completion								X[S]	X

Footnotes refer to Tables 6B, 6C, 6D and 6E

[A] Week 24 (Day 168) safety and efficacy evaluations from the initial double-blind period will serve as the baseline evaluations for the open-label extension treatment period. All additional baseline assessments and administration of the first dose of the extension treatment period should be completed on Day 168, if possible, and the patient will be released from the hospital following the 4-hr post-dose safety assessments. If necessary, the first dose administration and 4-hr safety follow-up in the clinic can be performed in the morning on the next day (Day 169).

[B] The Week 48 evaluations should be performed for all subjects, and should be done when a subject discontinues from the study prematurely during the initial open-label extension period. The Week 48 evaluations will be considered as the Final evaluation for subjects who discontinue prematurely between Week 24 and Week 48, as well as for those who complete the initial 24-week open-label treatment period, but do not continue additional open-label treatment.

[C] Safety follow-up to be performed 14 days after the final dose of study medication in the extension treatment period, or if the patient discontinues prematurely.

[D] Recording of dosing for the final dosing period.

[E] Patients will be contacted 30 days after their final dose of study medication to follow up on the occurrence of any SAEs.

[F] Patients will undergo cardiorespiratory monitoring in the clinic at each scheduled visit using a specialized data acquisition system (SOMNOtouch).

[G] Telephone contact with patient/caregiver will be done on Days 175 and 203 (± 1 day), and at Weeks 54, 66, 78, 90, 102, 114, 126, 138, 150, 162, 174 and 186 (± 1 week), to assess tolerability of the current dose of study medication; adverse events and concomitant medication use will be assessed.

[H] Patients completing 336 days (+/- 14 days) of treatment will be considered to have completed the initial 24-week extension treatment period of the study.

[I] On Day 168 (first dose at 5 mg bid) and Day 196 (first dose at 10 mg bid), vital signs, ECG and physical/neurological examinations will be performed pre-dose and 1 and 4 hr post-dose. At Baseline (Day 168), assessments performed as part of the final Week 24 evaluations in the preceding double-blind period of the study will serve as the pre-dose assessment.

[J] To be performed for all post-pubertal females.

[K] Ratings of the CGI-C and CIC for the open-label extension period should be based on the patient's condition at the Week 24 visit.

[L] The first dose (5 mg) at the starting dose (5 mg *bid*) on Day 168 (Week 24) and the first dose (10 mg) at the target dose (10 mg *bid*) on Day 196 (Week 28) will be administered in the clinic at least 6 hours after the previous dose.

[M] Informed consent must be obtained at Week 48, at Week 96, and again at Week 144, for the patient to continue open-label treatment for an additional 48 weeks. If in the Investigator's opinion, the patient is benefitting from treatment with sarizotan and is not experiencing any significant adverse events, they will be eligible to continue open-label treatment.

[N] All Week 96 (Final) evaluations should be performed for subjects who complete Part 1 of the additional open-label treatment period and do not continue open-label treatment, or when a subject discontinues from the study prematurely after Week 48 and prior to Week 96.

[O] Patients completing 672 days (+/- 14 days) of treatment will be considered to have completed Part 1 of the additional extension treatment period of the study.

[P] All Week 144 (Final) evaluations should be performed for subjects who complete Part 2 of the additional open-label treatment period, or when a subject discontinues from the study prematurely after Week 96 and prior to Week 144.

[Q] Patients completing 1008 days (+/- 28 days) of treatment will be considered to have completed Part 2 of the additional extension treatment period of the study.

[R] All Week 192 (Final) evaluations should be performed for subjects who complete Part 3 of the additional open-label treatment period, or when a subject discontinues from the study prematurely after Week 144 and prior to Week 192.

[S] Patients completing 1344 days (+/- 28 days) of treatment will be considered to have completed Part 3 of the additional extension treatment period of the study.

11.4 Visit Schedule and Assessments

Initial 24-Week Double-blind Treatment Period

11.4.1 Screening (Days -28 to -1)

At Screening (Days -28 to -1) each patient will report to the clinic where the following procedures will be performed to establish eligibility for the study:

a) Obtaining written informed consent (before any study procedures)

b) Demography and background information

c) MECP2 gene mutation test, if results not available from accredited laboratory

d) Medical history and current medical conditions

e) Physical examination

f) Neurological examination

g) Laboratory evaluations comprising the following tests:

- hematology - RBC, WBC, hemoglobin, hematocrit, platelets, neutrophils, eosinophils, basophils, lymphocytes, monocytes;
- biochemistry - total bilirubin, albumin, AST, ALT, γGT, BUN, creatinine, sodium, potassium, chloride, bicarbonate, calcium, LDH, alkaline phosphatase, total cholesterol, HDL, LDL, VLDL, triglycerides, glucose, and CPK;
- urinalysis – pH, glucose, ketones, specific gravity, nitrites, protein, bilirubin, hemoglobin and microscopic RBC, WBC and casts;
- thyroid function*: TSH, Free T4, Triiodothyronine (T3);
- virology tests* - hepatitis B antibody (core and surface) and surface antigen, and hepatitis C antibody screens, HIV;
- serum pregnancy test for post-pubertal females;

*screening only

h) Vital signs – body weight, height, temperature, pulse, systolic and diastolic BP, and respiratory rate

i) 12-lead standard ECG

j) Prior (previous 4 weeks) and concomitant medications and therapies

k) Adverse events (to be collected from the time of signing the consent form)

l) Respiratory monitoring at home (BioRadio) in Weeks 1, 2 and 3 of screening period

m) subject eligibility and inclusion/exclusion criteria checklist **(all laboratory results must be available before the baseline visit)**

11.4.2 Baseline (Day 0 through Day 1 pre-dose)

a) Overnight stay (hotel/hostel/housing) on Days 0 and 1;

b) Physical examination*

c) Neurological examination*

d) Ophthalmological examination (including visual acuity, lens, intraocular pressure and examination of the fundus, and OCT, if feasible for the patient)

e) Laboratory evaluations comprising the following tests:

- hematology*;
- biochemistry*;
- urinalysis*;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test for post-pubertal females.

f) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP; to be done at least 1 hour prior to the first dose and repeated 3 times with an interval of at least 10 minutes between readings;

g) 12-lead standard ECG - to be done at least 1 hour prior to the first dose and repeated 3 times with an interval of at least 10 minutes between readings;

h) Tanner Staging (see Appendix 7);

i) Suicidality assessment

j) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

k) Concomitant medication;

l) Respiratory monitoring in clinic;

m) Rett syndrome Clinical Severity Scale (RCSS);

n) Motor-Behavioral Assessment Scale;

o) Caregiver Top 3 Concerns;

p) Confirmation of subject eligibility and inclusion/exclusion criteria checklist (all necessary test results must be available before randomization and administration of the first dose).

*These tests do not need to be repeated if the screening assessment was done within **28** days of baseline.

11.4.3 Day 1 (Dose Level 1)

a) Randomization and first dose administration;

b) Blood samples for PK (blank): 0 (within 10 minutes prior to dosing), and 1 and 4 hours post-dose (first dose; window ±15 min);

c) Vital signs – body weight, temperature, respiratory rate, pulse, and systolic and diastolic BP at 1 and 4 hr post-dose (first dose);

d) 12-lead standard ECG – 1 and 4 hr post-dose (first dose);

e) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

f) Concomitant medication;

g) Administration of the evening dose in the clinic.

11.4.4 Day 2 (Dose Level 2)

a) Physical and neurological examinations;

b) Laboratory evaluations:

- plasma ACTH and cortisol;

c) Vital signs – body weight, temperature, pulse, systolic and diastolic BP, and respiratory rate;

d) 12-lead standard ECG;

e) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

f) Concomitant medication;

g) Leave hospital 4 hours after morning dose, if there are no safety issues.

11.4.5 Day 7 - Telephone Contact

A telephone contact will be made with the caregiver on Day 7 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Based on the information gathered on this call, a decision will be made whether or not to increase the patient's dose to Dose Level 3 on Day 8 and Dose Level 4 on Day 9, while the patient is residing at home.

11.4.6 Day 14

a) Respiratory monitoring at home (BioRadio) on any 3 days in the week preceding the scheduled visit;

b) Overnight stay (hotel/hostel/housing);

c) Physical and neurological examinations;

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

e) Vital signs – body weight, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) CGI-C – performed by the Investigator;

h) CIC – performed by the caregiver;

i) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

j) Concomitant medication.

11.4.7 Day 15

a) Vital signs – body weight, temperature, respiratory rate, pulse, and systolic and diastolic BP;

b) 12-lead standard ECG;

c) Blood samples for PK (Dose Level 4): 0 (within 10 minutes prior to morning dose – trough level), and 1 and 4 hours post-dose;

d) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

e) Concomitant medication;

f) Leave hospital 4 hours after morning dose, if there are no safety issues.

11.4.8 Week 8 (Day 56)

a) Respiratory monitoring at home (BioRadio) on any 3 days in the week preceding the scheduled visit;

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) Suicidality assessment

g) CGI-C – performed by the Investigator;

h) CIC – performed by the caregiver;

i) Motor-Behavioral Assessment Scale;

j) Caregiver Top 3 Concerns;

k) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

l) Concomitant medication.

11.4.9 Week 16 (Day 112)

a) Respiratory monitoring at home (BioRadio) on any 3 days in the week preceding the scheduled visit;

b) Physical and neurological examinations;

c) Ophthalmological examination;

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;

- serum prolactin;

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG

g) Respiratory monitoring in clinic;

h) CGI-C – performed by the Investigator;

i) CIC – performed by the caregiver;

j) Motor-Behavioral Assessment Scale;

k) Caregiver Top 3 Concerns;

l) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

m) Concomitant medication.

11.4.10 Week 24 (Day 168 or at early discontinuation) – Final Evaluation

a) Respiratory monitoring at home (BioRadio) on any 3 days in the week preceding the scheduled visit;

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females)

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) Respiratory monitoring in clinic;

j) CGI-C – performed by the Investigator;

k) CIC – performed by the caregiver;

l) RCSS;

m) Motor-Behavioral Assessment Scale;

n) Caregiver Top 3 Concerns;

o) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

p) Concomitant medication.

11.4.11 Safety Follow-up (Day 182 or 14 days after last dose of study medication)

For patients who complete the initial 24-week treatment period and do not continue treatment in the extension treatment period, as well as those patients who discontinue prematurely, the following assessments will be performed 14 days after the last dose of study medication:

a) Vital signs – body weight, temperature, pulse, systolic and diastolic BP, and respiratory rate

b) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator.

168-Week Open-label Extension Treatment Period

11.4.12 Week 24 (Day 168) – Baseline for open-label extension period

a) Obtaining written informed consent for the initial 24-week open-label extension treatment period;

b) Assessment of inclusion/exclusion criteria for the extension treatment period (see Section 9.2.7);

c) Final (Week 24) evaluations for the initial double-blind treatment period will serve as the baseline evaluations for all safety and efficacy measures for the open-label extension treatment period (see Section 11.4.10);

d) First dose (2 or 5 mg) at starting dose (2 or 5 mg *bid*), dependent on age and weight, given in clinic (minimally 6 hours after last dose in double-blind period);

e) Physical examination - at 1 and 4 hr post-dose;

f) Neurological examination - at 1 and 4 hr post-dose;

g) Vital signs – body weight, temperature, respiratory rate, pulse, and systolic and diastolic BP - at 1 and 4 hr post-dose;

h) 12-lead standard ECG –at 1 and 4 hr post-dose;

i) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

j) Concomitant medications and significant non-drug therapies;

k) Patient will leave hospital ~4 hours after first dose, if there are no safety issues.

11.4.13 Week 25 (Day 175) - Telephone Contact

A telephone contact will be made with the caregiver on Day 175 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.14 Week 28 (Day 196)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) First dose (2, 5 or 10 mg) at target dose (2, 5 or 10 mg *bid*), dependent on age and weight, or lower dose if there are tolerability issues, given in clinic (minimally 6 hours after prior dose);

c) Physical and neurological examinations – pre-dose and at 1 and 4 hr post-dose (AM dose);

d) Vital signs – body weight, temperature, respiratory rate, pulse, and systolic and diastolic BP – pre-dose and at 1 and 4 hr post-dose (AM dose);

e) 12-lead standard ECG– pre-dose and at 1 and 4 hr post-dose (AM dose);

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medications and significant non-drug therapies;

j) Patient will leave hospital ~4 hours after dosing, if there are no safety issues.

11.4.15 Week 29 (Day 203) - Telephone Contact

A telephone contact will be made with the caregiver on Day 203 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.16 Week 32 (Day 224)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.17 Week 40 (Day 280)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.18 Week 48 (Day 336 or early discontinuation)

These assessments will serve as the final evaluations for patients who are not continuing treatment in Part 1 of the additional open-label extension period (through Week 96).

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

For patients continuing in Part 1 of the additional open-label extension treatment period:

p) Obtaining written informed consent;

q) Medication dispensing for the first 12-week period.

11.4.19 Week 54 (Day 378) - Telephone Contact

A telephone contact will be made with the caregiver at Week 54 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.20 Week 60 (Day 420)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.21 Week 66 (Day 462) - Telephone Contact

A telephone contact will be made with the caregiver at Week 66 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.22 Week 72 (Day 504)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;

- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

11.4.23 Week 78 (Day 546) - Telephone Contact

A telephone contact will be made with the caregiver at Week 78 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.24 Week 84 (Day 588)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.25 Week 90 (Day 630) - Telephone Contact

A telephone contact will be made with the caregiver at Week 90 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate

CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.26 Week 96 (Day 672 or early discontinuation)

These assessments will serve as the final evaluations for patients who are not continuing treatment in Part 2 of the additional open-label extension period (through Week 144):

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

For patients continuing in Part 2 of the additional open-label extension treatment period:

p) Obtaining written informed consent;

q) Medication dispensing for the first 12-week period.

11.4.27 Week 102 (Day 714) - Telephone Contact

A telephone contact will be made with the caregiver at Week 102 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the

caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.28 Week 108 (Day 756)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.29 Week 114 (Day 798) - Telephone Contact

A telephone contact will be made with the caregiver at Week 114 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.30 Week 120 (Day 840)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

11.4.31 Week 126 (Day 882) - Telephone Contact

A telephone contact will be made with the caregiver at Week 126 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.32 Week 132 (Day 924)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.33 Week 138 (Day 966) - Telephone Contact

A telephone contact will be made with the caregiver at Week 138 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.34 Week 144 (Day 1008 or early discontinuation)

These assessments will serve as the final evaluation for patients who are not continuing treatment in Part 3 of the of the additional open-label extension period (through Week 192).

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

For patients continuing in Part 3 of the additional open-label extension treatment period:

p) Obtaining written informed consent;

q) Medication dispensing for the first 12-week period.

11.4.35 Week 150 (Day 1050) - Telephone Contact

A telephone contact will be made with the caregiver at Week 150 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.36 Week 156 (Day 1092)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.37 Week 162 (Day 1134) - Telephone Contact

A telephone contact will be made with the caregiver at Week 162 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.38 Week 168 (Day 1176)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

11.4.39 Week 174 (Day 1218) - Telephone Contact

A telephone contact will be made with the caregiver at Week 174 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.40 Week 180 (Day 1260)

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;

d) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

e) 12-lead standard ECG

f) CGI-C – performed by the Investigator;

g) CIC – performed by the caregiver;

h) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

i) Concomitant medication.

11.4.41 Week 186 (Day 1302) - Telephone Contact

A telephone contact will be made with the caregiver at Week 186 to inquire about the occurrence of any adverse events or use of concomitant medication; this information will be registered on the appropriate CRF page. Overall compliance with the study medication will also be assessed by questioning the caregiver. If tolerability issues are noted, appropriate adjustment of the dose will be performed, and the patient may be asked to return to the clinic for an unscheduled visit, if necessary.

11.4.42 Week 192 (Day 1344 or early discontinuation) – Final Evaluation

a) Respiratory monitoring in the clinic (SOMNOtouch);

b) Physical and neurological examinations;

c) Ophthalmological examination (including OCT, if feasible for the patient);

d) Laboratory evaluations comprising the following tests:

- hematology;
- biochemistry;
- urinalysis;
- plasma ACTH and cortisol;
- serum prolactin;
- serum pregnancy test (post-pubertal females);

e) Vital signs – body weight, height, temperature, respiratory rate, pulse, and systolic and diastolic BP;

f) 12-lead standard ECG;

g) Tanner Staging;

h) Suicidality assessment

i) CGI-C – performed by the Investigator;

j) CIC – performed by the caregiver;

k) RCSS;

l) Motor-Behavioral Assessment Scale;

m) Caregiver Top 3 Concerns;

n) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator;

o) Concomitant medication.

11.4.43 Safety Follow-up (14 days after last dose of study medication)

For patients who discontinue treatment prematurely or complete the initial 24-week open-label extension treatment period (Week 48), but don't continue open-label treatment, as well as those patients who complete the extension treatment period (Week 192), or discontinue treatment prematurely during open-label treatment, the following assessments will be performed 14 days after the last dose of study medication:

a) Vital signs – body weight, temperature, respiratory rate, pulse, and systolic and diastolic BP;

b) Adverse Events (AEs) – reported by the patient/caregiver or observed by the Investigator.

All patients will be followed up for 30 days after the last dose of study medication regarding the occurrence of Serious Adverse Events.

Visit Windows

A window of ± 2 days will be allowed on the Day 14 visit; a window of ± 7 days will be permitted for each of the visits at Weeks 8, 16 and 24 during the initial treatment period, and the visits at Weeks 40, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 during the extension treatment period; and a window of ± 3 days will be permitted for the visits at Weeks 28 and 32 during the extension treatment period. Telephone contacts in the extension treatment period on Days 175 and 203 should be made within ± 1 day of the scheduled date, while those scheduled for Weeks 54, 66, 78, 90, 102, 114, 126, 138, 150, 162, 174

and 186 should be performed within ± 7 days of the scheduled date. The overall window for the completion of each part of the additional extension period of the study is ± 14 days for Week 96 (end of Part 1), ± 28 days for Week 144 (end of Part 2), and ± 28 days for Week 192 (end of Part 3).

11.5 Concomitant Medications

Patients enrolled in the study must be on a stable medication regimen for 4 weeks prior to beginning the screening period (if receiving services - physical, occupational, or speech therapy - subjects must be on a stable regimen of these services for 3 months prior to beginning the study). Any medication (including over-the-counter medications), in addition to the study medication, that is administered during the study from the start of the screening period through to the final evaluations at Week 48, 96, 144 or 192 (Day 336, 672, 1008 or 1344, or at early discontinuation) must be recorded in the CRF. Patients/caregivers will be instructed to contact the Investigator for approval prior to taking any new medications, while residing at home and prior to their final evaluation.

The following guidelines regarding use of concomitant medications must be followed:

- Patients must not take any investigational agent within 2 months or 5 half-lives, whichever is longer, prior to screening;
- Use of other 5-HT1a agonists, e.g. buspirone, is permitted during the study, provided the patient is on a stable dose for at least 4 weeks prior to screening. However, addition of a 5-HT1a agonist during the treatment period is not permitted;
- Care should be taken when sarizotan is administered in patients taking warfarin or other medications that are highly bound to plasma albumin;
- Patients with a history of seizures who are receiving a stable dose of anti-seizure medication for at least 4 weeks prior to baseline will be eligible for the study, and will be allowed to continue on the medication.

12 SAFETY AND EFFICACY EVALUATIONS

12.1 Safety Assessments

The assessment of safety will be based on the following:

a) laboratory tests (biochemistry, hematology, urinalysis, serum prolactin, and plasma ACTH and cortisol levels),

b) 12-lead standard ECG,

c) vital signs (body weight, height, temperature, pulse, systolic and diastolic BP, and respiratory rate),

d) physical examinations,

e) neurological examinations,

f) routine ophthalmology examination (including OCT, if feasible for the patient);

g) 12-lead standard ECG,

h) subjective reporting of any AE by the patient/caregiver,

i) objective observation of any AE by the Investigator,

j) Tanner Staging,

k) Suicidality assessment.

The investigator will be asked to comment on any clinically significant abnormal test results.

12.1.1 Physical and Neurological Examinations

A physical examination will be performed according to the Schedule of Evaluations (Tables 6A, 6B, 6C, 6D and 6E). The findings will be entered on the Physical Examination section of the Case Report Form. The physical examination will include an examination of general appearance, skin, neck (including thyroid), eyes and ears, nose, mouth, throat, lungs, heart, abdomen, back, lymph nodes, extremities and nervous system. Genital, urinary tract and rectal examination will not be done on a routine basis.

A neurological examination will be performed according to the Schedule of Evaluations (Tables 6A, 6B, 6C, 6D and 6E). The findings will be entered on the Neurological Examination section of the Case Report Form. The neurological examination includes the following: evaluation of mental status, cranial nerves, muscle strength and tone, reflexes, the sensory system, coordination and gait.

12.1.2 Vital Signs

Vital signs assessments will be performed at all scheduled evaluations (Tables 6A, 6B, 6C, 6D and 6E). Vital signs will include, body weight, temperature, pulse, systolic and diastolic blood pressure, and respiratory rate. Pulse and blood pressure will be measured after the subject has been resting for at least 5 minutes. At the baseline visit, on Day 0 or at least 1 hour prior to the first dose of study medication on Day 1, measurements of blood pressure and pulse will be repeated 3 times, at least 10 minutes apart, and the values will be averaged to obtain the baseline values. In addition, height will be measured at Screening and Baseline, and at 8-week or 12-week intervals throughout the study.

In the extension treatment period, vital signs assessments will be performed prior to the first dose of study medication on Day 168 (final Week 24 assessment from the double-blind period), and repeated at 1 and 4 hours post-dose. Similarly, pre- and post-dose assessments will be performed on Day 196 (Week 28) for the first administration of the target dose (10 mg *bid*).

If a change *of clinical relevance* from pre-dose to post-dose is observed, the vital signs assessment should be repeated as often as needed, at the discretion of the Investigator. Findings should be documented on the Vital Signs section of the Case Report Form.

12.1.3 Electrocardiogram (ECG)

All subjects will have a standard 12-lead ECG performed as specified in the schedule of evaluations (see Tables 6A, 6B, 6C, 6D and 6E). At baseline (Day 1), At the baseline visit, on Day 0 or at least 1 hour prior to the first dose of study medication on Day 1, the ECG evaluation will be repeated 3 times, at least 10 minutes apart, and the values for the different parameters will be averaged to obtain the baseline values; the ECG will be repeated at 1 and 4 hours post-dose. Each ECG should be performed before collecting the scheduled blood sample for PK assessments.

In the extension treatment period, ECG assessments will be performed prior to the first dose of study medication on Day 168 (final Week 24 assessment from the double-blind period), and repeated at 1 and 4 hours post-dose. Similarly, pre- and post-dose assessments will be performed on Day 196 (Week 28) for the first administration of the target dose (10 mg *bid*).

To ensure consistency in the data analysis across subjects, all ECGs will be sent to a central ECG monitoring service (QECG) upon completion of each cohort; however, the 'real-time' review and interpretation of the 12-lead ECGs that will be used for determination of a subject's eligibility for enrollment in the trial, as well as post-dose safety monitoring, will be performed by a physician at the investigational site. One copy of the ECG tracing will be retained in the subject's records, one will be retrieved by the monitor, and a third will be provided to the central ECG reader for analysis. The ECG read out must be reviewed by the investigator, initialed and dated, and copies inserted in the subject's records and attached to the CRF.

Each ECG tracing must have the following information entered on it:

- Study number,
- Subject's number and initials,
- Date and time ECG obtained.

If clinically significant abnormalities are found, the subject's ECG should be repeated at regular intervals until it returns to normal.

Details of the procedures related to the centralized ECG monitoring service (QECG) will be provided in a separate manual prepared by the ECG Vendor.

12.1.4 Drug levels and pharmacokinetic assessments

Blood samples will be collected for PK evaluation at 0 min (before dosing), and 1 and 4 hours after the first dose of study drug is administered on Day 1. Additional blood samples for PK assessments will be taken on Day 15, just before the morning dose (steady state trough level) and at 1 and 4 hr after dosing. Samples will be frozen and stored at < -70°C pending analysis. A window of ± 15 min will be allowed on the timing of these samples.

12.1.5 Laboratory Evaluations

Blood and urine samples for laboratory tests will be collected at the visits specified in the schedule of evaluations (see Tables 6A, 6B, 6C, 6D and 6E). Evaluations of the hematology, biochemistry and urinalysis analytes listed in Table 7 will be performed at these scheduled visits. In addition, virology tests (hepatitis B core and surface antibodies and surface antigen, and hepatitis C antibodies, and a HIV test) and tests of thyroid function (TSH, free thyroxine [T_4] and triiodothyronine [T_3]) will be performed at screening only. Plasma ACTH and cortisol levels will be measured at Baseline and Day 2, and at Weeks 16 and 24

during the initial treatment period, and at Weeks 48, 72, 96, 120, 144, 168 and 192 in the extension treatment period. Serum prolactin levels will be measured at Baseline and at Weeks 16 and 24 during the initial treatment period, and at Weeks 48, 72, 96, 120, 144, 168 and 192 in the extension treatment period. A serum pregnancy test will be performed at Screening and Baseline and at Weeks 24, 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) for all post-pubertal females; a positive test result would exclude the patient from the study.

Table 7. Summary of laboratory analytes

Hematology	**Clinical Chemistry**		**Urinalysis**
Hematocrit	Sodium	Triglycerides	pH
Hemoglobin	Potassium	AST	Specific gravity
RBC count	Chloride	ALT	Protein
WBC count	Bicarbonate	Alkaline phosphatase	Glucose
Differential WBC count	Calcium	γGT	Ketones
Platelets	Glucose	LDH	RBC, WBC, casts
	BUN	Total cholesterol	Nitrites
	Creatinine	HDL, LDL, VLDL	Bilirubin
	Total bilirubin	CPK	Hemoglobin
	Albumin	Total protein	
Special Diagnostic Tests *Thyroid function*: TSH, free thyroxine (T_4), and triiodothyronine (T_3) (Screening only) *Virology*: Hepatitis B and C; HIV (Screening only) *HPA axis*: Plasma ACTH and cortisol levels (Baseline, Day 2 and Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192) *Prolactin*: Serum prolactin (Baseline, and Weeks 16, 24, 48, 72, 96, 120, 144, 168 and 192) *Serum pregnancy test:* Post-pubertal females (Screening, Baseline, and Weeks 24, 48, 72, 96, 120, 144, 168 and 192) *MECP2 genetic mutation testing* (optional): Only if prior test results from an accredited laboratory are not available (Screening)			

The Investigator must review screening laboratory values prior to the first administration of the study agent, to ensure that the subject meets the protocol's inclusion/exclusion criteria. The Investigator must review post-dose laboratory values within 24 hours of receipt of the laboratory report. After the review is completed, the Investigator must sign and date each laboratory report.

The laboratory will provide normal reference ranges for the laboratory tests on the laboratory results report. A value is **normal** when it falls on or within the upper and lower limits of the reference range. A value is **abnormal** when it exceeds the upper or lower limit of the reference range. The laboratory will flag all abnormal and clinically notable values (defined in Appendix 2) on the laboratory report, will provide the normal reference ranges for each parameter, and will verify that the result is not due to pre-analytical problems (e.g., sample taken improperly, sample stored incorrectly, sample labeled incorrectly) or to analytical problems (e.g., machine not accurately calibrated, technical problems with equipment or reagents, or deterioration of analyte).

In cases where a patient is unable to get to the clinic for a scheduled laboratory test, or when a follow-up laboratory test is required for safety reasons, the test may be performed by a local laboratory, rather than the central laboratory designated for the study. The results of the test(s) performed by the local laboratory will be reported to the Investigator, and a copy of the laboratory report will be included in the patient's study file.

The Investigator must evaluate any change *of clinical relevance* from pre-dose to post-dose in a laboratory test as to whether it meets the definition of an adverse event, and repeat, if needed, any clinically significant abnormal laboratory test. Any laboratory abnormalities meeting the definition of an adverse event should be recorded on the Adverse Events CRF.

Refer to Section 13.0, "Reporting Safety Information" for further directions.

12.1.6 Routine Ophthalmological Examination

A routine ophthalmological examination will be performed, preferably by a qualified ophthalmologist on each patient at Baseline and at Weeks 16 and 24 (or at early discontinuation) during the initial treatment period, and at Week**s** 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) in the extension treatment period. Any newly emergent findings noted after initiation of treatment with the study medication should be commented on by the ophthalmologist, in particular, the potential relationship of the findings to the study medication.

The battery of tests to be performed has been designed to ensure the patient's safety, take the least amount of physician time and conform to regulatory requirements. The following evaluations should be performed, if possible:

- *Visual acuity*: should be determined using tests appropriate for the patient's disability; both eyes should be assessed, and abnormalities should be listed.
- *Lens*
- *Intra-ocular pressure*
- *Fundus**:*** examination should be performed with dilation of the pupils and include determination of the presence of retinal pigmentation, changes in the vasculature, appearance of the fundus and the macula, as well as other adventitious changes. Each eye should be assessed as "normal" or "abnormal" and any abnormality should be listed. When possible, pictures of the fundus should be taken using 3 views (disc center, macula center and temporal to macula).
- *Optical Coherence Tomography (OCT):* OCT is an optical signal acquisition and processing method that allows for high-quality, micrometer-resolution retinal images to be obtained. OCT should be performed only if feasible, based on the patient's condition.

If any part of the planned battery of tests cannot be performed due to the patient's condition or inability to comply with the procedures, this should be documented in the CRF.

12.1.7 Adverse Events

All adverse events (AEs) experienced by the patient and observed by the caregiver while the patient is residing at home or observed by the Investigator during a hospital/clinic visit should be report on the AE CRF. Many patients with RTT experience seizures as part of their illness; therefore, collection of information on seizures will be a focus of AE reporting in this study. To ensure comprehensive collection of information related to seizures, the following questions should be asked of the caregiver at each visit:

At Screening:

- Does the patient have a history of seizures? (If the answer is 'No', there is no need to ask the remaining questions)
- Approximately how many seizures per day did the patient have over the last 2 weeks?
- What type of seizure did the patient have (if known)?
- Is the patient taking any medication to treat seizures?
- If the patient is taking medication for seizures, what is the medication and what is the daily dose?

At subsequent visits:

- Has there been any change in the frequency or type of seizure the patient is experiencing since the last visit?
- If the patient is taking medication for seizures, has there been any change in the medication (e.g. type of medication or dose) since the last visit?

The information collected from this questioning should be reviewed with the Caregiver by the Investigator prior to entering it on the appropriate pages of the eCRF.

12.1.8 Tanner Staging

The sexual maturity of patients enrolled in the study will be evaluated at baseline and at Weeks 24, 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) during the treatment period using the Tanner scale (Marshall and Tanner, 1969; Marshall and Tanner, 1970; see Appendix 7). The scale defines physical measurements of development in children, adolescents and adults based on external primary and secondary sex characteristics, such as the size of the breast, genitals, testicular volume and development of pubic hair. Due to natural variation, individuals pass through the Tanner stages at different rates, depending in particular on the timing of puberty. Tanner Staging will be used to assess potential effects of sarizotan on sexual development in the RTT patients.

12.1.9 Suicidality Assessment

An assessment of suicidality for patients enrolled in the study has been included, as requested by the FDA. Because of the limited abilities of the RTT patient population, this assessment consists of a simple questionnaire to assess suicidality based largely on caregiver reports. The Sponsor has created a structured suicidality assessment query, consisting of 10 questions requiring a Yes/No response, which will be administered to caregivers to assess the potential risk for suicide in each patient. The core principles underlying assessment of suicidality are addressed and are mixed with core features/ behaviors of patients showing depressed mood prior to suicidal acts. This assessment will be performed by the Investigator or other qualified staff at Baseline, and at scheduled visits at Weeks 8, 24, 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) and will be based on the behavior observed by the caregiver during the month prior to the visit. For each question for which the caregiver has given a positive response, the frequency of the behavior (i.e. rarely, regularly), as well as whether it was observed recently, should be indicated, and a brief text description provided.

The Investigator should carefully review the responses prior to deciding the next steps in the management of the patient.

12.2 Efficacy Assessments

12.2.1 Number of Apnea Episodes (Primary Efficacy Outcome)

Measurement of specific cardiorespiratory parameters for the assessment of primary and secondary efficacy outcomes during the initial 24-week treatment period will performed at home using respiratory inductive plethysmography (RIP) coupled with the BioRadio™ wireless physiological monitor (Great Lakes Neurotechnologies, Cleveland, Ohio). A brief description of RIP and use of the BioRadio is provided in Appendix 5. The cardio-respiratory data collected using the BioRadio will be analysed and summarized by an external vendor, Vivonoetics. A description of the analysis plan and procedures will be provided in a separate document.

The primary efficacy measure to be used in assessing the efficacy of sarizotan in treating the respiratory symptoms associated with RTT will be the percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour, during awake time, from baseline to endpoint (Week 24 or early discontinuation). This will be calculated from the data obtained during home respiratory monitoring.

Home cardiorespiratory monitoring will be performed during the screening period (three 3-day test periods during first 3 weeks) and for 3 days in the week prior to each scheduled clinic visit at Weeks 2, 8, 16 and 24. Monitoring will be performed when the patient is awake for 6 hours on each of the 3 days. Data from all of the quality recording time (minimally 3 hours) in the Screening period and from the week prior to each of the visits at Weeks 2, 8, 16 and 24 will be averaged and used in calculating the value for the primary efficacy measure. If the patient meets the criterion for number of apnea episodes, as determined by Vivonoetics, in any week of the Screening period, home monitoring in the subsequent week(s) would not be required, and the screening data would be used to calculate the baseline value for the analyses.

The caregiver should perform the home monitoring starting at approximately the same time each day. If the patient attends school, the monitoring should be performed when the patients is at home, if at all possible; otherwise, it should be noted when the monitoring is performed at school.

During home monitoring of respiratory function, caregivers should keep a diary and be instructed to record any problems that are encountered with the patient during the monitoring session, e.g., the patient is overly active or falls asleep, the RIP bands become loose or fall off, or the pulse oximeter becomes loose or falls off. The date and starting time of onset for these problems should be noted. The time at which the problem resolves should also be noted. The diary should be brought to the hospital/clinic at each scheduled visit, and the information from the diary will be used in the analysis of the BioRadio data.

Additional assessments of the respiratory outcomes will be performed at the hospital at Baseline and at the Week 16 and 24 visits, using the SOMNOtouch™ (SOMNOmedics GmBH, Randersacker, Germany), a stretch-sensitive resistance plethysmograph device similar to the BioRadio. A period of approximately 1 hour of cardiorespiratory monitoring using the SOMNOtouch will be performed at each of these visits. Data collected using the SOMNOtouch will be analysed and summarized by an external vendor, Vivonoetics.

During the open-label extension phase of the study, patients will return for visits every 4, 8 or 12 weeks, and respiratory assessments will be performed in the clinic using the SOMNOtouch at Weeks 28, 32, 40, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 (or at early discontinuation). Respiratory parameters derived from the SOMNOtouch data will be similar to those from the BioRadio, including apnea episodes. Efficacy data from the extension period from patients randomized to sarizotan at Baseline will be analysed to determine if there is any attenuation of the benefit noted during the first six months. In addition, a comparison will be made between patients who received sarizotan throughout both periods of the study

and those who were randomized to placebo in the double-blind period and switched to sarizotan in the open-label extension period.

12.2.2 Caregiver-rated Impression of Change (CIC)

The key secondary efficacy outcome will be the difference between sarizotan and placebo on the Caregiver-rated Impression of Change (CIC) from baseline, a 7-point Likert-type scale for which ratings range from 1 = very much improved to 7 = very much worse, with 4 = no change. This caregiver-rated measure will take into account activities, behavior, mood and functioning. This rating will be performed in consultation with the study Investigator, but should be based largely on the caregivers' evaluation during the reporting period. The rating of the CIC would be based on changes in the following domains:

- Activities (watching TV, interest in conversations around her, cooperation during toileting, dressing/bathing, etc.)
- Communication (verbal or by eye movements, hand movements, or head movements)
- Behavior (agitation, refusal to feed, scratching, social avoidance)
- Participation in family/outdoor/social events, etc.

The CIC assessment will be conducted on Day 14, and at Weeks 8, 16 and 24 (or at early discontinuation) during the initial double-blind treatment period, and will be based on the patient's condition at the Baseline visit. In the open-label extension treatment period, the CIC assessment will be performed at Weeks 28, 32, 40, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 (or at early discontinuation), and will be based on the patient's condition at the Week 24 visit.

12.2.3 Clinical Global Impression of Change (CGI-C)

The CGI (Guy 1976) is the general name for 2 scales: the CGI-Severity (CGI-S) measures global severity of illness at a given point in time, and the CGI-Change (CGI-C) measures change from the baseline state at each visit following the baseline visit. Whenever possible, the CGI scale must be completed by the same clinician for every assessment; if this is not possible, the rating clinician should review the subject's presentation (along with review of clinical notes) with the rater who completed the initial evaluation.

In the current study the CGI-C rating scale permits a global evaluation of the subject's improvement or worsening over time. At each post-baseline visit, the rater will assess the change in the subject's symptoms of RTT relative to the symptoms at baseline using a 7-point scale, ranging from 1 (very much improved) to 7 (very much worse), with a score of 4 indicating no change. The CGI-C assessment will be conducted on Day 14, and at Weeks 8, 16 and 24 (or at early discontinuation) during the initial double-blind treatment period, and will be based on the patient's condition at the Baseline visit. In the open-label extension treatment period, the CGI-C assessment will be performed at Weeks 28, 32, 40, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156, 168, 180 and 192 (or at early discontinuation), and will be based on the patient's condition at the Week 24 visit. It is recommended that recently published guidelines for rating the CGI-C in RTT patients are followed for completing this assessment (Neul et al, 2015).

12.2.4 Other Respiratory Symptoms of RTT

The effect of sarizotan on the following respiratory symptoms of RTT will be evaluated:

- Percent time spent with breathing dysrhythmia (% time apnea + % time hyperventilation) per hour;
- Number of hyperventilation episodes (≥10 seconds each) per hour;
- Oxygen saturation (number of episodes of oxygen desaturation below 90% per hour).

- *Respiratory Distress Index* - The sum of the following parameters calculated per hour of wakefulness: 1) number of breath-holding episodes, 2) number of episodes of hyperventilation [1 and 2 are as defined in inclusion criteria; each would have to be ≥10 seconds in duration], and 3) number of drops in oxygen values to < 90%.

Data for determination of these additional respiratory outcome parameters will be collected during home cardiorespiratory recording using the BioRadio.

12.2.5 Motor-Behavioral Assessment Scale

The Motor-Behavioral Assessment Scale (Fitzgerald, Jankovic and Percy, 1990) is comprised of three sub-scales:

I. Behavioral/Social Assessment (16 items): regression of motor skills; regression of communication skills; poor eye/social contact; lack of sustained interest; irritability, crying tantrums; overactive or over-passive; does not reach for objects or people; does not respond to spoken words when addressed by examiners or parents, acts as deaf even to loud noises; feeding difficulties; chewing difficulties; lack of toilet training; masturbation; self-mutilation/pulling hair or ears, scratching, etc.; aggressive behavior; seizures; apparent insensitivity to pain;

II. Orofacial/Respiratory Assessment (7 items): speech disturbance; bruxism; breath holding; hyperventilation; air-saliva expulsion/drooling; mouthing of hands and objects; biting self and others;

III. Motor Assessment/Physical Signs (14 items): hand clumsiness; stereotypic hand washing, rubbing, clapping, stroking, kneading; ataxia/apraxia (gait and trunk) or no gait; truncal rocking/shifting weight; oculogyric movements; bradykinesia; dystonia; hypomimia; scoliosis; myoclonus; dyskinesias including chorea/athetosis or oromotor; hypertonia/rigidity; hyperreflexia; vasomotor disturbance.

Each item is rated on a scale from 0 to 4, with '0' indicating normal or never, and '4' indicating very severe or constant. This assessment will be performed at Baseline and at Weeks 8, 16, and 24 during the initial treatment period, and at Weeks 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) in the extension treatment period.

12.2.6 Caregiver Top 3 Concerns

The severity of patient symptoms will be assessed using the Caregiver Top 3 Concerns scale, which uses a 100-mm visual analogue scale (VAS). The caregiver will select the 3 symptoms that are most problematic at baseline from a list of 12 possible concerns, and will rate each one using the VAS to indicate the severity on a scale of 0 to 100. The same 3 symptoms will be rated using the VAS at the scheduled visits at Weeks 8, 16, and 24 in the initial treatment period, and Weeks 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) in the extension treatment period.

The concerns from which the caregivers will select their top 3 are listed alphabetically below:

- Aggression/frustration (head banging, screaming, spitting)
- Anxiety
- Difficulties with activities of daily living (toileting, feeding, grooming)
- GI problems – constipation, bloating
- Inappropriate social contact/avoids eye to eye contact, etc.
- Involuntary/repetitive/non-purposeful hand movements, hand wringing, mouthing, etc.

- Lack of communication
- Lack of hand use
- Motor problems; difficulty with ambulation
- Respiratory difficulties (breath holding - apnea, hyperventilation, air/saliva expulsion/ drooling)
- Seizures
- Self-mutilation.

12.2.7 Rett Syndrome Clinical Severity Scale (RCSS)

The RCSS is a scale specifically designed to assess the severity of symptoms of RTT (Neul et al, 2008). This 13-item measure provides a clinician rating of core symptoms of Rett syndrome on a Likert scale of either 0 to 4 or 0 to 5 with a maximum total score of 58. The Investigator will use the scale to rate the following: frequency and manageability of seizures, respiratory irregularities, scoliosis, ability to walk (gait apraxia), hand use, speech and sleep. A total score, as well as feature-specific scores will be calculated. This scale will be assessed at Baseline and Week 24 (or early discontinuation) in the initial treatment period, and Weeks 48, 72, 96, 120, 144, 168 and 192 (or early discontinuation) in the extension treatment period.

12.2.8 Rater Training

A multicenter Investigators' Meeting will be held to standardize test procedures and consistency of ratings across centers for each of the rating scales, except the CIC and Caregiver Top 3 Concerns, which will be rated by the caregiver.

To ensure the sensitivity and reliability of the assessments, it is requested that the same evaluator at the site conduct the CGI-C, and the same caregiver perform the rating of the Caregiver Top 3 Concerns and CIC, at every visit. It is recognized that it will sometimes not be possible to meet this condition, but every reasonable effort should be made to ensure uniform conditions across evaluations.

13 REPORTING SAFETY INFORMATION

13.1 Adverse Events

Adverse Event evaluations will be performed during the screening period, at baseline and at each visit of the study. Untoward medical events will be collected, from the time of signing of Informed Consent to the end of the safety follow-up period (14 days after last dose of study medication). All Adverse Events will be recorded in the CRF. In addition, all subjects will be followed up for 30 days after their last dose of study medication for the occurrence of any Serious AE.

13.1.1 Glossary

Adverse Drug Reaction (ADR)

In the pre-approval clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not be established, all noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions. The phrase "response to a medicinal product" means that a causal relationship between a medicinal product and an adverse event is at last a reasonable possibility, i.e., the relationship cannot be ruled out.

Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Serious Adverse Event (SAE) or Serious Adverse Drug Reaction (Serious ADR)

Any untoward medical occurrence that at any dose:

- Results in death,
- Is life-threatening, i.e., an event which, in the view of the Investigator, places the subject at immediate risk of death from the event as it occurred (it does not include an event which hypothetically might have caused death if it were more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect (if exposure to product just before conception or during pregnancy resulted in an adverse outcome in the child), or
- Is an important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Symptoms or medically significant laboratory or instrumental (e.g., electrocardiographic) abnormalities of a pre-existing disease, such as cancer or other disease, should not be considered an adverse event. However, occurrence of new symptoms, or laboratory or instrumental abnormalities, as well as worsening of pre-existing ones, are considered adverse events. Planned procedures that require hospitalization will not be considered SAEs.

Unexpected Adverse Drug Reaction

An Unexpected Adverse Drug Reaction is adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product).

Non-Serious Adverse Event

A non-serious adverse event is any adverse event that does not meet the criteria listed above for a serious adverse event.

Treatment-Emergent Adverse Events

Adverse events that are newly occurring or worsened in severity after administration of the study medication are considered to be treatment-emergent AEs (TEAEs). In the open-label extension period, only newly occurring AEs and AEs that were ongoing at Week 24 and worsen in severity will be considered TEAEs.

13.1.2 Data Collection

For each event, record the following information on the Adverse Event section of the Case Report Form:

- **Classification of the Event:** Classify the event as either serious or non-serious
- **Description of Signs or Symptoms:** Whenever possible, record a specific diagnosis for the event. If a diagnosis cannot be made, then record each sign or symptom separately, e.g., record nausea and vomiting as two events. If multiple episodes of an event occur, separated by an appropriate time interval to justify considering the subsequent episodes as a repeat occurrence, record each episode separately on the Case Report Form.
- **Onset Date and Time:** Record the date and time the event started. If a change from baseline/previous evaluation in a laboratory test is reported as an adverse event, record the start date as the date of collection of the first lab sample that shows the change.

Stop Date and Time: Record the date and time the event resolved. If a change from baseline/previous evaluation in a laboratory test is reported as an adverse event, record the stop date as the date of collection of the first sample that shows a return to the previous level.

Intensity:

1. **Mild:** Event not resulting in disability/incapacity, which resolves without treatment.
2. **Moderate:** Event not resulting in disability/incapacity, which requires treatment.
3. **Severe:** Event resulting in temporary and mild disability/incapacity, which requires treatment.

Relationship to the Study Agent: Every effort should be made to determine the cause of each adverse event. The correlation between the study agent and the adverse event should be classified as follows:

1. **Probable**
   a) The event follows a reasonable temporal sequence from administration of the study agent;
   b) The event follows a known response pattern to the study agent;
   c) The event cannot be reasonably explained by:
      - the known characteristics of subject's clinical state, or
      - by other therapy administered, or
      - by the diagnostic/interventional procedure;
   d) There is evidence of partial or complete disappearance of the event after withdrawal of the product (positive de-challenge)

2. **Possible**
   a) The event follows a reasonable temporal sequence from administration of the study agent;
   b) Causation of the event by the study agent cannot be excluded;
   c) The event follows a known response pattern to the study agent but the event could have been produced by:
      - the subject's clinical state, or
      - other therapy administered, or
      - a diagnostic/interventional procedure.

3. **Unlikely**
   a) The adverse event follows a reasonable temporal sequence from administration of the study agent;
   b) Other reasons are more likely to be the cause of the adverse event, based on the present knowledge of the
      - disease under treatment, or
      - other therapy administered, or
      - study drug;
   c) A causal relationship between the adverse event and the study drug can't be ruled out with certainty.

4. **Not Related**
   The event is either a pre-dose event or is definitely due to causes separate from the administration of the study agent, i.e.,
      - documented pre-existing condition
      - technical and manual procedural problems
      - concomitant medication
      - subject's clinical state.

5. **Unknown**
   The event does not meet any of the above criteria because of:
      - conflicting data and/or
      - dubious or insufficient/poor evidence
      - the event is not judged as related or not related.

Action Taken in Relation to the Adverse Event:

1. **None**
2. **Drug treatment required** (a medication was prescribed or changed; record on the Concomitant Medication section of the Case Report Form)
3. **Change in dose of concomitant medication**
4. **Non-drug treatment required** (a non-drug treatment was prescribed or changed, record under "Comments" in the Adverse Event section of the Case Report Form)
5. **Hospitalization or prolonged hospitalization**
6. **Diagnostic or clinical test(s) conducted** (attach a copy of the results to the Case Report Form)
7. **Subject discontinued from the study**

Action Taken with the Study Medication:

1. **Study medication dose interruption**
2. **Study medication dose reduction**
3. **Study medication discontinuation**
4. **None**

Subject Outcome:

1. **Recovered without sequelae**
2. **Recovered with sequelae** (describe the sequelae under "Comments" in the Adverse Event section of the Case Report Form)
3. **Not Recovered, event on-going** (follow the subject until a definite outcome can be determined. When follow-up data are collected, report follow-up information under "Comments" in the Adverse Event section of the Case Report Form; if event is serious, fill in a follow-up Serious Adverse Event Report)
4. **Died** (list primary cause of death under "Event Description" of the Adverse Event section of the Case Report Form; if available, attach a copy of the autopsy report to the Case Report Form and send a copy to the Sponsor)

Comments:

Provide other pertinent clinical information and observations under "Comments" in the Adverse Event section of the Case Report Form. For example, record predisposing or contributing conditions, such as previous history, concomitant diseases or medications, and/or procedural risks.

13.1.3 Subject Follow-up

Every attempt should be made to follow the subject until the adverse event has resolved or until the Investigator determines the subject has returned to an acceptable state of health.

13.1.4 Reporting Serious Adverse Events

The Investigator must report **all serious adverse events within 24 hours**, irrespective of the relationship to study medication, to the CRO, automatically through the eCRF or by telephone or fax. The CRO will then forward this information to NEWRON within 24 hours of receipt. The names of the CRO contact will be communicated to the investigators by the CRO prior to the start of subject enrollment.

The minimum information required for an initial report of a Serious AE is as follows:

- Sender of report (name, address of Investigator, site number),
- Subject identification (screening/randomization number),
- Protocol number,
- Reportable event.

In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to the CRO by mail together with the Serious AE Form, retaining a copy on-site. If an autopsy is performed, a copy of the autopsy report should be actively sought by the Investigator and sent to the CRO as soon as available. A copy of the autopsy report will be retained on-site.

A follow-up Serious Adverse Event Report will be completed by the Investigator if important follow-up information (i.e., diagnosis, outcome, causality assessment, results of specific investigations) are made available after submission of the initial form. The follow-up form will be sent to the CRO as described above.

If the Investigator becomes aware of any deaths or serious adverse events after the end of the 30-day follow-up window established in the protocol following investigational product administration, they will be reported to the CRO as described above.

13.1.5 Safety Reporting to Investigators, IRBs, IECs and Regulatory Authorities

The Sponsor or their designee will be responsible for reporting all SAEs to regulatory authorities, investigators, Institutional Review Boards (IRBs) and Independent Ethics Committees (IECs), as applicable, in accordance with national regulations for the United States, Italy, India, the United Kingdom and Australia. For all active investigators located in the United States, the Sponsor or their designee will prepare an expedited report for all SAEs that are unexpected and potentially related to the study drug, and copies will be distributed according to all applicable laws and regulations. The investigational site also will forward a copy of all expedited reports to their IRB.

13.1.6 Reporting of Overdose

If the investigational site staff administering the study medication or the study Pharmacist reports that a subject took more than the requisite number of capsules or a higher dose than was assigned, this will be considered an overdose and must be reported immediately to the Investigator. Any instance of overdose, whether symptomatic or not, must be communicated to the CRO within 24 hours and be fully documented as a Serious Adverse Event. Details of any signs or symptoms and their management should be recorded including details of any antidote(s) administered.

13.1.7 Breaking of the Study Blind by the Investigator

The blind should be broken by the Investigator only in the case of emergency and if knowledge of the administered compound is deemed necessary for the medical treatment of the subject. Should it be necessary to break the blind, the Investigator will be able to do this using the IWRS. If possible, the Sponsor

should be notified before the study drug blind is broken. If the blind is broken the Investigator must immediately notify the CRO and provide the subject's number. The reason for breaking the blind should be noted on the Adverse Event (or Serious Adverse Event) section of the Case Report Form. Newron will inform the Safety Monitoring Board of the unblinding.

13.1.8 Pregnancy

Females of childbearing potential who are not using a contraception method judged to be adequate by their Health Care Provider, will not be eligible for the study. [*UK only*: Adequate contraception is defined as the use of a hormonal contraceptive or an Intra Uterine Device (IUD), preferably in conjunction with a spermicidal substance. Appropriate guidelines for use of the specific contraceptive should be followed. For patients whose illness is severe, i.e. advanced stage of physical and mental incapacitation, and are sexually inactive, the caregiver and the healthcare provider should discuss the most appropriate option, including abstinence. Adequate contraception should be continued until 4 weeks after stopping study medication.

As a further precaution, a serum pregnancy test will be performed at screening and baseline for all post-pubertal females. In addition, a serum pregnancy test will be performed at Weeks 24, 48, 72, 96, 120, 144, 168 and 192 (or at early discontinuation) for post-pubertal females; additional serum pregnancy tests may be performed, based on local requirements.

If a patient becomes pregnant during the study, she will be discontinued from the study immediately. The Investigator must report all pregnancies, within 24 hours of notification by the patient, to the CRO by email or by fax using the Pregnancy Reporting form. The timelines and other reporting requirements are the same as for a Serious AE. The patient (or her parent or legal guardian/representative) should be instructed to notify the Investigator within 24 hours if it is determined, after completion of the study, that the patient has become pregnant, either during the treatment phase of the study or within 30 days of completing the study. Whenever possible, a pregnancy should be followed to term and for 1 year after delivery of the baby, and any premature terminations reported. The status of the mother and child should be reported to the CRO or NEWRON within 24 hours after delivery, and one year later.

13.2 Safety Monitoring Board

An Independent Safety Monitoring Board (ISMB) will be established by Newron to review the safety of all patients enrolled in the Sarizotan 001/II/2015 study. It will be made up of at least 3 voting members. All of these members will have taken part in other international safety monitoring boards. Ravi Anand, MD, Newron's Chief Medical Officer, will serve as the Sponsor's primary contact for the ISMB.

The ISMB will review data from all patients enrolled at specified intervals throughout the trial. The ISMB will receive unblinded data; however, the blind will be maintained to the investigators, Sponsor, and CRO until after the final analysis has been completed. The CRO and Sponsor will compile subject data by treatment group and provide to the ISMB. The ISMB will have access to safety data, including adverse events, dropouts, SAEs, clinically significant abnormal laboratory tests, vital signs and ECGs, The ISMB will be regularly notified of the occurrence of any fatal/life threatening event within 24 hours and other serious adverse events within 72 hours. The ISMB will also receive detailed information on any adverse dropouts occurring in the study.

The ISMB is empowered to review all of the safety data on an ongoing basis, with special emphasis on serious AEs and deaths, in addition to the standard safety parameters. Based on their review of the safety data the ISMB will make a recommendation to Newron to (a) amend the ongoing study (e.g., increase safety monitoring), (b) terminate the study (e.g., the sarizotan safety profile is unacceptable), or (c) continue the

study as designed. The current study protocol will not be amended or changed (this includes the study design and entry criteria) unless mandated by the emerging (unblinded) safety profile of sarizotan.

Details of the ISMB charter (separate document) will be submitted to regulatory authorities, and will be available to IECs/IRBs upon request.

14 SUBJECT COMPLETION AND DISCONTINUATION

A subject will be considered to have completed the initial randomized treatment period of the study if the subject returns for the final visit at Week 24. 'Discontinuation' will refer to any subject who does not complete the full 24 weeks (168 ± 7 days) of the initial treatment period of the study. A subject will be considered to have completed the initial 24-week open-label extension treatment period of the study if the subject returns for the final visit at Week 48 (336 ± 14 days). A subject will be considered to have completed Part 1 of the additional extension treatment period of the study if the subject returns for the final visit at Week 96 (672 ± 14 days). A subject will be considered to have completed Part 2 of the additional extension treatment period of the study if the subject returns for the final visit at Week 144 (1008 ± 28 days). A subject will be considered to have completed Part 3 of the additional extension treatment period of the study if the subject returns for the final visit at Week 192 (1344 ± 28 days). The end of the study for an individual patient is defined as completion of the Week 48 evaluation (for patients not entering the additional extension period), the Week 96 evaluation (end of Part 1; for patients not continuing open-label treatment after Week 96), the Week 144 evaluation (for patients enrolled in Part 2 of the open-label extension), or the Week 192 evaluation (for patients enrolled in Part 3 of the open-label extension), and the Safety Follow-up assessment 2 weeks after the last dose of study medication.

In the absence of a medical contraindication or significant protocol deviation, every effort should be made by the investigator to keep the subject in the study; however, should the subject be withdrawn, all efforts will be made to complete and report the observations as thoroughly as possible, including post-treatment evaluation at the time of the subject's withdrawal, with an explanation of why the subject is withdrawing from the study.

The criteria for discharging a subject from the study prior to a post-study examination are listed below. The reason and time of all discontinuations will be specified on the 'final overall assessment' form.

A subject may be withdrawn from the study participation if:

- Any hypersensitivity or allergic reaction, clearly linked to the study medication, has occurred;
- Subject experiences an AE sufficiently severe, in the opinion of the investigator, that contraindicates continuing in the study;
- Subject wishes to withdraw (e.g. subject refuses to have any more PK blood samples taken; in this instance a specific reason must be recorded by the investigator)
- A subject is afflicted with a systemic illness, unrelated to the study medication but during the study period, for which a concomitant medication is required;
- A major protocol deviation has occurred, e.g., the subject failed to meet protocol entry criteria or did not adhere to protocol requirements, and continued participation poses an unnecessary risk to the subject's health.
- The subject is lost to follow-up, i.e., the subject did not return to the clinic, and attempts to contact the subject were unsuccessful. Attempts to contact the subject must be documented.
- The Sponsor, Institutional Review Board / Independent Ethics Committee (IRB/IEC), or regulatory agency terminates the study.

Dropouts will not be replaced.

14.1 Procedures for handling withdrawals

The Investigator may terminate a subject's study participation at any time during the study if a subject meets the study termination criteria described in Section 14. In addition, a subject may discontinue his/her participation without giving a reason at any time during the study. Should a subject's participation be

discontinued, the primary reason for termination must be recorded. If a subject is withdrawn from the study after receiving a dose of study medication, he/she will not be replaced. An attempt should be made to perform all final (Week 24 [double-blind period], Week 48 [initial 24-week open-label extension period], Week 96 [Part 1 of additional open-label extension period], Week 144 [Part 2 of additional open-label extension period], or Week 192 [Part 3 of additional open-label extension period]) assessments on the subject, and to follow up on any safety issues until resolution. Subjects who discontinue prematurely, as well as those subjects completing the study, should return to the clinic for a Safety Follow-up assessment 2 weeks after the last dose of study medication, and must be followed up for 30 days after the last dose of study medication regarding the occurrence of Serious Adverse Events.

15 PHARMACOKINETIC AND STATISTICAL METHODS

15.1 Pharmacokinetics and Determinants of Pharmacokinetic Variability

The concentrations of sarizotan in plasma samples will be determined using a validated LC-MS/MS method (see Section 11.2).

Appropriate pharmacokinetic parameters of sarizotan will be calculated for each dose level (Dose Levels 1 and 4 only) using non-compartmental methods, e.g. C_{max}, t_{max}, $t_{1/2}$, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$. The derived pharmacokinetic parameters will be listed and plotted by subject and summarized by treatment. An analysis will be performed to evaluate the multiple-dose pharmacokinetics (PK) of sarizotan at the doses tested, and determine if the PK parameters are dose proportional.

15.2 Statistical methods

This is a prospective, 24-week, randomized, double-blind, placebo-controlled study, with a 168-week open-label extension, designed to evaluate the safety, tolerability, and efficacy of two oral doses of sarizotan in patients with RTT with respiratory abnormalities. At least 129 patients will be randomized (approximately 43/group) to receive 24 weeks of double-blind treatment with either low dose sarizotan [sarizotan 2 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg) or 5 mg *bid* (age ≥13 years, weight ≥25 kg)], high dose sarizotan [sarizotan 5 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg), or 10 mg *bid* (age ≥13 years, weight ≥25 kg)], or placebo *bid*.

At the start of the extension treatment period, all patients, including those previously randomized to placebo, will receive treatment with sarizotan and undergo dose titration, starting at a dose of 2 or 5 mg *bid*, dependent on age and body weight (see Section 10.2). The dose will be maintained at 2 mg *bid*, increased to 5 mg *bid*, or increased to 10 mg *bid* after 4 weeks if there are no tolerability issues.

Data from the initial 24-week double-blind period will be used for comparing the efficacy and safety of low dose sarizotan (2 and 5 mg *bid*) and high dose sarizotan (5 and 10 mg *bid*) vs. placebo. The data from the open-label extension period, during which all patients will receive sarizotan, will be analyzed separately to evaluate long-term effects of treatment. The Week 24 efficacy and safety assessments will serve as the baseline assessments for the extension treatment period.

15.2.1 Populations

The safety population will consist of all subjects who took a dose of study medication. The efficacy analyses will be performed on an Intent-to-Treat (ITT) population comprising all patients who were randomized to treatment.

15.2.2 Background and demographic characteristics

The background and demographic characteristics (age, race, ethnicity, weight, height, education, past and current medical conditions, etc.), disease characteristics, and prior/concomitant medication use for randomized patients will be summarized by treatment group. Continuous variables will be summarized by minimum, maximum, mean, median, and standard deviation, and discrete variables will be summarized using frequencies and percentages.

15.2.3 Study medication

The number of subjects receiving each dose level and the number of capsules taken will be reported.

15.2.4 Concomitant therapy

A listing of concomitant medications administered from the time of the first dose of the study medication through completion of the final (Week 192) evaluations will be summarized by treatment group for the double-blind period (Days 1-168), and overall for the open-label extension period (Days 169-1344).

15.2.5 Safety evaluations

All subjects who receive a dose of study medication will be included in the safety analyses. All adverse events will be listed and summarized by body system and preferred term. The incidence of AEs (%) and their relatedness to the study drug, as assessed by the Investigator, will be reported. Serious adverse events (SAEs) and events which are newly occurring or worsening after administration of the study medication (TEAEs) will be summarized. In addition, adverse events that result in death or discontinuation (ADOs) of the study medication will be listed separately.

Results for other safety parameters such as vital signs, laboratory parameters, ECGs, physical, neurological and ophthalmological examinations, and the suicidality assessment, will be listed and summarized accordingly. Changes from baseline in vital signs, ECG and laboratory values, and physical, neurological and ophthalmological examination findings will be summarized, with abnormal and clinically notable values/findings being identified. In the initial 24-week, double-blind, placebo-controlled period, a comparison will be made between the sarizotan and placebo groups in the proportion of subjects experiencing SAEs, ADOs, and TEAEs and any clinically significant abnormalities in other safety parameters. Where feasible, mean changes from baseline will be examined using a paired t-test. Safety data for the open-label extension period will be analyzed separately and will use the Week 24 evaluations from the double-blind period as the baseline assessments.

15.2.6 Efficacy evaluations

Primary Efficacy Outcome

The primary efficacy outcome will be the percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour, during awake time. This will be calculated from the data obtained during home respiratory monitoring by means of an ambulatory data acquisition system (BioRadio™) designed to record specific respiratory and cardiac parameters. Assessment of the respiratory outcomes will be performed at home on any 3 days in each of the first 3 weeks of the Screening period, and in the week prior to each of the visits at Weeks 2, 8, 16, and 24. Monitoring will be performed when the patient is awake and should be attempted for 6 hours on each of the 3 days. Data from all of the quality recording time (minimally 3 hours) in the Screening period, and from the week prior to each of the visits at Weeks 2, 8, 16, and 24, will be averaged and used in calculating the value for the primary efficacy measure. If the patient meets the criterion for number of apnea episodes, as determined by Vivonoetics, in any week of the Screening period, home monitoring in the subsequent week(s) would not be required, and the screening data would be used to calculate the baseline value for the analyses.

The primary efficacy analysis for the initial, 24-week, double-blind, treatment period will be performed on the Intent-to-Treat (ITT) population, consisting of all patients who were randomized to treatment. The percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour will be compared between treatment groups using a Mixed Model Repeated Measures (MMRM) analysis. The MMRM model will include percent change from baseline as response, the fixed, categorical effects of treatment group, visit, treatment group-by-visit interaction, and the continuous terms age and baseline value as covariate. A statistically significant difference between high dose sarizotan (5 or 10 mg *bid*) and placebo will be considered evidence of efficacy in reducing apnea.

Key Secondary Efficacy Outcome

The mean rating of the caregiver-rated CIC at Week 24/endpoint will be compared between treatment groups using the Van Elteren test (a stratified Cochran Mantel Haenszel test with modified ridit scores). The following age strata will be considered in the analysis: <13 years and ≥ 13 years. In addition to the above analysis, comparisons between treatments for the proportion of patients rated improved at Week 24 will be done using logistic regression.

Other Secondary Efficacy Outcomes

The analysis of the Caregiver Top 3 Concerns will be performed first, comparing the change from baseline in the total score on the VAS for each of the 3 symptoms identified as most concerning at baseline using an ANCOVA-LOCF analysis. The model will include treatment group and the baseline value along with age as covariates. Change will be estimated using the least-squares mean derived from the ANCOVA. Comparisons between treatment groups at Week 24 will be made using the difference in least-squares mean, 95% confidence interval (CI) and p-values from the ANCOVA model.

Descriptive statistics will be presented for the other secondary efficacy outcomes. The proportion of patients rated improved, as well as the mean score, on the Investigator-rated CGI-C at each of the scheduled visits will be presented. Actual values and the change from baseline in other secondary efficacy variables (Motor Behavioral Assessment Scale and subscales, other respiratory parameters, and RCSS) at all scheduled visits will be presented. An additional analysis of efficacy will be performed for patients with a sarizotan plasma concentration of >400 ng/mL.

Multiple Comparisons/ Multiplicity

The study level error rate will be controlled by following a sequential multiple testing procedure, where testing will begin with the primary efficacy endpoint. The effects of the high dose sarizotan versus placebo will be analyzed first for all parameters in a pre-specified sequence.

The sequence of testing will be as presented below:

- Percentage change from baseline in the number of apnea episodes,
- Mean rating of CIC,
- Mean change from baseline in the Top Three Concerns Total Score.

Initial 24-week Double-blind Treatment Period

The primary efficacy assessment for the study will be performed using data from the initial 24-week double-blind, placebo-controlled treatment period. A statistical "Win" will be claimed if the following results are obtained in the primary and key secondary efficacy analyses:

- Statistically significant difference between high dose sarizotan (5 or 10 mg *bid*) and placebo in the percent reduction (change) from baseline in the number of apnea episodes (each ≥10 seconds in duration) per hour, during awake time;
- Directional change on the CIC in favor of high dose sarizotan (5 or 10 mg *bid*) group compared to placebo.

168-week Open-label Extension Treatment Period

Efficacy data from the extension period obtained from patients randomized to sarizotan at Baseline will be analyzed to determine if there is any attenuation of the benefit noted during the first six months. The response in placebo patients switched to sarizotan in the extension period will be compared with that in patients who were treated with sarizotan throughout the study. Data for the primary and secondary

respiratory parameters will be derived from data collected in the clinic using the SOMNOtouch. The statistical methodology to be used in the analyses conducted for the open-label extension treatment period will be described in detail in the Statistical Analysis Plan.

15.2.7 Interim analysis

There will be no interim analysis in this study.

15.2.8 Sample size and power considerations

A minimum of 129 patients with RTT will be included in this study, with approximately equal numbers of patients randomly assigned to each of the following three treatment groups:

- low dose sarizotan [sarizotan 2 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg) or 5 mg *bid* (age ≥13 years, weight ≥25 kg)],
- high dose sarizotan [sarizotan 5 mg *bid* (age 4 to <13 years; or age ≥13 years, weight <25 kg), or 10 mg *bid* (age ≥13 years, weight ≥25 kg)],
- or placebo *bid*.

The sample size estimate was based on the following assumptions:

- 3 treatment groups (2 active + placebo)
- Primary endpoint: Percent reduction in the number of apnea episodes (≥10 sec each) per hour
- H_0: Percent reduction in apnea episodes in placebo and active groups is the same
- H_A: There is a difference in the % reduction in apnea episodes between high dose sarizotan and placebo.

The assumptions for the estimated standard deviation (SD) are based on limited data from a study by Khwaja et al. (2014), in RTT patients. There were only 5 patients with data reported for apnea index (for apnea episodes ≥ 10 seconds). Pilot observational data collected using the BioRadio device in 26 patients with RTT indicated that the number of episodes of apnea may approach 60 per hour. Based on the range of 10-60 episodes per hour, ranges of 10-30, 10-45 and 10-60 apnea episodes per hour indicate SDs of 5.0, 8.75 and 12.5, respectively.

Sample sizes were computed to determine the number of patients required to detect a minimum difference in mean percent reduction of 20% (high dose sarizotan 30%, placebo 10%; see Table 8) and 10% (high dose sarizotan 20%, placebo 10%; see Table 9), based on differences between high dose sarizotan and placebo.

Table 8. Sample Size Estimates Assuming 90% Power and – 20% Difference

Parameter	**1**	**2**	**3**	**4**
Test significance level, α	0.050	0.050	**0.050**	0.050
1 or 2 sided test?	2	2	**2**	2
High Dose mean % Chg, μ_1	-30.00	- 30.00	**- 30.00**	-30.00
Placebo mean % Chg, μ_2	-10.00	-10.00	**-10.00**	-10.00
Difference, $\mu_1 - \mu_2$	-20.00	-20.00	**-20.00**	-20.00
Common St. Dev., σ	12.50	15.00	**20.00**	25.00
Effect size, $\delta = \lvert \mu_1 - \mu_2 \rvert / \sigma$	1.60	1.33	**1.00**	0.80
Power (%)	90	90	**90**	90
n per group	10	13	**23**	34

Table 9. Sample Size Estimates Assuming 90% Power and – 10% Difference

Parameter	1	2	3	4
Test significance level, α	0.050	**0.050**	0.050	0.050
1 or 2 sided test?	2	**2**	2	2
High Dose mean % Chg, μ_1	-20.00	**- 20.00**	- 20.00	-20.00
Placebo mean % Chg, μ_2	-10.00	**-10.00**	-10.00	-10.00
Difference, $\mu_1 - \mu_2$	-10.00	**-10.00**	-10.00	-10.00
Common St. Dev., σ	20.00	**12.50**	8.75	5.00
Effect size, $\delta = \lvert \mu_1 - \mu_2 \rvert / \sigma$	0.50	**0.80**	1.14	2.00
Power (%)	90	**90**	90	90
n per group	86	**34**	18	7

Sample sizes of 23 or 34 in each treatment group will have 90% power to detect a difference in mean percent reduction of -20% or -10%, respectively, assuming a common SD of 20 (for the 20% reduction) and 12.5 (for the 10% reduction), using a two group t-test with a 0.050 two-sided significance level. Assuming an attrition rate of 25% (7 or 9 patients) per group, a total of 30 or 43 patients would need to be enrolled in each group to get 23 or 34 patients completing the study. As this is the first large-scale study evaluating the effect of sarizotan on respiratory symptoms in patients with RTT, a conservative estimate of 10% reduction in apnea episodes was used to estimate the sample size required, i.e. approximately 43 patients per group. The sample size may be revised based on a prospective assessment of variability in the frequency of apnea episodes of 10 seconds or greater duration, from data collected from the first 20 patients who complete screening.

Details of all statistical analyses will be provided in a separate Statistical Analysis Plan, which will be completed prior to unblinding of the data at the Week 24 endpoint.

16 ETHICS

16.1 Ethical Considerations

The study will be carried out in accordance with the Declaration of Helsinki, as amended by the 64th World Medical Association General Assembly, Fortaleza, Brazil, October 2013 (Appendix 1A). However, where applicable, the principles of the 1996 version of the Declaration of Helsinki will be adhered to (Appendix 1B).

Subject Information and Informed Consent

The Informed Consent Form, as well as the Subject Information Sheet must be approved by the Ethics Committee together with the Study Protocol, before the start of the study.

The subject's parent, legal guardian, or representative must sign and personally date an approved Informed Consent Form after receiving detailed written and verbal information about the reason, the nature, the required procedures, the intended duration and the possible risks and benefits and any discomfort for the subject associated with the study. The subject may provide assent, if capable.

He/She should be informed that the subject's participation in the study is voluntary and that he/she may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.

The language used in the oral and written information about the trial, including the written Informed Consent Form should be as non-technical as practical and should be understandable to the subject.

The subject must be given ample time to read and to understand the Subject Information Sheet and opportunity to inquire and ask any clarification about the trial before signing the Informed Consent Form.

No study procedure can be performed (including the screening visit) before the informed consent form has been signed. The informed consent procedure must be done according to the guidelines provided in the Declaration of Helsinki and the ICH E6 Guideline for Good Clinical Practice.

The subject must be made aware and agree that personal information may be scrutinized during audit / inspection by competent authorities and properly authorized persons. However, personal information will be treated as strictly confidential and will not be publicly available.

By signing the Investigator Statement (Appendix 3), the Investigator assures NEWRON Pharmaceuticals that Informed Consent will be obtained.

Original signed Informed Consent Forms will be filed with the Investigator's File.

16.2 Institutional Review Board / Independent Ethics Committee Approval

The protocol, Investigator's Brochure, Subject Information Sheet, Informed Consent Form and any advertisement for the recruitment of subjects must be reviewed and approved by an appropriately constituted IRB/IEC, as required in Chapter 3 of the ICH E6 Guideline. A copy of the Committee's dated approval and a list of the members of the IRB/IEC will be given to the Sponsor for the Sponsor's files. A copy will be also included in the Final Report. Written IRB/IEC approval must be obtained by the Sponsor prior to shipment of study agent or subject enrollment. Any substantial amendments to the protocol, Informed Consent Form or Subject Information Sheet, must be approved by this committee, [*UK only*: as well as the Competent Regulatory Authority,] prior to implementation.

17 ADMINISTRATIVE CONSIDERATIONS

17.1 Regulatory Requirements–Sponsor/Investigator Obligations

This study will be conducted in accordance with the Declaration of Helsinki and the ICH E6 Guideline (Good Clinical Practice). To ensure compliance the Investigator agrees, by written consent to this protocol, to fully cooperate with compliance checks by allowing access to all documentation, including subjects' hospital files (the source documents), by authorized individuals.

17.2 Curriculum Vitae

The Investigator and any co-Investigator(s) must provide the Sponsor with current copies of their own curriculum vitae.

17.3 Investigators and Study Administrative Structure

The administrative structure of the study (e.g., Investigators, monitoring and evaluation personnel, laboratory facilities, clinical trial supply management) is presented in the Trial Master File.

The listing should include:

a) The Investigators.

b) Any other person carrying out observations of primary or other major efficacy variables, such as a nurse, physician's assistant, clinical psychologist, clinical pharmacist or house staff physician.

17.4 Investigator's Statement

This document, signed and dated by the Principal Investigator, describes the Investigator's obligations. The standard text is appended to the protocol (Appendix 3)

17.5 Monitoring Procedures

17.5.1 Study Monitoring

A CRO will be selected by the Sponsor to oversee the conduct of the trial. An appropriate representative of the CRO (Study Monitor) will maintain contact with the Investigator and will visit the study site for the purpose of discussing and/or reviewing data. An initiation visit will be made by the Study Monitor to discuss with the Investigator the protocol and the obligations of both the Sponsor and the Investigator. The Investigator must allow the Study Monitor to perform periodic, interim monitoring visits. The purposes of these visits are:

- To verify that written Informed Consent was obtained prior to each subject's participation in the trial.
- To assess the progress of the study.
- To review the compliance with the study protocol.
- To determine whether all adverse events were appropriately reported.
- To determine whether the Investigator is maintaining the essential documents.
- To discuss any emergent problem.
- To check the Case Report Forms for accuracy and completeness.
- To validate the contents of the Case Report Forms against source documents.
- To assess the status of drug storage, dispensing and retrieval.

The Investigator will make available the source documents for inspection. This information will be considered as confidential. *Deviations from the protocol must be notified to the Study Monitor as soon as possible.*

The Study Monitor will perform a closeout visit at the time when all CRFs have been completed and all queries have been answered.

17.5.2 Case Report Form

All subject data generated during the study will be recorded on the electronic Case Report Form (eCRF). The eCRF must be completed for all subjects for whom signed Informed Consent has been obtained. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's source document/eCRF. The Investigator, or designated representative, should complete the source document/eCRF as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. No section of the eCRF is to be left blank without an appropriate explanation by the Investigator, since the lack of such explanation may necessitate discarding an otherwise usable observation.

Paper copies of the eCRFs for the patient rating scales (e.g. CGI-C, CIC, RCSS) may be printed and used by the Investigator or designated staff during the assessment of the patient. Data recorded on these paper forms must be transcribed into the eCRF, and the original forms stored in the patient's file as source documents.

If requested, copies of the Case Report Forms are to be made available to the appropriate regulatory agencies.

The eCRFs will only be considered complete when each eCRF has been reviewed and electronically confirmed by the Investigator, indicating his/her assurance of the accuracy of all recorded data. It is expected that the Investigator and his/her staff will cooperate with the monitoring team and provide any missing data in a timely manner.

17.5.3 Auditing/Inspecting

The Investigator will make all pertinent records available, including source documentation, for inspection by regulatory authorities and for auditing by the Sponsor. This information will be considered as confidential. Audits/Inspections may occur any time from start to after conclusion of the study. When an Investigator signs the protocol, he/she agrees to allow regulatory authorities and Newron auditors to inspect his/her study records.

17.6 Archiving of Records

Copies of the protocol, subject identification codes, Case Report Forms, source data, Informed Consent Forms and other documents pertaining to the study conduct must be kept for the maximum period of time as required by the study center. This time period must be at least two years after the last approval of the marketing application of the study agent in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least two years have elapsed since the formal discontinuation of clinical development of the study agent.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Originals of all documentation and copies of outgoing correspondence concerning the study will be stored and retained by the Sponsor in a safe area in the Trial Master File for the lifetime of the product. In particular, the final report must be retained by the Sponsor, or the subsequent owner, for five years beyond the lifetime of the study agent.

17.7 Final Report

The Final Report will be written by the CRO, according to specifications to be defined by Newron.

17.8 Study Documentation and Publication of Study Results

Study Documentation

All unpublished documentation (including the protocol, Case Report Form and Investigator's Brochure) given to the Investigator is strictly confidential. All recipients must agree not to disclose the information herein contained to any person not connected with the study without the prior written authorization of NEWRON Pharmaceuticals. The submission of these documents to the IRB/IEC is expressly permitted. The involved parties agree that the results of this study will be used in their original form and/or in a global report for submission to governmental and regulatory authorities of any country.

All information communicated to the investigator(s) by Newron is the exclusive property of Newron Pharmaceuticals S.p.A. The Principal Investigator will ensure this information shall be kept strictly confidential by him/her or any other person connected with the study and shall not be disclosed to any third party without the prior written consent of Newron.

Publication of Results

Any formal presentation or publication of the data from this trial will be considered as a joint publication by the Investigator(s) and Newron. Authorship will be determined by a Publication Committee consisting of the lead investigator(s) from the trial, representatives from Newron, and an external consultant with expertise in the field. For multi-center studies, it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol by a statistician designated by Newron. Investigators participating in this study agree not to present or publish data gathered from a single center or sub-group of centers before the full initial publication, unless agreed to by all other investigators and Newron. Authorship of any publications resulting from pooled data will include members of each of the contributing centers, as well as Newron personnel.

Newron will form a study publication committee to coordinate and develop a publication policy and help in its implementation. The publication committee will be comprised of the Principal Investigator(s), a representative of Newron and an external consultant. Members of the publication committee cannot serve as first authors of more than one primary publication.

Any publication, abstract, or paper of any information or material relating to or arising out of the present clinical study shall be sent to Newron for review at least sixty (60) days before presentation at any congress, or publication of the final form(s) by any journal. Newron will inform the Investigator of any changes or deletions necessary to preserve Newron's confidential and proprietary technical information. All rights and interests worldwide in any inventions, know-how or other intellectual or industrial property rights, which arise during the course and/or as a result of the present clinical study or which arise from the information or materials supplied under this Agreement, shall be assigned to, vest in and remain the property of Newron Pharmaceuticals S.p.A.

17.9 Financial Agreement

A financial agreement (separate from the protocol) will be made and signed by NEWRON Pharmaceuticals or their designee and by a representative of the Institution where the study will be conducted.

17.10 Termination of the Study

In the event that the Investigator is unable to continue the study, another suitable sub-Investigator at the site will be designated to serve as the Investigator in the interim, until a new Investigator can be identified. This interim Investigator, if approved by the site IRB/IEC will carry out the responsibilities of the Investigator. Documentation testifying to this will be submitted to the Study Monitor within 10 days of the change. Within 6 months of the appointment of the interim Investigator, the new Principal Investigator must be identified and approved by both NEWRON Pharmaceuticals and the IRB/IEC for the study to continue at the site.

If the Sponsor and/or the Investigator should discover conditions arising during the study that indicate it should be terminated, an appropriate schedule for termination will be instituted. If the Investigator terminates the study, an explanatory letter will be provided to NEWRON Pharmaceuticals. Should the study be discontinued due to a decision by NEWRON Pharmaceuticals, the Investigator will be reimbursed for reasonable expenses incurred and for the subjects actually treated according to the study protocol.

17.10.1 Study discontinuation by the Sponsor

The Sponsor may terminate the entire study, or the study at an individual site, at any time, for any of the following reasons:

- failure to enroll subjects;
- protocol violations or deviations;

- inaccurate or incomplete data;
- non GCP compliance;
- completion of enrolment;
- administrative reasons.

17.10.2 Study discontinuation by the Clinical Investigator

The Investigator may terminate his/her participation in the study in consultation with the Sponsor due to the occurrence of significant adverse events and/or adverse drug reactions endangering the health of subjects, which make it ethically unacceptable to continue.

17.11 Insurance Policy

NEWRON or its designee will provide insurance coverage for damages emerging from the trial and involving the subjects treated with the test compound, provided that the Investigator(s) have adhered to the terms and provisions of the protocol. The principal Investigator will be supplied with all data concerning the insurance company and policy number for a maximum sum insurable.

17.12 Financial Disclosure

The study will be performed under a US IND; therefore, all Investigators/sub-Investigators, contractors, etc., are expected to comply with the obligations as specified in the CFR (21 CFR part 54) by the US FDA, including requirements for full Financial Disclosure (Guidance for Clinical Investigators, Industry and FDA Staff: Financial Disclosure by Clinical Investigators, February 2013).

The Principal Investigator and sub-Investigators will provide the Sponsor with adequate and accurate financial information (PD35) to ensure that the Sponsor can make complete and accurate financial certification of disclosure statements to concerned regulatory authorities. It is the duty of the Investigator to promptly update previous information provided to the Sponsor if there are salient changes that occur during the course of the study, and for a period of one year following its completion (last patient last visit).

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19 APPENDICES

19.1 APPENDIX 1: Declaration of Helsinki

Appendix 1A: 2013 Version of Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continual y through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

19. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

20. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

21. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

22. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

24. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

25. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without

reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

26. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

30. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient- physician relationship.

31. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no

intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

33. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

34. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

35. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

36. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

Appendix 1B: 1996 Version of Declaration of Helsinki

World Medical Association Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964 and amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, 35th World Medical Assembly, Venice, Italy, October 1983, 41st World Medical Assembly, Hong Kong, September 1989, and the 48th General Assembly, Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated the fulfilment of this mission. The Declaration of Geneva of the World Medical Assembly binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civic and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interest of the subject must always prevail over the interests of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is a liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

Whenever the minor child is in fact able to give consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH CLINICAL CARE
(Clinical research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, reestablishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient -- including those of a control group, if any -- should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.

4. The refusal of the patient to participate in a study must never interfere with the physician-patient relationship.

5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I,2).

6. The physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS
(Non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subject should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well being of the subject.

19.2 APPENDIX 2: Clinically Notable Values

The following guidelines, originally established by the Division of Neuropharmacological Drug Products of the FDA**,** will be used for determining clinically notable values for laboratory and vital signs parameters.

NOTABLE VALUES FOR LABORATORY PARAMETERS AND VITAL SIGNS

CLINICALLY NOTABLE BLOOD CHEMISTRY VALUES

Parameter	Synonym	S.I. units			Other units		
		Unit	Lower Limit	Upper Limit	Unit	Lower Limit	Upper Limit
Albumin		g/L	≤ 14	≥ 62	g/dL	≤ 1.4	≥ 6.2
Alk. Phosphatase	ALP	U/L	NA	≥ 3.0*URL			
Amylase		U/L	≤ 15	≥ 350			
ALAT, SGPT	ALT,GPT	U/L	NA	≥ 3.0*URL			
ASAT, SGOT	AST,GOT	U/L	NA	≥ 3.0*URL			
Bicarbonate		mmol/L	≤ 10	NA			
Bilirubin total		µmol/L	NA	≥ 34	mg/dL		≥ 2.0
Calcium total		mmol/L	≤ 2.1	≥ 3.0	mg/dL	≤ 8.2	≥ 12
Chloride		mmol/L	≤ 80	≥ 125			
Cholesterol		mmol/L	NA	≥ 141	mg/dL		≥ 500
Creatinine kinase	CPK	U/L	NA	≥ 3.0*URL			
Creatinine		µmol/L	NA	≥ 177	mg/dL		≥ 2.0
Gamma-GT Male		U/L	NA	≥ 100			
Female		U/L	NA	≥ 90			
Globulin total		g/L	≤ 10	NA			
Glucose		mmol/L	≤ 1.7	≥ 13.9	mg/dL	≤ 30	≥ 250
LDH		U/L	NA	≥ 3.0*URL			
Phosphate		mmol/l	NA	≥ 2			
Potassium		mmol/L	≤ 2.5	≥ 6.5			
Protein total		g/L	≤ 45	NA			
PTT		sec	NA	≥ 80			
Sodium		mmol/L	≤ 120	≥ 165			
Triglycerides		mmol/L	NA	≥ 5.6	mg/dL		≥ 500
Urea		mmol/L	NA	≥ 16.6	mg/dL		≥ 100
Urea nitrogen	BUN	mmol/L	NA	≥ 84.0	mg/dL		≥ 30
Uric acid Male		µmol/L	NA	≥ 624	mg/dL		≥ 10.5
Female		µmol/L	NA	≥ 505	mg/dL		≥ 8.5

CLINICALLY NOTABLE HEMATOLOGY VALUES

Parameter	Synonym	S.I. units			Other units		
		Unit	Lower Limit	Upper Limit	Unit	Lower Limit	Upper Limit
Basophils		L/L	NA	≥ 0.15	%		≥ 15
Eosinophils		L/L	NA	≥ 0.10	%		≥ 10
Erythocytes Male	RBC	10^{12}/L	≤ 2.5	NA			
Female		10^{12}/L	≤ 2.0	NA			
ESR Male		Mm	NA	≥ 25			
Female		Mm	NA	≥ 35			
Hematocrit Male	EVF	L/L	≤ 0.37	NA	%	≤ 37	
Female		L/L	≤ 0.32	NA	%	≤ 32	
Hemoglobin Male		g/L	≤ 115	NA	g/dL	≤ 11.5	
Female		g/L	≤ 95	NA	g/dL	≤ 9.5	
Leukocytes	WBC	10^{9}/L	≤ 2.8	≥ 16.0			
Lymphocytes		L/L	NA	≥ 0.80	%		≥ 80
MCHC		mmol/L	≤ 12.4	≥ 27.9	g/dL	≤ 20	≥ 45
MCV		10^{-15}L	≤ 60	≥ 120			
Monocytes		L/L	NA	≥ 0.40	%		≥ 40
Neutrophils		L/L	≤ 0.15	NA	%	≤15	
Thrombocytes	Platelets	10^{9}/L	≤ 75	≥ 700			

CLINICALLY NOTABLE URINALYSIS VALUES

Variable	Clinically Notable Values
Protein	Increase of 2 units
Glucose	Increase of 2 units

CLINICALLY NOTABLE VITAL SIGNS VALUES

Parameter	Unit	Decrease	Increase
Sitting/Supine SBP	mmHg	Value ≤ 90 and ≥ 20 decrease from Baseline.	Value ≥ 180 and ≥ 20 increase from Baseline
Sitting/Supine DBP	mmHg	Value ≤ 50 and ≥ 15 decrease from Baseline.	Value ≥ 105 and ≥ 15 increase from Baseline
Orthostatic Hypotension (based on standing SBP/DBP)	mmHg	Decrease in SBP/DBP from Supine to Standing position exceeding > 30mmHg	NA
Sitting pulse rate	bpm	Value ≤ 50 and ≥ 15 decrease from Baseline	Value ≥ 120 and ≥ 15 increase from Baseline
Weight	kg	≥ 7% decrease from Baseline.	≥ 7% increase from Baseline.
Respiration rates*	Breaths/ minute	< 12	> 25
Temperature	ºC	NA	Value ≥ 38.3 and ≥ 1.1 increase from Baseline.
Temperature	˚F	NA	Value ≥ 101.0 and ≥ 2.0 increase from baseline

* For respiration rate values are relevant, not decrease/increase.

19.3 APPENDIX 3: Investigator Statement

Investigator Statement

Compound:	**Sarizotan**
Protocol Number:	Sarizotan/001/II/2015
Title:	A Randomized, Double-Blind, Placebo-Controlled, Six-Month Study to Evaluate the Efficacy, Safety and Tolerability of Sarizotan in Patients with Rett Syndrome with Respiratory Symptoms
Trial Center Number:	
Principle Investigator:	
Address:	
Telephone:	
Fax:	
Email:	

COMMITMENTS

By signing this document, I agree to conduct the trial as outlined in the protocol and in accordance with (as applicable) the Declaration of Helsinki, the ICH GCP Guideline as well as all applicable government regulations.

I declare:

1. I am well qualified by scientific training and experience to conduct investigational studies in the clinical area of the proposed trial and I am affiliated with a recognized medical school or with an independent institution recognized for its excellence.
2. I have received and understood the information about pharmacology, toxicology and possible risks and side effects of the investigational compound (e.g., as described in the Investigator's Brochure).
3. I shall provide information to all staff members involved in the trial about their obligations as described in this document.
4. I shall submit the protocol, Informed Consent form/subject information sheet and other required documentation to the IRB/IEC for review and approval.

5. I shall make no changes to the protocol without formal amendment (prepared in agreement with the Sponsor), except when necessary to protect the safety, the rights or welfare of subjects. In this last case I will inform the Sponsor of the change.
6. I shall require Informed Consent from each subject prior to enrollment into the study. The Informed Consent shall be documented by use of a written consent form approved by the Sponsor and the IRB/IEC.
7. I shall use the investigational compound only in compliance with the trial protocol and I shall be responsible for the security and accountability of clinical study supplies.
8. I shall notify NEWRON immediately or no later than 24 hours by telephone and/or by fax of serious or unexpected adverse events and submit written reports of adverse events, as outlined in the protocol, to NEWRON, the IRB/IEC and to Regulatory Authorities (when appropriate).
9. I shall complete the Sponsor's Case Report Form (CRF) in a timely and legible manner.
10. I shall maintain accurate source records (hospital or other institutional records), which will support the data entered into Case Report Forms and I shall maintain these as specified in the protocol.
11. I shall allow monitoring visits by NEWRON's representatives at predetermined frequency.
12. I shall allow the authorized Sponsor representative and any Regulatory Authorities to inspect the facilities and pertinent records at reasonable times and in a manner which ensure subject confidentiality.
13. I shall maintain confidentiality about all information concerning the investigational compound, such as patent applications, formulas, manufacturing process, basic scientific data and formulation information supplied by the Sponsor and not previously published and I shall not disclose this information to a third party without the written consent of the Sponsor.
14. I shall permit the information developed in the clinical trial to be used by the Sponsor in connection with the development of the compound and may be disclosed to the IRB/IEC and Regulatory Authorities.

Following completion of the study, the data may be considered for reporting at a scientific meeting and/or for publication in a scientific journal. A copy of the manuscript or abstract will be provided to the Sponsor for review before submission to a scientific journal for publication and/or a scientific meeting selection committee for oral or poster presentation. Subgroup or individual Investigator publications must not interfere or compromise publication of the multi-center results of this clinical trial.

_____ _____

Investigator Signature Date

_____

Investigator (Printed Name)

19.4 APPENDIX 4: RTT Diagnostic Criteria

Revised Diagnostic Criteria for Rett Syndrome (Neul et al, 2010)

RTT Diagnostic Criteria 2010

Consider diagnosis when postnatal deceleration of head growth observed.

Required for typical or classic RTT

1. A period of regression followed by recovery or stabilization*
2. All main criteria and all exclusion criteria
3. Supportive criteria are not required, although often present in typical RTT

Required for atypical or variant RTT

1. A period of regression followed by recovery or stabilization*
2. At least 2 out of the 4 main criteria
3. 5 out of 11 supportive criteria

Main Criteria

1. Partial or complete loss of acquired purposeful hand skills.
2. Partial or complete loss of acquired spoken language**
3. Gait abnormalities: Impaired (dyspraxic) or absence of ability.
4. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms

Exclusion Criteria for typical RTT

1. Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems***
2. Grossly abnormal psychomotor development in first 6 months of life#

Supportive Criteria for atypical RTT##

1. Breathing disturbances when awake
2. Bruxism when awake
3. Impaired sleep pattern
4. Abnormal muscle tone
5. Peripheral vasomotor disturbances
6. Scoliosis/kyphosis
7. Growth retardation
8. Small cold hands and feet
9. Inappropriate laughing/screaming spells
10. Diminished response to pain
11. Intense eye communication - "eye pointing"

* Because *MECP2* mutations are now identified in some individuals prior to any clear evidence of regression, the diagnosis of "possible" RTT should be given to those individuals under 3 years old who have not lost any skills but otherwise have clinical features suggestive of RTT. These individuals should be reassessed every 6–12 months for evidence of regression. If regression manifests, the diagnosis should then be changed to definite RTT. However, if the child does not show any evidence of regression by 5 years, the diagnosis of RTT should be questioned.

** Loss of acquired language is based on best acquired spoken language skill, not strictly on the acquisition of distinct words or higher language skills. Thus, an individual who had learned to babble but then loses this ability is considered to have a loss of acquired language.

Revised Diagnostic Criteria for Rett Syndrome (continued)

*** There should be clear evidence (neurological or ophthalmological examination and MRI/CT) that the presumed insult directly resulted in neurological dysfunction.

Grossly abnormal to the point that normal milestones (acquiring head control, swallowing, developing social smile) are not met. Mild generalized hypotonia or other previously reported subtle developmental alterations[16] during the first six months of life is common in RTT and do not constitute an exclusionary criterion.

If an individual has or ever had a clinical feature listed it is counted as a supportive criterion. Many of these features have an age dependency, manifesting and becoming more predominant at certain ages. Therefore, the diagnosis of atypical RTT may be easier for older individuals than for younger. In the case of a younger individual (under 5 years old) who has a period of regression and ≥2 main criteria but does not fulfill the requirement of 5/11 supportive criteria, the diagnosis of "probably atypical RTT" may be given. Individuals who fall into this category should be reassessed as they age and the diagnosis revised accordingly.

19.5 APPENDIX 5: Cardiorespiratory Measurements using the BioRadio

Validation of the BioRadio System for Use in Remote Monitoring of Respiratory Function

The BioRadio™ system will be used in the first sarizotan study in Rett syndrome patients (Study Sarizotan/001/II/2015) to assess respiratory function, including changes in the frequency of apnea episodes, which will be the primary efficacy parameter.

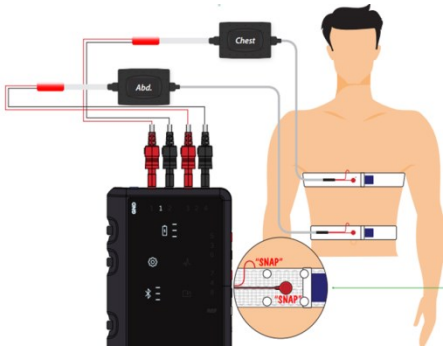
What is the BioRadio?

The BioRadio is a wireless data acquisition system developed by Great Lakes NeuroTechnologies (Cleveland, OH) that is capable of recording, displaying and analyzing physiological signals in real time, e.g. measuring electrocardiogram (ECG), respiratory, electro-encephalogram (EEG), electromyogram (EMG) and pulse oximetry parameters. Physiological signals are amplified, sampled and digitized, and can be wirelessly transmitted to a computer via Bluetooth receiver and/or recorded to onboard memory for post-analysis.

What does it consist of?

The BioRadio consists of a Primary Module, which has 4 (differential) or 8 (single-ended) channels, allowing measurement of multiple parameters simultaneously, and an optional Sensor Pod which has an input for a pulse oximeter and another sensor. A differential channel on the Primary Module can be configured for respiratory inductance plethysmography (RIP) using inductive bands. This configuration will be used in the sarizotan study; an additional channel will be used for pulse oximetry. The BioRadio is worn by the patient and uses wireless technology to transfer data from sensors attached to the body, allowing the patient to move about freely during the recording process.

How does it work?



The BioRadio system employs RIP technology to measure respiratory functional parameters, including apnea index, hyperventilation index, epoch apnea, respiratory rate, respiratory cycle time, and respiratory irregularity. Dual-band RIP is a technique that uses two elastic bands containing inductive coils, which wrap around the chest and abdomen of the patient, to calculate chest and abdominal expansion, respiratory rate, respiratory pattern and tidal volume. An alternating current is passed through the belt, generating a magnetic field. The act of breathing changes the cross-sectional area of the patient's body, which in turn changes the shape of the magnetic field generated by the belt, inducing an opposing current that can be measured. The signal produced is linear and related to the change in cross-sectional area. The utility of dual-band RIP in measuring respiratory function is based on the premise that volume changes at the orofacial orifices are equal to the sum of the volume changes of the rib cage and abdominal compartments (Konno and Mead, 1967), thus measurements done using RIP are equivalent to those made using spirometry, when the RIP system is properly calibrated (Sackner et al, 1989).

How does it measure respiration?

Impedance pneumography, using low amplitude, high frequency, alternating currents between two recording electrodes has been extensively used to record thoracic movements during respiration. However impedance pneumography use is constrained by findings that indicate the electrical resistance of the rib cage tissues is less than air, and therefore the AC passing through the thoracic cavity reflects tissue impedance and provides an erroneous value for thoracic volume. Impedance pneumography is affected by

motion and cardiogenic artifacts, and as the rib cage (RC) signal is dependent on posture, tidal volumes are difficult to estimate and the polarity of signals changes suddenly. This has led to increasing use of inductive plethysmography.

Respiratory inductive plethysmography has been used extensively as a non-invasive method for measuring respiratory volumes, particularly in the diagnosis of sleep-related breathing disorders (Cantineau et al, 1992; Bloch et al, 1997) and upper airway resistance syndrome (Loube et al, 1999). RIP has a number of advantages over impedance pneumography, which employs alternating current between two surface electrodes to record thoracic movements or volume changes at the rib cage (Landon, 2003). These advantages of RIP include greater accuracy and sensitivity, the ability to assess thoraco-abdominal coordination, and the minimization of motion artifact.

Validation studies of the BioRadio

The older RIP model was validated in patients and healthy volunteers for measurement of tidal volumes and respiratory lung volume by comparing it with data obtained with a pneumotachometer and spirometer as references (Valta et al 1992). A series of validation studies were performed for newer models (Respitrace Plus™) of the RIP, where it was first calibrated using qualitative diagnostic calibration by comparison of signals obtained by the pneumotachometer and from the sum of the rib and abdominal cages (RC and AC). The newer model has also been validated by comparing its performance to the older RIP.

The Respitrace Plus™ was evaluated both under clinical and laboratory conditions and compared with an earlier validated version (Respigraph™) in the measurement of tidal volume, long-term end-expiratory lung volume (EELV) and changes in EELV induced by positive end-expiratory pressure (PEEP) in a study done by Leino et al (2001). In this study no significant differences were noted between RIP and the pneumotachometer with regard to measurements of tidal volume in spontaneously breathing volunteers and mechanically ventilated patients with acute lung injury, or in measurements of PEEP-induced changes in EELV in acute lung injury patients.

A portable RIP has been tested in monitoring of ventilation during exercise in unrestrained subjects, including 20 healthy volunteers, 6 patients with COPD, and 5 patients with congestive heart failure (Clarenbach et al, 2005). An elastic body garment (LifeShirt™; VivoMetrics) containing the inductance sensors encircling the rib cage and abdomen was used and connected to a portable, battery-powered device which was connected to sensors measuring pulse oximetry, ECG and body movements, in addition to RIP. A comparison between measurements obtained with the portable RIP and a flowmeter (pneumotachograph) over the course of progressive exercise to exhaustion (treadmill) showed good agreement for respiratory cycle time, tidal volume, and minute ventilation for both healthy volunteers and patients with breathing difficulties. The RIP monitor was able to identify differences in breathing patterns between healthy volunteers and patients with pulmonary or cardiac disease.

The new RIP was also validated during controlled ventilation post operatively in patients with chronic obstructive pulmonary disease (COPD) and in acute lung injury, by comparing results with those obtained from a pneumotachometer (Neumann et al, 1998).

Use of the BioRadio to measure respiratory indices in patients

The guidelines for the use of unattended portable monitors in the diagnosis of obstructive sleep apnea published by the Portable Monitoring Task Force of the American Academy of Sleep Medicine, recommend the use of RIP, as opposed to other non-invasive techniques, e.g. piezo sensors, strain gauges and thoracic impedance, for measuring respiratory effort, in particular for scoring hypopnea, in the laboratory setting (Collop et al, 2007).

A study in newborn infants found that thoracic impedance monitors were less sensitive than RIP in monitoring respiratory events related to obstructive sleep apnea (Brouillette et al, 1987). Similarly, a study

performed comparing RIP and other methods, e.g. strain gauges and impedance pneumography, to a pneumotachograph in measuring breath amplitude and tidal volume in newborn infants demonstrated that RIP showed more accurate and consistent measurements in both the supine and prone positions, compared with the other methods (Adams et al, 1993).

A non-calibrated RIP has been used in a study in monkeys to measure phase angle (relationship of thoracic to abdominal movements during breathing) to assess the effects of inspiratory load on thoraco-abdominal ansynchrony, as is observed in infants and small children with acute upper airway obstruction (Hammer et al, 1995). A good correlation was observed between the phase angle measurements, calculated based on data from the RIP, and inspiratory load, i.e., phase angle increased continuously with higher inspiratory loads.

Use of the BioRadio in patients with Rett Syndrome

Recently, a study was performed in 12 female patients with Rett syndrome, ages 3-10, to evaluate the safety, pharmacokinetics and efficacy of mecasermin (Khwaja et al, 2014). Eight of the 12 patients were diagnosed with breathing abnormalities (e.g. air expulsion, breath holding, hyperventilation, apnea) at baseline. Automated measurements of cardiorespiratory parameters were performed using RIP and ECG, and recorded using the BioRadio. Data collected with this system was used to calculate various respiratory parameters, including total respiratory cycle times (Ttot) and inspiratory peaks (amplitude >20% above the typical amplitude). Ttot was categorized as normal, apneic or tachypneic based on aged-defined norms. Clinically significant apnea was defined as a pause in inspiration ≥10 seconds, and the number of these events per hour (apnea index) was calculated for each patient at baseline (mean ± SE: 10.11 ± 19.34) and at subsequent visits. In addition, hyperventilation was defined as ≥5 consecutive tachypneic breaths, with amplitude greater than the typical amplitude, and the number of these events per hour (hyperventilation index) was calculated for each patients at baseline (mean ± SE: 3.55 ± 6.71) and at subsequent visits. These measurements, which were made using the BioRadio and RIP, showed good agreement with clinicians' assessments using plethysmography.

Regulatory status of the BioRadio

The BioRadio complies with FCC Part 15 rules and conforms to FDA guidelines for use as a research tool in capturing electronic data in clinical trials. The Quality Management System for the device complies with the requirements of ISO13485:2003, and it is CE marked in Europe for research purposes. The BioRadio is not a medical device, but it complies with the Radio and Telecommunications Terminal Equipment (R&TTE) Directive 1999/5/EC and the Electromagnetic Compatibility (EMC) Directive 2004/108/EC, which are part of the product electrical safety requirements stated within IEC 60601-1.

The BioRadio is available for sale in the US, Europe and Canada. The development of the device has been funded in part by the NIH and 25 collaborating institutions have been involved in its testing. Results of studies using the BioRadio have been presented in over 65 peer-reviewed publications and presentations.

Summary

Respiratory inductive plethysmography has been used extensively over the past 25 years as a reliable method for non-invasive measurement of respiratory functional parameters. Good agreement has been demonstrated in a large number of studies between measurements taken using RIP and direct measurements using spirometry or pneumotachography. Coupling of RIP with a portable wireless data acquisition system such as the BioRadio allows respiratory measurements to be made in freely moving subjects. The utility of this combination has been demonstrated in a recent study in Rett syndrome patients, where cardiorespiratory monitoring was performed using the BioRadio and RIP, including measurement of apnea and hyperventilation indexes (Khwaja et al, 2014).

19.6 APPENDIX 6: Procedure for Preparing and Administering Sarizotan as a Solution

Administration of sarizotan as an oral solution

If the patient has difficulty swallowing whole capsules, the whole capsule may be dissolved in apple juice, cranberry juice, orange juice, tea (with or without milk), or water. Do not use a liquid other than one of these for mixing this medication. The procedure to prepare and administer a drinkable solution is as follows:

- Place the capsule(s) into a cup.
- Add 1 fluid ounce (2 tablespoons or 30 milliliters) of near boiling (80^0C or higher) hot apple juice, cranberry juice, orange juice, tea (with or without milk), or water to the cup.
- Stir the mixture with a spoon for 5-10 minutes or until all parts of the capsule have dissolved. The solution may appear cloudy and have a pink to orange color.
- Allow the solution to cool to room temperature.
- The patient should drink all of the medication solution immediately. Use a straw to sip if necessary.
- Add 1 more ounce (2 tablespoons or 30 milliliters) of warm or room temperature apple juice, cranberry juice, orange juice, tea, water or other liquid to the cup, swirl to rinse the walls of the cup, and have the patient drink immediately. This step is to make sure all of the medication is taken by the patient.
- Add 1 more ounce (2 tablespoons or 30 milliliters) of warm or room temperature apple juice, cranberry juice, orange juice, tea, water or other liquid to the cup, swirl to rinse the walls of the cup, and have the patient drink immediately. This step is to make sure all of the medication is taken by the patient.
- Discard any unused solution. Do not save solution or leftovers for later use.

Patients following a ketogenic diet may use low sugar or no-added sugar apple juice or cranberry juice product.

Dose differences are not anticipated between administering sarizotan in an oral capsule, or in a drinkable solution. The pharmacokinetics (PK) of sarizotan are linear in the dose range of 0.5 to 25 mg. Rapid absorption is indicated by Cmax occurring within 1 hour after administration. The absolute bioavailability is about 90%, so complete absorption is expected with both modes of administration.

These procedures can be performed by the caregiver during outpatient treatment or by the Investigator when the patient is being dosed in the hospital.

Administration of sarizotan as a solution through a gastrostomy tube (G-tube)

To give the medication via an enteral feeding tube (such as gastrostomy tube):

- Follow the standard procedures for enteral administration to ensure that the whole dose is given. Use aseptic techniques. Make sure to use a clean medicine cup, syringe, spoon and gauze. Follow this procedure to prepare and administer a solution via an enteral feeding tube:
- Place the capsule into a cup.

- Add 1 fluid ounce (2 tablespoons or 30 milliliters) of near boiling (80°C or higher) hot water to the cup.
- Stir the mixture with a spoon for 5-10 minutes or until all parts of the capsule have dissolved. The solution may appear cloudy and have a pink to orange color.
- Allow the solution to cool to room temperature.
- Following proper techniques, administer the solution to the patient via the feeding tube using an appropriate syringe or other approved device.
- Add 1 fluid ounce (2 tablespoons or 30 milliliters) of warm or room temperature water to the cup, swirl to rinse the walls of the cup, and use the syringe or approved device to administer the solution via the feeding tube immediately. This step is to make sure all of the medication is taken by the patient.
- Add another 1 fluid ounce (2 tablespoons or 30 milliliters) of warm or room temperature water to the cup, swirl to rinse the walls of the cup, and use the syringe or approved device to administer the solution via the feeding tube immediately.
- This additional step is to make sure all of the medication is taken by the patient.
- Discard any unused solution. Do not save solution or leftovers for later use.

19.7 APPENDIX 7: Tanner Staging

SEXUAL MATURITY RATING (TANNER STAGING) IN ADOLESCENTS

Stage	Female				Male				
	Age range (years)	Breast growth	Pubic hair growth	Other changes	Age range (years)	Testes growth	Penis growth	Pubic hair growth	Other changes
I	0–15	Pre-adolescent	None	Pre-adolescent	0–15	Pre-adolescent testes (≤2.5 cm)	Pre-adolescent	None	Pre-adolescent
II	8–15	Breast budding (thelarche); areolar hyperplasia with small amount of breast tissue	Long downy pubic hair near the labia, often appearing with breast budding or several weeks or months later	Peak growth velocity often occurs soon after stage II	10–15	Enlargement of testes; pigmentation of scrotal sac	Minimal or no enlargement	Long downy hair, often appearing several months after testicular growth; variable pattern noted with pubarche	Not applicable
III	10–15	Further enlargement of breast tissue and areola, with no separation of their contours	Increase in amount and pigmentation of hair	Menarche occurs in 2% of girls late in stage III	1½–16.5	Further enlargement	Significant enlargement, especially in diameter	Increase in amount; curling	Not applicable
IV	10–17	Separation of contours; areola and nipple form secondary mound above breasts tissue	Adult in type but not in distribution	Menarche occurs in most girls in stage IV, 1–3 years after thelarche	Variable: 12–17	Further enlargement	Further enlargement, especially in diameter	Adult in type but not in distribution	Development of axillary hair and some facial hair

Stage	Female				Male				
	Age range (years)	**Breast growth**	**Pubic hair growth**	**Other changes**	**Age range (years)**	**Testes growth**	**Penis growth**	**Pubic hair growth**	**Other changes**
V	12.5–18	Large breast with single contour	Adult in distribution	Menarche occurs in 10% of girls in stage V.	13–18	Adult in size	Adult in size	Adult in distribution (medial aspects of thighs; linea alba)	Body hair continues to grow and muscles continue to increase in size for several months to years; 20% of boys reach peak growth velocity during this period