Official Title: An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age who Have Rett Syndrome

NCT Numbers: NCT04988867

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CLINICAL STUDY PROTOCOL

An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age who Have Rett Syndrome

Protocol Number: ACP-2566-009

Amendment 2

Original Protocol Date: 08 December 2020

Protocol Amendment 1 Date: 30 June 2021

Protocol Amendment 2 Date: 19 December 2022

Protocol Template Version: 0.2

SPONSOR SIGNATURE PAGE

Title: An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age who Have Rett Syndrome

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Signature Page for ACP-2566-009 Protocol Amendment 2

Approve	[redacted]
Approve	[redacted]

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DECLARATION OF INVESTIGATOR

I confirm that I have read the above protocol. I understand it, and I will work according to the moral, ethical, and scientific principles governing clinical research as set out in the principles of Good Clinical Practice, as required by International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline E6 and as described in the United States (US) Code of Federal Regulations (CFR) 21 CFR parts 50, 54, 56, 312, and according to applicable local requirements.

Confidentiality Statement

The confidential information in this document is provided to you as an Investigator or Consultant for review by you, your staff, and the applicable institutional review board/ethics committee. Your acceptance of this document constitutes agreement that you will not disclose the information contained herein to others without written authorization from the Sponsor.

Investigator

Signature	Date
Name (printed)	

PROTOCOL SYNOPSIS[1]

Protocol Number	ACP-2566-009
EudraCT Number	Not applicable
Protocol Title	An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age who Have Rett Syndrome
Name of Investigational Product	Trofinetide oral solution
Indication	Rett syndrome
Phase of Development	2/3
Sponsor	Acadia Pharmaceuticals Inc. [redacted]

Primary Objectives	**Primary Endpoints**
• To investigate the safety and tolerability of treatment with oral trofinetide in girls two to five years of age who have Rett syndrome • To characterize the pharmacokinetics of oral trofinetide in girls two to five years of age who have Rett syndrome	The safety endpoints are as follows: • treatment-emergent adverse events (TEAEs) • serious adverse events (SAEs) • withdrawals due to adverse events (AEs) • potentially clinically important changes in other safety assessments The pharmacokinetic (PK) endpoints are as follows: • whole blood concentration of trofinetide • trofinetide PK parameters using the population PK approach

[1] NOTE: In this protocol, as in FDA guidance documents, the use of the word ”should” means that something is suggested or recommended, but not required. If something “may be” done, the meaning is that something is allowed, but not required. The terms “must” and “will” mean that something is required.

Exploratory Efficacy Objectives • To investigate the efficacy of treatment with oral trofinetide in girls two to five years of age who have Rett syndrome • To investigate the benefit of treatment with oral trofinetide on overall quality of life for girls two to five years of age who have Rett syndrome	**Exploratory Efficacy Endpoints** • Clinical Global Impression–Improvement (CGI-I) score • Change from Baseline in Clinical Global Impression–Severity (CGI-S) score • Caregiver Global Impression–Improvement (CaGI-I) score • Change from Baseline in Overall Quality of Life Rating of the Impact of Childhood Neurologic Disability (ICND) Scale
Number of Study Sites	Approximately 10 sites will participate in this study.
Number of Subjects Planned	Approximately 10 to 15 subjects are expected to be enrolled, at least 1 subject who weighs ≥9 to <11 kg, and at least 4 subjects who are less than 4 years of age at Screening, including at least one subject who is 2 years of age.
Test Product, Dose, and Administration	**Note**: Subjects who began treatment with trofinetide under the original protocol dated 08 December 2020 will continue to have trofinetide titration according to the dosing schedule in the original protocol. **Subjects Who Began Treatment Under Original Protocol Dated 08 December 2020** Subjects will begin treatment with trofinetide 2 g (in 10 mL) twice daily (BID). The dose will be increased to 3 g (in 15 mL) BID at the Week 2 visit, 4 g (in 20 mL) BID at the Week 4 visit, and 5 g (in 25 mL) BID at the Week 8 visit. In each case, the dose will be increased only if the Investigator judges that the subject is showing acceptable tolerability of the treatment. Doses will be administered orally or by gastrostomy (G) tube (G-tube) (doses administered via gastrojejunal [GJ] tubes must be administered through the G-port). At any point in the study, if the subject is not able to tolerate administration of the assigned dose, the Investigator may instruct the caregiver to reduce the dose of study drug to a dose as low as 1 g BID. In addition, up to four doses (in total, consecutive or non-consecutive) may be withheld within the first 6 weeks. After the subject is able to tolerate treatment, the Investigator will increase the dose as tolerated and will continue treatment on the highest dose the subject can tolerate (up to 5 g BID). This final assigned dose, i.e., the highest tolerated dose, must be given BID and the morning and evening doses must be identical.

	Subjects Enrolled Under Protocol Amendment 1 Dated 30 June 2021 Subjects participating under this amended protocol will begin treatment with trofinetide 2 g (in 10 mL) twice daily (BID). The dose will be increased to 4 g (in 20 mL) BID at the Week 2 visit. At the Week 4 visit, the dose will be increased to 5 g (in 25 mL) BID for subjects who weigh ≥9 to <12 kg (based on weight at Baseline), or 6 g (in 30 mL) BID for subjects who weigh 12 to <20 kg. In each case, the dose will be increased only if the Investigator judges that the subject is showing acceptable tolerability of the treatment. Doses will be administered orally or by gastrostomy (G) tube (G-tube) (doses administered via gastrojejunal [GJ] tubes must be administered through the G-port). At any point in the study, if the subject is not able to tolerate administration of the assigned dose, the Investigator may instruct the caregiver to reduce the dose of study drug to a dose as low as 1 g BID. In addition, up to four doses (in total, consecutive or non-consecutive) may be withheld within the first 6 weeks. After the subject is able to tolerate treatment, the Investigator will increase the dose as tolerated and will continue treatment on the highest dose the subject can tolerate (up to 5 g BID for subjects who weigh ≥9 to <12 kg, or up to 6 g BID for subjects who weigh 12 to <20 kg). This final assigned dose (i.e., the highest tolerated dose) must be given BID and the morning and evening doses must be identical.

Study Design	This is a multicenter, open-label study of trofinetide for the treatment of girls 2 to 5 years of age with RTT. Participating girls 2 to 4 years of age must weigh ≥9 kg and <20 kg. Girls 5 years of age who weigh ≥9 kg and <12 kg can also be enrolled.
	
	The duration of the study will be 26 months and will have three main periods:
	
	• Screening period: up to 4 weeks
	• Treatment period
	  • Period A: 12 weeks
	  • Period B: up to approximately 21 months
	• Safety follow-up period: 30 days
	
	Screening Period (Up to 4 Weeks)
	
	During the Screening period, subjects will be assessed for study eligibility. Only those subjects who meet all inclusion and no exclusion criteria will be eligible for the study.

	Subjects will be evaluated for the diagnosis of RTT. In addition, there must be verified documentation of a *MECP2* (methyl-CpG–binding protein 2 gene) mutation. Investigators should not withdraw a subject's prohibited medication for the purpose of enrolling them into the study. Medications should be discontinued only if it is deemed clinically appropriate to do so and in consultation with the treating physician. Caregivers will begin to keep a semi-structured caregiver diary during the Screening period. **Treatment Period A (12 Weeks)** Treatment Period A is designed for evaluation of the dosing, tolerability, pharmacokinetics, and exploratory efficacy of trofinetide in this population. The treatment period is approximately 12 weeks, the same length as the double-blind, placebo controlled study in girls and women 5 to 20 years of age (Table S–1). The Baseline visit (Visit 2) may occur after screening procedures are completed and have not ruled the subject out of eligibility for the study. Dosing is twice a day, once in the morning and once in the evening. The first dose of study drug will be administered after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The day the first dose is taken will be considered Day 1. A triplicate ECG must be performed 2 hours (±15 minutes) after the first dose. Two PK samples will also be collected after the first dose: the first, 2 hours (±15 minutes) after dosing, as close as possible to the postdose ECG, and the second, at least 1 hour after the first. Study drug must be discontinued in the event that a post-Baseline QTcF (QT interval corrected using Fridericia's correction method) duration of ≥500 ms or an increase of ≥60 ms compared to the average QTcF interval at Baseline (before dosing) is observed. After Treatment Period A (and the 30-day follow-up period, if applicable) is completed by all participants, an interim clinical study report of those data will be written. A final clinical study report will be written after the entire study is completed. **Treatment Period B (Up to Approximately 21 Months)** Treatment Period B is designed to assess the safety and exploratory efficacy of long-term treatment with trofinetide. Treatment Period B is similar in design and objectives to the open-label extension studies in girls and women 5 to 20 years of age.

	During Treatment Period B, subjects will have assessments completed at 24 weeks and 52 weeks after the Baseline visit and then every 26 weeks (6 months) thereafter until the study concludes or is terminated. Assessments may be completed in the clinic or off-site. Study visits may be done off-site at the discretion of the Principal Investigator and only with the prior approval of the Sponsor or Medical Monitor. **Safety Follow-Up Period (30[+4] Days)** A 30(+4)-day safety follow-up telephone or telemedicine contact is to be completed for subjects who complete the treatment period of the study or discontinue prematurely from the study (and do not begin to take marketed trofinetide within the 30 days after completing the treatment period of the study). The study schematic is provided in Figure S–1. The schedule of assessments is provided in Table S–1 (Screening, Baseline, and Treatment Period A) and Table S–2 (Treatment Period B, and Safety Follow-Up).
Study Duration	The duration of participation for individual study subjects will be approximately 26 months, consisting of a screening period of up to 4 weeks, a 12-week initial treatment period, an approximately 21-month extended treatment period, and a safety follow-up period of 30 days. The study start date is defined as the date the first subject is enrolled, which is the baseline visit date for the first subject. The primary completion date is the last date that subject data was collected for the primary outcome measure. The study completion date is defined as the last date that subject data was collected for the primary outcome measure, secondary outcome measure(s), and adverse events, which includes the safety follow-up telephone call visit.
Main Criteria for Inclusion and Exclusion	To be eligible for this study, subjects must meet all of the inclusion criteria and none of the exclusion criteria. **Inclusion Criteria:** 1. Informed consent prior to the conduct of any study procedures is required as follows: a. Written informed consent will be obtained from the legally acceptable representative (LAR). The process of obtaining informed consent will be conducted in accordance with institutional review board (IRB) or ethics committee (EC) policy and applicable local law.

	b. The subject's caregiver must also provide written informed consent regarding their participation in the study prior to participating in any study procedures. 2. Female subject a. 2 to 4 years of age and body weight ≥9 kg and <20 kg at Screening OR b. 5 years of age and body weight ≥9 kg and <12 kg at Screening 3. Can swallow the study medication provided as a liquid solution or can take it by gastrostomy tube 4. The subject's caregiver is English-speaking and has sufficient language skills to complete the caregiver assessments ***Diagnosis*** 5. Has classic/typical Rett syndrome (RTT) or possible RTT according to the Rett Syndrome Diagnostic Criteria (Appendix A) 6. Has a documented disease-causing mutation in the *MECP2* gene 7. Has a CGI-S score of ≥4 at Screening and Baseline ***Concomitant Treatment*** 8. If the subject is taking or was taking an anticonvulsant or any other CNS-active medication (including cannabinoids): a. the treatment regimen has been stable for at least 4 weeks before Baseline and there is no current plan to change the dose, OR b. if the medication was discontinued, the discontinuation has occurred no fewer than 2 weeks or 5 half-lives (whichever is greater) before Baseline 9. If the subject is taking or was taking any other medication daily for a chronic illness (not including antibiotics, pain relievers, or laxatives): a. the treatment regimen of the medication has been stable for at least 2 weeks before Baseline and there is no current plan to change the dose, OR b. if the medication was discontinued, the discontinuation has occurred no fewer than 2 weeks or 5 half-lives (whichever is greater) before Baseline 10. If the subject is receiving or was receiving a non-pharmacologic somatic treatment (e.g., a ketogenic diet or vagal nerve stimulation):

a. the treatment regimen has been stable for at least 2 weeks before Baseline and there is no current plan to change the treatment, OR
b. if the treatment was discontinued, the discontinuation has occurred no fewer than 2 weeks before Baseline

Seizures

11. Has a stable pattern of seizures, or has had no seizures, within 8 weeks prior to Screening

Place of Residence

12. Subject and caregiver(s) must reside at a location to which study drug can be delivered and have been at their present residence for at least 4 weeks prior to Screening

Exclusion Criteria:

Concomitant Treatment

1. Has been treated with growth hormone within 12 weeks of Baseline
2. Has been treated with IGF-1 within 12 weeks of Baseline
3. Has been treated with insulin within 12 weeks of Baseline

Medical Conditions Other Than RTT

4. Has current clinically significant cardiovascular, endocrine (such as hypo- or hyperthyroidism, Type 1 diabetes mellitus, or uncontrolled Type 2 diabetes mellitus), renal, hepatic, respiratory or gastrointestinal disease (such as celiac disease or inflammatory bowel disease) or has major surgery planned during the study
5. Has a history of, or current, cerebrovascular disease or brain trauma
6. Has significant, uncorrected visual or uncorrected hearing impairment
7. Has a history of, or current, malignancy

Laboratory Studies, Vital Signs, and Electrocardiogram

8. Has one or more clinical laboratory test value(s) outside the range specified below at Screening:
   a. hemoglobin below the normal range
   b. aspartate aminotransferase (AST) or alanine aminotransferase (ALT) value more than 1.5 × the upper limit of the normal range (ULN) for that subject's age and gender

	c. total bilirubin value ≥1.5 × ULN d. serum creatinine ≥ULN e. serum potassium below the normal range; serum potassium may be repeated during the Screening period with the agreement of the Medical Monitor f. thyroid stimulating hormone (TSH) value outside the normal range 9. Has a clinically significant abnormal laboratory value at Screening. Laboratory testing may be repeated during the Screening period with agreement of the Medical Monitor. 10. Has clinically significant abnormality in vital signs at Screening or Baseline 11. Has any of the following: a. QTcF interval of >450 ms at Screening or Baseline b. History of a risk factor for torsades de pointes (e.g., heart failure or family history of long QT syndrome) c. History of clinically significant QT prolongation that is deemed to put the subject at increased risk of clinically significant QT prolongation d. Other clinically significant finding on ECG at Screening or Baseline ***Other Criteria*** 12. Has a significant sensitivity or allergic reaction to trofinetide or its excipients 13. Has participated in another interventional clinical study within 30 days prior to Screening 14. Is judged by the Investigator or the Medical Monitor to be inappropriate for the study for any reason
Pharmacokinetic Assessments	PK blood samples will be collected at each visit during Treatment Period A. At Baseline (Visit 2), a PK sample will be obtained before dosing. After study drug administration, two PK samples will be collected, the first one approximately 2 hours after dosing and the second approximately 1 hour later. In addition to the two PK samples taken at Baseline, two PK samples will be taken at each subsequent visit (or upon early termination [ET]) in Treatment Period A, for a total of ten postdose PK samples over the duration of the study. At Visit 3, Visit 4, Visit 5, and Visit 6, the two PK samples will be collected at <u>one</u> of the following time intervals: • 1-3 hours after dosing • 4-7 hours after dosing

	• 8-11 hours after dosing Every subject should provide at least two PK samples from each of the three specified time intervals over the course of Visits 3 through 6 (1-3 hours after dosing, 4-7 hours after dosing, and 8-11 hours after dosing). The two PK samples taken within a time interval should be collected at least one hour apart. Pharmacokinetic samples will also be collected, if possible, at any ET visit or the visit immediately following any SAE or following any AE leading to discontinuation. For all scheduled PK samples (and for unscheduled samples if possible), the dates and times of administration of the study drug, over the 2 days prior to and on the morning of the PK sample draw, as well as the date and time of the sample draw, will be recorded. For samples collected from subjects who experience any SAE or experience an AE leading to discontinuation, the date and time of the last dose of study drug prior to the SAE or AE leading to discontinuation will also be recorded.
Optional Caregiver Interview	Participation of caregivers in an interview is an optional component of the study. Caregivers will be interviewed by a 3rd party central agency about the study subject's and caregivers' experience of treatment with trofinetide. Only caregivers who sign a separate informed consent will be interviewed. The results of the caregiver interviews will be summarized. The caregiver interview will be administered during Treatment Period B, at or before the final study visit (Week 104 [EOT/ET]).
Sample Size Calculations	Approximately ten (10) to fifteen (15) subjects will be enrolled in this study, at least 1 subject who weighs ≥9 to <11 kg, and at least 4 subjects who are less than 4 years of age at Screening, including at least one subject who is 2 years of age. The sample size is not based on statistical considerations and is believed to be adequate to characterize the PK of trofinetide in this population.
Statistical Methods	**Analysis Sets** The following populations will be defined and used in the analysis: Safety Analysis Set The Safety Analysis Set will consist of all enrolled subjects who received at least one dose of study medication. This analysis set will be used for all safety as well as any descriptive efficacy analyses. Pharmacokinetic (PK) Analysis Set The PK Analysis Set will consist of all subjects who receive at least 1 dose of study drug and have sufficient blood concentration data to

	calculate at least one PK parameter. **Safety Analyses** Safety results will be summarized using descriptive statistics. Adverse events will be coded into standard terminology using the Medical Dictionary for Regulatory Activities (MedDRA). Treatment-emergent adverse events (TEAEs), TEAEs leading to discontinuation, TEAEs considered related to study drug, TEAEs by maximum severity, and serious adverse events (SAEs) reported after study medication start, will all be summarized. Descriptive statistics for ECG, vital signs and weight, and clinical laboratory parameters, including changes from Baseline, will be tabulated by timepoint. Additionally, categorical analyses will be conducted on the incidence of subjects with prolonged QTc (QT interval of ECG corrected for heart rate) intervals and changes in QTc intervals in accordance with International Council on Harmonisation (ICH) guidelines. Additional safety analysis details will be specified in the statistical analysis plan (SAP). **Pharmacokinetic Analyses** Pharmacokinetic (PK) and exploratory efficacy measures will be collected from all subjects at the Baseline visit before dosing, at the Baseline visit after dosing, and after dosing at Weeks 2, 4, 8 and 12. Whole blood concentration data for trofinetide will be listed and summarized using descriptive statistics. Population PK analyses will be performed to characterize the PK in children 2 to 5 years of age. The details of the PK analysis will be presented in a separate population PK report in accordance with a separate data analysis plan (DAP). **Efficacy Analyses** Descriptive summary statistics for the CGI-I and CaGI-I (observed values only), and for the CGI-S and ICND QoL score (observed values and change from Baseline) will be summarized by visit using the Safety Analysis Set.
Date	19 December 2022

Figure S–1 Schematic of Study Design for ACP-2566-009

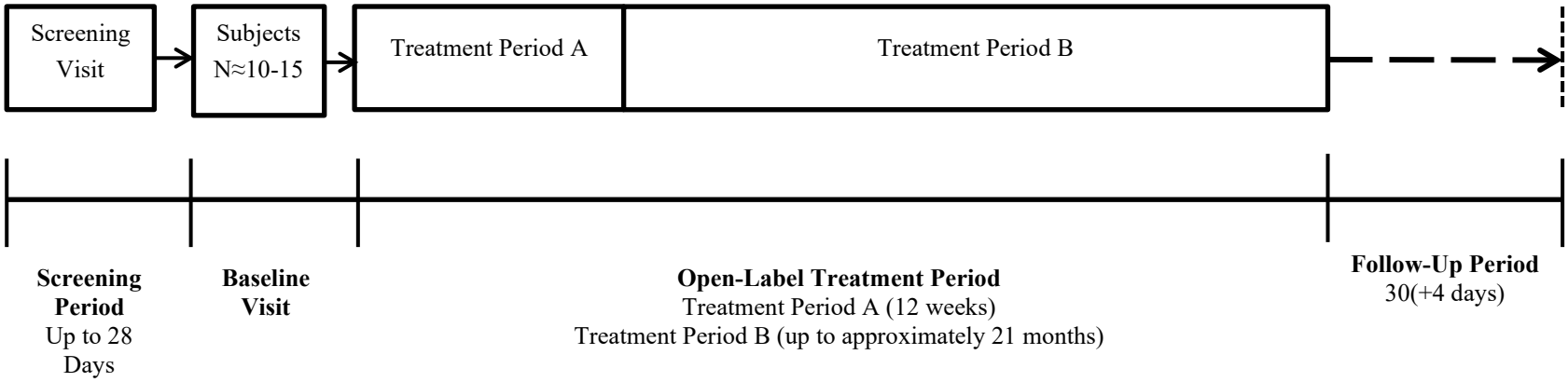

Table S–1 Schedule of Events and Assessments for Study ACP-2566-009 (Screening, Baseline, and Treatment Period A)

Period	Screening	Baseline[a]	Treatment Period A			
Visit			Week 2[b]	Week 4	Week 8	Week 12
Visit Number	1	2	3	4	5	6
Visit window (days)	N/A	N/A	±3	±3	±3	+3
Type of Visit[c]	Clinic or Off-site	Clinic	Clinic or Off-site			Clinic
Informed consent	X					
Inclusion/exclusion criteria	X	X				
Medical history and demographics	X					
Confirm documented diagnosis of RTT or possible RTT	X					
Confirm documented *MECP2* mutation[d]	X					
Rett syndrome history	X					
Rett Syndrome Clinical Severity Scale	X					
Physical examination[c]	X	X	X	X	X	X
Vital signs[e]	X	X	X	X	X	X
Height	X					X
Weight[c]	X	X	X	X	X	X
Electrocardiogram (ECG)[f]	X[f]	X[f]	X	X	X	X[f]
Clinical laboratory tests	X	X	X	X	X	X
Urinalysis	X	X	X			X
TSH, free T3, free T4	X					
Blood samples for pharmacokinetics[g]		X	X	X	X	X
Clinical Global Impression–Improvement (CGI-I)			X	X	X	X
Clinical Global Impression–Severity (CGI-S)	X	X	X	X	X	X
Caregiver Global Impression–Improvement (CaGI-I)						X
Overall Quality of Life Rating of the Impact of Childhood Neurologic Disability (ICND) Scale		X				X
Dispensing and review of semi-structured caregiver diary	X	X	X	X	X	X
Prior medications	X					
Concomitant medications		X	X	X	X	X
Assessment of adverse events	X	X	X	X	X	X
Authorization of study drug dispensation[h]		X----------X				
Study drug return[h]			X----------X			
Study drug accountability[h]			X	X	X	X

Abbreviations: AE=adverse event; EOT=end of treatment; *MECP2*=methyl-CpG–binding protein 2 gene; N/A=not applicable; PK=pharmacokinetic; RTT=Rett syndrome; SAE=serious adverse event; T3=triiodothyronine; T4=thyroxine; TSH=thyroid stimulating hormone

[a] The first dose of study drug will be administered after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. In that case, the second day is still considered part of the Baseline visit. The day the first dose is taken will be considered Day 1.

[b] Timing of post-Baseline visits will be calculated from the first day of dosing (Day 1) (i.e., the Week 2 visit will occur 2 weeks [14±3 days] after the first day of dosing).

[c] The Baseline and Week 12 visits must be done at the clinic. All other study visits may be done in the clinic or off-site. Study visits may be done off-site at the discretion of the Principal Investigator and only with the prior approval of the Sponsor or Medical Monitor. When a study visit takes place off-site, the physical examination will not be required. Weight should be measured whenever possible at off-site visits.

[d] If documentation of the mutation is not adequate, genomic testing for a mutation in the *MECP2* gene may be conducted as part of Screening.

[e] Vital signs will include body temperature, resting respiration rate, sitting systolic and diastolic blood pressure, and pulse rate. The sitting blood pressure should be measured after the subject has been sitting or supine for ≥3 minutes.

[f] ECGs will be completed in triplicate at Visit 1 (Screening), at Visit 2 (Baseline), both predose and at 2 hours (±15 minutes) after dosing, and at Visit 6 (Week 12). A single ECG will be completed at all other designated visits.

[g] A predose PK blood sample must be collected before administration of study drug at Baseline (Visit 2). After the first dose of study drug is administered, two PK samples will be collected: the first, 2 hours (±15 minutes) after dosing, as close as possible to the postdose ECG, and the second, at least 1 hour after the first.

In addition to the two PK samples taken at Baseline, two PK samples will be taken at each subsequent visit (or upon early termination [ET]) in Treatment Period A, for a total of ten postdose PK samples over the duration of the study. At Visit 3, Visit 4, Visit 5, and Visit 6, the two PK samples will be collected at one of the following time intervals: 1-3 hours after dosing, 4-7 hours after dosing, 8-11 hours after dosing. Every subject should provide at least two PK samples from each of the specified time intervals over the course of Visits 3 through 6 (1-3 hours after dosing, 4-7 hours after dosing, and 8-11 hours after dosing). The two PK samples taken within a time interval should be collected at least one hour apart.

When possible, an additional PK sample will be collected from subjects who experience any SAE or experience an AE leading to discontinuation as soon as possible after the occurrence of that event.

[h] Study drug will be dispensed at the site during the Baseline visit when the visit is conducted in the clinic. For the remainder of the study, investigational product will be shipped directly to the subject. Study drug shipment, return, and accountability will be performed in accordance with the drug distribution plan.

Table S–2 Schedule of Events and Assessments for Study ACP-2566-009 (Treatment Period B, and Safety Follow-Up)

Period	Treatment Period B				Safety Follow-Up[g]
Visit	Week 24	Week 52 (12 months)	Week 78 (18 months)	Week 104 EOT/ET (24 months)	EOT/ET +30 days
Visit Number	7	8	9	10	
Visit window (days)	±7	±7	±7	±7	+4
Type of Visit[a]	Clinic or Off-site				Telephone or Telemedicine
Physical examination[a]	X	X	X	X	
Vital signs[b]	X	X	X	X	
Height				X	
Weight[a]	X	X	X	X	
Electrocardiogram (ECG)[a]	X	X	X	X	
Clinical laboratory tests	X	X	X	X	
Urinalysis	X	X	X	X	
Blood samples for pharmacokinetics[c]				X[c]	
Clinical Global Impression–Improvement (CGI-I)	X	X	X	X	
Clinical Global Impression–Severity (CGI-S)	X	X	X	X	
Caregiver Global Impression–Improvement (CaGI-I)	X	X	X	X	
Overall Quality of Life Rating of the Impact of Childhood Neurologic Disability (ICND) Scale	X	X	X	X	
Dispensing and review of semi-structured caregiver diary	X	X	X	X[d]	
Concomitant medications	X	X	X	X	X
Assessment of adverse events	X	X	X	X	X
Optional caregiver interview[e]	X				
Authorization of study drug dispensation[f]	X----------X				
Study drug return[f]	X----------X				
Study drug accountability[f]	X	X	X	X	

Abbreviations: AE=adverse event; EOT=end of treatment; ET=early termination; SAE=serious adverse event

[a] Study visits may be done in the clinic or off-site. Study visits may be done off-site at the discretion of the Principal Investigator and only with the prior approval of the Sponsor or Medical Monitor. The EOT visit should be done in the clinic whenever possible. When a study visit takes place off-site, the physical examination will not be required. Weight should be measured whenever possible at off-site visits.

[b] Vital signs will include body temperature, resting respiration rate, sitting systolic and diastolic blood pressure, and pulse rate. The sitting blood pressure should be measured after the subject has been sitting or supine for ≥3 minutes.

[c] Pharmacokinetic samples will not be collected at the Week 104 visit or at an ET visit during Treatment Period B. Pharmacokinetic samples will be collected, if possible, at the visit immediately following any SAE or following any AE leading to discontinuation.

[d] At the Week 104 EOT/ET visit, the caregiver diary will be returned and reviewed, but no diary will be dispensed.

[e] Caregiver interviews will be conducted remotely via telephone during Treatment Period B, at or before the final study visit (Week 104 [EOT/ET]). Participation in the caregiver interview is an optional component of the study requiring a separate informed consent.

[f] Study drug will be dispensed at the site during the Baseline visit when the visit is conducted in the clinic. For the remainder of the study, investigational product will be shipped directly to the subject. Study drug shipment, return, and accountability will be performed in accordance with the drug distribution plan.

[g] A 30(+4)-day safety follow-up telephone or telemedicine contact is to be completed for subjects who complete the treatment period of the study or discontinue prematurely from the study (and do not begin to take marketed trofinetide within the 30 days after completing the treatment period of the study).

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Term	Definition
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCRP	breast cancer resistance protein
BID	bis in die; twice daily
CaGI-I	Caregiver's Global Impression–Improvement
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression–Improvement
CGI-S	Clinical Global Impression–Severity
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic case report form
EOT	end of treatment
ET	early termination
EU GDPR	European Union General Data Protection Regulation
FXS	fragile X syndrome
GCP	Good Clinical Practice
GJ	gastrojejunal
GPE	glycine-proline-glutamate
ICF	informed consent form
ICH	International Council for Harmonisation
ICND	Impact of Childhood Neurologic Disability
IGF-1	insulin-like growth factor 1
IRB	institutional review board
LAR	legally acceptable representative
MATE2-K	multidrug and toxin extrusion protein 2-K
MECP2	gene encoding methyl-CpG binding protein 2 (in humans)
PD	pharmacodynamic
P-gp	P-glycoprotein
PK	pharmacokinetic(s)
QT interval	QT interval for heart rate of ECG
QTc	QT interval of ECG corrected for heart rate
QTcB	QT interval corrected using Bazett's correction method
QTcF	QT interval corrected using Fridericia's correction method

Term	Definition
RSBQ	Rett Syndrome Behaviour Questionnaire
RTT	Rett syndrome
RTT-CBI	Rett Syndrome Caregiver Burden Inventory
RTT-CSS	Rett Syndrome Clinical Severity Scale
RTT-DSC	RTT Domain Specific Visual Analog Scale
SAE	serious adverse event
TBI	traumatic brain injury
TEAE	treatment emergent adverse event
ULN	upper limit of normal
US	United States

1 INTRODUCTION

This document is a research protocol and the described study will be conducted in compliance with the protocol and the International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guideline and applicable regulatory requirements.

1.1 Background Information

Rett syndrome (RTT) is a rare, seriously debilitating neurodevelopmental disorder which manifests in early childhood and affects primarily females. The main clinical features include a loss of acquired hand skills and spoken language, characteristic repetitive hand stereotypies, and either gait problems or an absence of gait. Patients with RTT cannot feed themselves and walk only with assistance. RTT patients require lifelong 24-hour care. There are no medicines currently approved for the treatment of RTT.

RTT is characterized by a distinctive pattern of disease onset and progression: (1) relatively normal initial development; (2) regression of acquired skills; (3) a plateau or "pseudostationary" period; and (4) a late motor decline (Neul and Chang 2020). Psychomotor development may appear normal for the first six months of life, followed by failure to reach normal developmental milestones. Developmental regression, especially loss of purposeful hand movements and spoken language, occurs between 18 and 30 months. After regression, people with RTT enter a plateau or "pseudostationary" period in which skills are no longer lost. Social interaction improves but the characteristic hand stereotypies become prominent (Neul and Chang 2020). In adulthood, a late motor deterioration stage occurs, characterized by worsening dystonia, rigidity, and in some cases, deterioration in the ability to walk and parkinsonian symptoms (Samaco and Neul 2011).

1.2 Investigational Product

Trofinetide is a novel synthetic analog of the tripeptide glycine-proline-glutamate (GPE), a product of the naturally occurring cleavage of insulin-like growth factor 1 (IGF-1), intended to treat the core symptoms of RTT by reducing neuroinflammation and supporting synaptic function. In the central nervous system (CNS), IGF-1 is produced by neurons and microglia. IGF-1 in the brain is critical for both normal development and for response to injury and disease. Trofinetide crosses the blood-brain barrier following oral administration and is thought to normalize insufficient bioavailability of IGF-1. Furthermore, it is believed that trofinetide inhibits the production of inflammatory cytokines as well as the overactivation of microglia and astrocytes. Both the inflammatory cytokines and pathologically activated microglia contribute to deficits in synaptic development and functional maturation of synaptic plasticity that are fundamental to the wide-ranging effects of RTT.

In the US, trofinetide for the treatment of RTT was granted Fast Track designation on 04 June 2013, Orphan Drug designation on 11 February 2015, and Rare Pediatric Disease designation on 02 March 2020.

1.3 Previous Clinical Experience

1.3.1 Clinical Pharmacology

The pharmacokinetics (PK) of trofinetide was assessed in healthy subjects, subjects with RTT, fragile X syndrome (FXS), and traumatic brain injury (TBI).

1.3.1.1 Linearity and Dose Proportionality

Trofinetide exhibits linear kinetics with no time- or dose-dependent effect on PK parameters. Systemic exposure to trofinetide was dose-proportional across the studied dose range, with no metabolic auto-inhibition or auto-induction.

1.3.1.2 Comparison of Single-Dose and Multiple-Dose Pharmacokinetics

Following oral administration of trofinetide, decline from peak concentration occurred in a biphasic manner. Minimal to no accumulation was observed following multiple-dose administration, and as such the single-dose PK profile is considered representative of the steady-state profile.

1.3.1.3 Absorption

Following oral administration, trofinetide was rapidly absorbed (T_{max} [time to maximum drug concentration], approximately 2 hours). There is no clinically relevant food effect and no difference in PK following morning or evening dosing.

1.3.1.4 Distribution

In vitro, trofinetide has shown low protein binding in human plasma (<6%). The volume of distribution at steady state was approximately 60 L, which suggests that trofinetide reaches beyond the extracellular fluid, with limited distribution into tissues (intracellularly). Nonclinical data demonstrated increased penetration of trofinetide into rat brain in the absence of injury.

1.3.1.5 Metabolism

In contrast to GPE, trofinetide displayed resistance to proteolytic degradation in human or rat plasma, and in human Caco-2 cells. Nonclinical data indicated that metabolism is not likely to be an important route of trofinetide disposition.

1.3.1.6 Elimination

Trofinetide is primarily excreted unchanged in urine, and hepatic metabolism is not likely to be an important route of trofinetide disposition. Trofinetide has an initial short half-life of approximately 1.2 hours, followed by a slower terminal elimination phase (half-life of approximately 13 hours). The initial half-life is considered the effective half-life for trofinetide.

1.3.1.7 Intrinsic and Extrinsic Factors

Intrinsic Factors

Population PK analysis in pediatric patients identified body weight as a significant factor, with effect on clearance and volume of distribution and consequently on the overall systemic exposure to trofinetide.

Formal clinical studies assessing the effect of renal or hepatic impairment on the PK of trofinetide have not been performed.

Extrinsic Factors

Coadministered drugs that are inhibitors or inducers of CYP450 are not likely to significantly affect the plasma concentrations of trofinetide.

At the target clinical concentrations, the potential of trofinetide to affect the PK of coadministered drug by inhibition or induction of CYP450 enzymes is low. Because of the relatively high clinical dose, the intestinal concentration of trofinetide is anticipated to be significantly high; trofinetide has the potential to inhibit CYP3A4 in the intestine, thus affecting coadministered drugs that have low bioavailability and are CYP3A4 substrates, such as midazolam.

Coadministered drugs that are inhibitors or inducers of drug transporter are not likely to significantly affect the plasma concentrations of trofinetide.

At the target clinical concentrations, the potential of trofinetide to affect the PK of coadministered drug by inhibition or induction of hepatic drug transporter is low. Because the intestinal concentration of trofinetide is anticipated to be significantly high, trofinetide has the potential to inhibit P-glycoprotein (P-gp) or breast cancer resistance protein (BCRP) transporter in the intestine. Similarly, because of the projected high renal concentration of trofinetide, it has the potential to inhibit multidrug and toxin extrusion protein 2-K (MATE2-K), which may decrease the renal excretion of endogenous and exogenous organic cations, such as metformin.

1.3.1.8 Overview of Clinical Pharmacokinetic/Pharmacodynamic Relationships

Exposure-response relationships were assessed in subjects with RTT using selected measures of efficacy endpoints. In subjects with RTT, a significant relationship was observed between systemic exposure and changes in the Rett Syndrome Behaviour Questionnaire (RSBQ), the RTT Domain Specific Visual Analog Scale (RTT-DSC), and the Clinical Global Impressions–Improvement (CGI-I) scale, where improvements were observed with higher exposure. Pharmacokinetic/pharmacodynamic (PK/PD) analysis of the drug exposure and efficacy data from all trofinetide-treated subjects demonstrated a relationship between exposure (AUC) and magnitude of response for the RSBQ, RTT-DSC, and CGI-I.

1.3.2 Phase 2 Studies

Two Phase 2, double-blind, randomized, placebo-controlled, dose-escalation clinical studies were conducted in girls and women with RTT: Study Neu-2566-RETT-001 and Study Neu-2566-RETT-002.

In Study Neu-2566-RETT-001 (Study 001), 56 adolescent and adult RTT subjects (15 to 44 years of age) received oral administration of placebo, trofinetide 35 mg/kg twice daily (BID), or trofinetide 70 mg/kg BID for up to 28 days. Results from the group-level analysis by individual cohort showed that trofinetide at 70 mg/kg BID demonstrated biological activity and preliminary evidence of efficacy. Trofinetide was associated with evidence of benefit over placebo based on prespecified criteria (p-value <0.2 in three core variables from three different efficacy domains): the Rett Syndrome Motor Behavior Assessment (RTT-MBA) change index (p=0.146), suggesting likely improvement in major signs and symptoms of Rett syndrome; the CGI-I score (p=0.164), suggesting improvement in illness and general clinical presentation compared to baseline; and the Caregiver Top 3 Concerns total visual analogue scale (VAS) score (p=0.076), suggesting improvement in the most concerning aspects of the Rett syndrome symptomology, as identified by caregivers.

Study Neu-2566-RETT-002 (Study 002) was subsequently conducted to assess 42 days of double-blind treatment with trofinetide 50 mg/kg BID, 100 mg/kg BID, and 200 mg/kg BID versus placebo in 82 female children and adolescents with RTT (5 to 15 years of age). For the 200 mg/kg BID dose group, three of the five core endpoints showed a statistically significant difference from placebo: the RSBQ total score (p=0.042), the clinician-rated RTT-DSC total score (p=0.025), and the CGI-I scale (p=0.029). The remaining two core outcome endpoints, the Caregiver Top 3 Concerns assessment and the RTT-MBA were directionally positive. The 50 mg/kg BID and 100 mg/kg BID groups showed some improvement but did not reach statistical significance. Across the measures, improvement was seen in a number of symptom areas of the RTT phenotype that seriously impair the quality of life of women and girls with RTT: breathing problems, repetitive movements (including hand function), mood dysfunction

(including nighttime behaviors), ambulation, and seizures. On the RSBQ, all but one of the subscales were directionally in favor of the 200 mg/kg BID treatment group, with notable improvement in the General Mood subscale (p=0.007), the Breathing subscale (p=0.095), and the Repetitive Face Movement subscale (p=0.047). On the RTT-DSC, improvements were notable in two domains, Ambulation (p=0.040) and Seizures (p=0.057), and were directionally in favor of the 200 mg/kg BID treatment for Attentiveness and Social Interaction.

Overall, these two Phase 2 studies demonstrated an acceptable safety and tolerability profile across the doses and treatment durations studied and a proof-of-concept efficacy profile to support initiation of Phase 3 clinical development.

Phase 2 studies of the oral formulation of trofinetide in adolescent and adult subjects with Fragile X syndrome and of the intravenous formulation in adult subjects with moderate to severe TBI have also been completed and are described in the Investigator's brochure.

1.3.3 Phase 3 Studies

Study ACP-2566-003 (Study 003) is a 12-week, multicenter, randomized, double-blind, placebo-controlled, parallel group Phase 3 study to evaluate efficacy and safety of a single trofinetide treatment group relative to placebo in girls and women 5 to 20 years of age with RTT. The starting dose of study drug is based on the individual's weight as the population PK (PopPK) model included body weight as a significant covariate on both clearance (CL) and volume of distribution (Vd). Dosing is 6 g BID, 8 g BID, 10 g BID, or 12 g BID depending the weight ranges (12-20 kg, >20-35 kg, >35-50 kg, and > 50 kg, respectively).

The co-primary efficacy endpoints in Study 003 are change from Baseline to Week 12 in RSBQ total score and CGI-I Score at Week 12. The key secondary endpoint is change from Baseline to Week 12 in Communication and Symbolic Behavior Scales Developmental Profile™ Infant-Toddler Checklist–Social Composite Score (CSBS-DP-IT Social). Approximately 184 subjects will be randomized in a 1:1 ratio to trofinetide or placebo. Subjects will be stratified according to age (5-10 years old, 11-15 years old, and 16-20 years old) and Baseline RSBQ severity (<35 total score and ≥35 total score). A minimum of 12 subjects are required to be randomized for each age stratum.

Additionally, ACP-2566-004 (Study 004) is a 40-week, multicenter, open-label extension (OLE) to Study 003 that gives RTT patients who received placebo in the antecedent study a chance to receive treatment with trofinetide, allows subjects who received treatment with trofinetide in the antecedent study to continue treatment, and assesses the long-term safety, tolerability, and efficacy of trofinetide in this population. Approximately 180 RTT patients are expected to be enrolled in Study 004. Upon completion of Study 004, subjects will have

the option to continue trofinetide open-label treatment in Study ACP-2566-005 (Study 005) for a further 32 months, or until such time as trofinetide may be approved and becomes commercially available, or development is discontinued in which case the study would be terminated.

Always refer to the latest version of the trofinetide Investigator's Brochure for the overall benefit/risk assessment and the most accurate and current information regarding drug metabolism, pharmacokinetics, efficacy, and safety.

1.4 Study Rationale

Study 003 evaluates trofinetide treatment in girls and women with RTT who are 5 to 20 years of age and weigh ≥12 kg. However, In the US the diagnosis of RTT is commonly made as early as 18 months to 2 years of age. Study 009 would provide data on safety, tolerability, and PK that could inform trofinetide dosing recommendations in these patients as well as data on long-term safety and efficacy.

The primary reason for maintaining an age cut-off of ≥5 years of age in Study 003 was in consideration of the variable clinical phenotype and developmental regression in the early presentation of RTT that could confound evaluation of the efficacy of interventional treatment. Enrollment of patients <5 years of age into clinical studies could not begin before analysis of animal juvenile toxicity data. Those data are now available and support administration across the intended pediatric age range. Please refer to the trofinetide Investigator's Brochure for further details.

1.4.1 Rationale for Study Design

A placebo-controlled study design is not feasible in this population because of the small numbers of patients with Rett syndrome in this restricted age range. It would be necessary to randomize a large number of patients in order to make any conclusions about differences between patients treated with active drug and those treated with placebo. In addition, interpretation of the efficacy data for an interventional treatment during the regression phase of RTT would present a challenge. The pattern and timing of the regression period varies among patients and would be difficult to characterize even in a large controlled study. The design of the Phase 3 Study 003 was deliberate in attempting to reduce the impact of disease variability in the evaluation of efficacy of trofinetide in RTT: an age cut-off of ≥5 years was selected for both the Phase 2 Study 002 and the Phase 3 Study 003 and both studies have an inclusion criterion requiring the patients to be post-regression at Screening.

Because demonstrating efficacy is not the primary focus of this study, the starting dose is relatively low and increases toward the target dose are made each week in a stepwise fashion, taking into account how well the subject tolerates the medication (see Section 1.4.2). PK data

collected during treatment will provide information on the exposure to drug achieved by the doses the subjects reach, and can later be compared to the exposures associated with benefit in the other studies of trofinetide in RTT. Data from 10 patients is sufficient to provide the PK and tolerability data that may be compared to the data from studies of older patients with RTT.

Period A is 12 weeks long to mirror the 12-week length of Study 003. Period B mirrors the open-label studies, Study 004 and Study 005.

Like Study 005, Period B is designed to give subjects in the study the ability to receive open-label treatment with trofinetide until it becomes commercially available in the US, or until development of trofinetide for RTT is discontinued.

It is estimated that the length of the study will provide enough time on treatment from the time the first subject enters the study until trofinetide is available, or until it becomes apparent that trofinetide will not be marketed in the US.

1.4.2 Rationale for Dose Selection

As was done to determine the appropriate doses for the Phase 3 program based on the Phase 2 study experience in girls and women with RTT, virtual patient simulations have been used to predict doses that would achieve the “target exposure” for the population in this study.

As in the ongoing Phase 3 studies, PK simulations indicated a dose of 6 g BID would achieve the target exposure expected to achieve efficacy for patients who weigh 12 to <20 kg. PK simulations indicated a dose of 5 g BID would achieve the targeted exposure in patients who weigh ≥9 to <12 kg. This study will initiate with a lower trofinetide dose of 2 g BID to best ensure initial tolerability. The trofinetide dose will be increased to 5 g BID or 6 g BID for the respective weight ranges. The dose will be increased to 6 g BID only for subjects who weigh 12 to <20 kg and who enroll under the present amended protocol.

In each case, the dose will be increased only if the Investigator deems that the subject is showing acceptable tolerability of the treatment. If the assigned dose is not tolerated, the dose may be reduced to as low as 1 g BID. In addition, up to four doses (in total, consecutive or non-consecutive) may be withheld within the first 6 weeks. The Investigator will increase the dose as tolerated and the subject will continue treatment on the highest tolerated dose.

1.5 Benefit/Risk Assessment

1.5.1 Known Potential Risks

Individuals with RTT frequently experience chronic constipation, and many have taken medications to treat constipation for a long time as well. Gastrointestinal side effects such as

diarrhea and vomiting are the most notable tolerability issue in clinical studies with oral trofinetide.

In Study 002, in which subjects 5 to 15 years of age were enrolled, diarrhea was the most frequently reported TEAE and was more frequently reported as a TEAE in trofinetide-treated subjects than in the placebo group, and frequency appeared to be dose-related (Table 1–1). This included diarrhea, intermittent diarrhea, and loose stools. The majority of cases were mild or moderate in severity and resolved on their own or with treatment per standard clinical practice (such as treatment with loperamide). In addition, the incidence of vomiting was higher in the highest-dose trofinetide group than with placebo. Diarrhea was not as frequent in the trofinetide treatment groups in the other Phase 2 study of oral trofinetide, but it was generally more frequently reported as a TEAE in trofinetide-treated subjects than in placebo-treated subjects. Adverse events (AEs) of diarrhea have also occurred frequently in Study 003, the ongoing double-blind, placebo-controlled, study and Study 004, the ongoing open-label extension.

A detailed summary of the safety data from clinical studies is available in the trofinetide Investigator's Brochure.

Table 1–1 Gastrointestinal TEAEs Reported in ≥2 Trofinetide-Treated Subjects During or Following Double-Blind Treatment: Study Neu-2566-RETT-002 – ITT Population

MedDRA System Organ Class Preferred Term	Placebo BID (N=24) n (%)	Trofinetide 50 mg/kg BID (N=15) n (%)	Trofinetide 100 mg/kg BID (N=16) n (%)	Trofinetide 200 mg/kg BID (N=27) n (%)
Gastrointestinal disorders	4 (16.7)	5 (33.3)	4 (25.0)	15 (55.6)
Diarrhoea	1 (4.2)	4 (26.7)	2 (12.5)	15 (55.6)
Vomiting	3 (12.5)	1 (6.7)	2 (12.5)	6 (22.2)
Constipation	- -	- -	- -	2 (7.4)
Gastroenteritis	- -	- -	1 (6.3)	1 (3.7)

Source: Neu-2566-RETT-002 CSR, Table 12-3
Abbreviations: BID=twice daily; ITT=Intention-to-treat; MedDRA= Medical Dictionary for Regulatory Activities

At the target clinical concentrations, the potential of trofinetide to affect the PK of coadministered drug by inhibition or induction of cytochrome P450 (CYP) enzymes, or by inhibition or induction of hepatic drug transporters is low. Because of the relatively high clinical dose, the intestinal concentration of trofinetide is anticipated to be significantly high; trofinetide has the potential to inhibit CYP 3A4 enzyme (CYP3A4) in the intestine. Similarly,

trofinetide has the potential to inhibit P-gp or BCRP transporter in the intestine. A physiologically based PK (PBPK) model showed that concomitant administration of 12 g oral trofinetide increases the exposure of orally administered midazolam, a CYP3A4 probe substrate, by 1.37- and 1.24-fold for AUC and C_{max}, respectively. Therefore trofinetide is considered a weak inhibitor of CYP3A4.

Similarly, because of the projected high renal concentration of trofinetide, it has the potential to inhibit MATE2-K, which may decrease the renal excretion of endogenous and exogenous organic cations, such as metformin.

Coadministered drugs that are inhibitors or inducers of CYP enzymes, or that are inhibitors or inducers of a drug transporter are not likely to significantly affect the blood concentrations of trofinetide.

Trofinetide is considered unlikely to adversely affect the cardiovascular system following oral dosing in humans.

No clinically significant QTc prolongation was observed following administration of trofinetide to healthy subjects and there was no signal of increased risk of QT prolongation with trofinetide treatment in patients with RTT or FXS.

Based on the results of the nonclinical and clinical studies, the risk of clinically significant QTc prolongation in humans is estimated to be low. However, the Investigator should be cautious when subjects in a trofinetide study take a medication that may prolong the QT interval.

1.5.2 Known Potential Benefits

Consistent with the evidence from nonclinical studies of GPE and trofinetide, there is evidence from clinical trials that trofinetide may be effective in the treatment of Rett syndrome (see Section 1.3). Trofinetide is not authorized for use in any indication.

A detailed summary of the potential risks and benefits is available in the trofinetide Investigator's Brochure.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives

The primary objectives of this study are:

- To investigate the safety and tolerability of treatment with oral trofinetide in girls two to five years of age who have Rett syndrome
- To characterize the pharmacokinetics of oral trofinetide in girls two to five years of age who have Rett syndrome

2.1.1 Primary Endpoints

The safety endpoints are as follows:

- treatment-emergent adverse events (TEAEs)
- serious adverse events (SAEs)
- withdrawals due to adverse events (AEs)
- potentially clinically important changes in other safety assessments

The pharmacokinetic (PK) endpoints are as follows:

- whole blood concentration of trofinetide
- trofinetide PK parameters using the population PK approach

2.2 Exploratory Efficacy Objectives

The exploratory efficacy objectives of this study are:

- To investigate the efficacy of treatment with oral trofinetide in girls two to five years of age who have Rett syndrome
- To investigate the benefit of treatment with oral trofinetide on overall quality of life for girls two to five years of age who have Rett syndrome

2.2.1 Exploratory Efficacy Endpoints

The exploratory efficacy endpoints for this study are:

- Clinical Global Impression–Improvement (CGI-I) score at Weeks 2, 4, 8, 12, 24, 52, 78, and end of treatment (EOT)
- Change from Baseline to Weeks 2, 4, 8, 12, 24, 52, 78, and EOT in Clinical Global Impression–Severity (CGI-S) score
- Caregiver Global Impression–Improvement (CaGI-I) score at Weeks 12, 24, 52, 78, and EOT
- Change from Baseline to Weeks 12, 24, 52, 78, and EOT in Overall Quality of Life Rating of the Impact of Childhood Neurologic Disability (ICND) Scale

3 STUDY DESCRIPTION

3.1 Overview of Study Design

This is a multicenter, open-label study of trofinetide for the treatment of girls 2 to 5 years of age with RTT. Participating girls 2 to 4 years of age must weigh ≥9 kg and <20 kg. Girls 5 years of age who weigh ≥9 kg and <12 kg can also be enrolled.

Approximately 10 to 15 subjects are expected to be enrolled, at least 1 subject who weighs ≥9 to <11 kg, and at least 4 subjects who are less than 4 years of age at Screening, including at least one subject who is 2 years of age. Approximately 10 sites will participate in this study.

The duration of the study will be 26 months and will have three main periods (Figure S–1):

- Screening period: up to 4 weeks
- Treatment period
    - Period A: 12 weeks
    - Period B: up to approximately 21 months
- Safety follow-up period: 30 days

The schedule of events and assessments is provided in Table S–1 (Screening, Baseline, and Treatment Period A) and Table S–2 (Treatment Period B, and Safety Follow-Up).

Study drug will be dispensed at the site during the Baseline visit when the visit is conducted in the clinic. For the remainder of the study, investigational product will be shipped directly to the subject. Study drug shipment, return, and accountability will be performed in accordance with the drug distribution plan.

The study start date is defined as the date the first subject is enrolled, which is the baseline visit date for the first subject.

The primary completion date is the last date that subject data was collected for the primary outcome measure.

The study completion date is defined as the last date that subject data was collected for the primary outcome measure, secondary outcome measure(s), and adverse events, which includes the safety follow-up telephone call visit.

Procedures for when a subject is lost to follow-up are provided in Section 4.5.

The Sponsor may discontinue the study for any reason.

3.1.1 Screening Period (Up to 4 Weeks)

During the Screening period, subjects will be assessed for study eligibility and prohibited medications will be discontinued if medically appropriate. Subject eligibility will be assessed by the site and the Sponsor through an eligibility review process. Only those subjects who meet all inclusion and no exclusion criteria will be eligible for the study.

Subjects will be evaluated for the diagnosis of RTT. In addition, there must be verified documentation of a *MECP2* mutation.

Investigators should not withdraw a subject's prohibited medication for the purpose of enrolling them into the study. Medications should be discontinued only if it is deemed clinically appropriate to do so and in consultation with the treating physician. The investigator should consider decreasing or discontinuing laxatives before the baseline visit to mitigate possible gastrointestinal side effects from trofinetide administration.

Caregivers will begin to keep a semi-structured caregiver diary during the Screening period.

3.1.2 Treatment Period A (12 Weeks)

Treatment Period A is designed for evaluation of the dosing, tolerability, and pharmacokinetics of trofinetide in this population. Exploratory efficacy assessments will also be conducted. The duration of Treatment Period A is approximately 12 weeks (Table S–1), the same length as the double-blind, placebo controlled study in girls and women 5 to 20 years of age.

The Baseline and Week 12 visits must be done at the clinic. All other study visits may be done in the clinic or off-site. Study visits may be done off-site at the discretion of the Principal Investigator and only with the prior approval of the Sponsor or Medical Monitor. When a study visit takes place off-site, the physical examination will not be required. Weight should be measured whenever possible at off-site visits.

The Baseline visit (Visit 2) may occur after screening procedures are completed and have not ruled the subject out of eligibility for the study.

Dosing is twice a day, once in the morning and once in the evening. The study drug must not be mixed with any food or liquid, including water. Immediately after administration of the study drug, food or liquid may be consumed if required.

The first dose of study drug will be administered after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. In that case, the second day is still considered part of the Baseline visit.

The day the first dose is taken will be considered Day 1. A triplicate ECG must be performed 2 hours (±15 minutes) after the first dose. Two PK samples will also be collected after the first dose: the first, 2 hours (±15 minutes) after dosing, as close as possible to the postdose ECG, and the second, at least 1 hour after the first. Study drug must be discontinued in the event that a post-Baseline QTcF (QT interval corrected using Fridericia's correction method) duration of ≥500 ms or an increase of ≥60 ms compared to the average QTcF interval at Baseline (before dosing) is observed.

After Treatment Period A (and the 30-day follow-up period, if applicable) is completed by all participants, an interim clinical study report of those data will be written. A final clinical study report will be written after the entire study is completed.

3.1.3 Treatment Period B (Up to Approximately 21 Months)

Treatment Period B is designed to assess the safety and exploratory efficacy of long-term treatment with trofinetide. Treatment Period B is similar in design and objectives to the open-label extension studies in girls and women 5 to 20 years of age.

During Treatment Period B, subjects will have assessments completed at 24 weeks and 52 weeks after the Baseline visit and then every 26 weeks (6 months) thereafter until the study concludes or is terminated. Assessments may be completed in the clinic or off-site. Study visits may be done off-site at the discretion of the Principal Investigator and only with the prior approval of the Sponsor or Medical Monitor.

3.1.4 Safety Follow-Up Period (30[+4] Days)

A 30(+4)-day safety follow-up telephone or telemedicine contact is to be completed for subjects who complete the treatment period of the study or discontinue prematurely from the study (and do not begin to take marketed trofinetide within the 30 days after completing the treatment period of the study). The telephone or telemedicine contact includes assessment of concomitant medications and treatments and assessment of AEs.

The study schematic is provided in Figure S–1.

The schedule of assessments is provided in Table S–1 (Screening, Baseline, and Treatment Period A) and Table S–2 (Treatment Period B, and Safety Follow-Up).

4 SUBJECT ELIGIBILITY AND WITHDRAWAL CRITERIA

To be eligible for this study, subjects must meet all of the inclusion criteria and none of the exclusion criteria.

4.1 Inclusion Criteria

A subject must meet all of the following inclusion criteria to be eligible for participation in the study:

1. Informed consent prior to the conduct of any study procedures is required as follows:
    a. Written informed consent will be obtained from the legally acceptable representative (LAR). The process of obtaining informed consent will be conducted in accordance with institutional review board (IRB) or ethics committee (EC) policy and applicable local law.

b. The subject's caregiver must also provide informed consent regarding their participation in the study prior to participating in any study procedures.

2. Female subject
   a. 2 to 4 years of age and body weight ≥9 kg and <20 kg at Screening OR
   b. 5 years of age and body weight ≥9 kg and <12 kg at Screening
3. Can swallow the study medication provided as a liquid solution or can take it by gastrostomy tube
4. The subject's caregiver is English-speaking and has sufficient language skills to complete the caregiver assessments

Diagnosis

5. Has classic/typical Rett syndrome (RTT) or possible RTT according to the Rett Syndrome Diagnostic Criteria (Appendix A)
6. Has a documented disease-causing mutation in the *MECP2* gene
7. Has a CGI-S score of ≥4 at Screening and Baseline

Concomitant Treatment

8. If the subject is taking or was taking an anticonvulsant or any other CNS-active medication (including cannabinoids):
   a. the treatment regimen has been stable for at least 4 weeks before Baseline and there is no current plan to change the dose, OR
   b. if the medication was discontinued, the discontinuation has occurred no fewer than 2 weeks or 5 half-lives (whichever is greater) before Baseline
9. If the subject is taking or was taking any other medication daily for chronic illness (not including antibiotics, pain relievers, or laxatives):
   a. the treatment regimen of the medication has been stable for at least 2 weeks before Baseline and there is no current plan to change the dose, OR
   b. if the medication was discontinued, the discontinuation has occurred no fewer than 2 weeks or 5 half-lives (whichever is greater) before Baseline
10. If the subject is receiving or was receiving a non-pharmacologic somatic treatment (e.g., a ketogenic diet or vagal nerve stimulation):
    a. the treatment regimen has been stable for at least 2 weeks before Baseline and there is no current plan to change the treatment, OR
    b. if the treatment was discontinued, the discontinuation has occurred no fewer than 2 weeks before Baseline

Seizures

11. Has a stable pattern of seizures, or has had no seizures, within 8 weeks prior to Screening

Place of Residence

12. Subject and caregiver(s) must reside at a location to which study drug can be delivered and have been at their present residence for at least 4 weeks prior to Screening

4.2 Exclusion Criteria

A subject must meet none of the following exclusion criteria to be eligible for the study:

Concomitant Treatment

1. Has been treated with growth hormone within 12 weeks of Baseline
2. Has been treated with IGF-1 within 12 weeks of Baseline
3. Has been treated with insulin within 12 weeks of Baseline

Medical Conditions Other Than RTT

4. Has current clinically significant cardiovascular, endocrine (such as hypo- or hyperthyroidism, Type 1 diabetes mellitus, or uncontrolled Type 2 diabetes mellitus), renal, hepatic, respiratory or gastrointestinal disease (such as celiac disease or inflammatory bowel disease) or has major surgery planned during the study
5. Has a history of, or current, cerebrovascular disease or brain trauma
6. Has significant, uncorrected visual or uncorrected hearing impairment
7. Has a history of, or current, malignancy

Laboratory Studies, Vital Signs, and Electrocardiogram

8. Has one or more clinical laboratory test value(s) outside the range specified below at Screening:
    a. hemoglobin value below the normal range
    b. aspartate aminotransferase (AST) or alanine aminotransferase (ALT) value more than 1.5 × the upper limit of the normal range (ULN) for that subject's age and gender
    c. total bilirubin value ≥1.5 × ULN
    d. serum creatinine ≥ULN
    e. serum potassium below the normal range; serum potassium may be repeated during the Screening period with the agreement of the Medical Monitor
    f. thyroid stimulating hormone (TSH) value outside the normal range

9. Has a clinically significant abnormal laboratory value at Screening. Laboratory testing may be repeated during the Screening period with agreement of the Medical Monitor.
10. Has clinically significant abnormality in vital signs at Screening or Baseline
11. Has any of the following:
    a. QTcF interval of >450 ms at Screening or Baseline
    b. History of a risk factor for torsades de pointes (e.g., heart failure or family history of long QT syndrome)
    c. History of clinically significant QT prolongation that is deemed to put the subject at increased risk of clinically significant QT prolongation
    d. Other clinically significant finding on ECG at Screening or Baseline

Other Criteria

12. Has a significant sensitivity or allergic reaction to trofinetide or its excipients
13. Has participated in another interventional clinical study within 30 days prior to Screening
14. Is judged by the Investigator or the Medical Monitor to be inappropriate for the study for any reason

4.3 Subject Withdrawal of Consent

In accordance with the Declaration of Helsinki and other applicable regulations, legally acceptable representatives (LARs) consenting on behalf of subjects have the right to withdraw the subject from the study at any time, and for any reason, without prejudice to her future medical care.

If the LAR decides to withdraw consent on behalf of the subject from all components in the study, this must be documented and no additional assessments will be performed. The Sponsor may retain and continue to use any data collected before such a withdrawal of consent. The LAR may request destruction of any samples taken and not tested, prior to their withdrawal of consent, and the investigator must document this in the site study records.

If the LAR wants to discontinue treatment on behalf of the subject and agrees to the evaluations specified at the EOT/ET visit and/or at safety follow up (whichever is applicable), as outlined in Table S–2, the agreed assessments should be conducted. The subject's reason for wanting to discontinue treatment and the agreement to continue with the applicable assessments for study termination must be documented.

4.4 Subject or Study Discontinuation

Subjects may be discontinued from the study for a number of reasons, including, but not limited to, those listed below:

- Adverse event
- Death
- Increase in post-Baseline QTcF interval (defined below and in Section 6.3.4)
- Lack of efficacy
- Lost to follow-up (Section 4.5)
- Non-compliance with study drug
- Physician decision
- Protocol deviation
- Study terminated by sponsor
- Use of prohibited medication
- Withdrawal of consent by LAR
- Other

The Sponsor reserves the right to discontinue the study at any time for any reason. Such reasons may be any of, but not limited to, the following:

- Occurrence of AEs unknown to date in respect of their nature, severity, and duration or the unexpected incidence of known AEs
- Medical, ethical or business reasons affecting the continued performance of the study

Regulatory authorities also have the right to terminate the conduct of the study in their region for any reason.

4.4.1 Post-Baseline QTcF Interval Stopping Criteria

Study drug must be discontinued in the event that a post-Baseline QTcF duration of ≥500 ms or an increase of ≥60 ms compared to the average QTcF interval at Baseline (before dosing) is observed. For visits at which more than one ECG is completed, the average QTcF interval of all legible ECGs will be used to determine the QTcF interval for that visit.

4.4.2 Handling of Subject Discontinuation During the Treatment Period

Unless the LAR has withdrawn consent on behalf of the subject to be contacted for this study, every reasonable effort should be made to complete Visit 10 or the early termination (ET) visit and the safety follow-up (as outlined in Table S–2) if a subject discontinues during the treatment period of the study for any reason. All information will be reported on the applicable pages of the electronic case report form (eCRF).

If a subject is discontinued from the study because of an AE, every reasonable attempt should be made to follow and appropriately treat the subject until the AE resolves or until the Investigator deems the AE to be chronic or stable. For subjects who continue to be followed for safety, SAEs should continue to be reported as described in Section 7.4.2. All SAEs will continue to be followed and appropriately treated until such events have resolved or the Investigator deems them to be chronic or stable.

Pharmacokinetic samples will also be collected, if possible, at any ET visit or the visit immediately following any SAE or following any AE leading to discontinuation, even if it is an unscheduled visit.

4.5 Subject Lost to Follow-Up

A subject will be considered lost to follow-up if they fail to attend a scheduled visit (including the safety follow-up visit) and the study site is unable to contact the caregiver.

Every reasonable effort should be made to contact the caregiver and will include a minimum of 3 documented telephone calls (each performed at different times of the day) and, if necessary, a certified letter to the caregiver's last known mailing address or local equivalent methods. All contact attempts are to be documented in the source documents.

4.6 Prior and Concomitant Therapy

All medications used up to 12 weeks prior to Baseline through completion of the safety follow-up visit or ET are to be recorded.

4.6.1 Prior Medication

Prior medication is defined as any medication taken before the date of the first dose of study drug.

4.6.2 Concomitant Medication

Concomitant medication is defined as any medication taken on or after the date of the first dose of study drug.

In order to ensure that appropriate concomitant therapy is administered, it is essential that caregivers be instructed not to administer any medication to the subject without prior

consultation with the Investigator (unless the subject is receiving treatment for a medical emergency).

The Investigator may prescribe appropriate medication to treat AEs.

Every effort should be made to maintain stable regimens of concomitant medications and allowed non-medicine based therapies throughout the course of the study, with the understanding that there will be some changes to a treatment regimen that are due to school schedules or are otherwise seasonally related. Special cases are medications taken for the treatment of constipation or that have diarrhea as an acute side effect. These may be adjusted as needed if diarrhea occurs.

4.6.2.1 Permitted and Prohibited Medications

Prohibited medications are IGF-1, growth hormone, and insulin. Prohibitions for concomitant medications will be followed between Visit 2 and Visit 10. Medications that can prolong QT interval are not prohibited but must be used with caution. Any use of medications that could interfere with study conduct must be discussed with the Medical Monitor.

CNS-active concomitant medications should remain at a stable dose throughout the study if possible. Any non-pharmacologic somatic treatment regimen (e.g., a ketogenic diet or vagal nerve stimulation) that has CNS effects should remain stable throughout the study if possible. Treatment of constipation may be changed as needed.

Subjects who require initiation of treatment with a prohibited medication will be withdrawn from the study.

Subjects who are discovered to have previously taken a prohibited medication during the study will be withdrawn from the study unless:

- the prohibited medication has been discontinued AND
- withdrawal from the study presents an unacceptable medical risk to the subject

The justification to allow the subject to continue in the trial will be made by the Sponsor/Medical Monitor, with medical input from the Investigator, and will be documented. If a subject is allowed to remain in the trial, this will be reported as a major protocol deviation and not a waiver.

5 INVESTIGATIONAL PRODUCT

5.1 Investigational Product Description

Trofinetide oral solution will be provided in a ready-to-use aqueous solution for oral administration. Doses will be administered orally or by gastrostomy (G) tube (G-tube) (doses administered by gastrojejunal [GJ] tubes must be administered through the G-port).

5.1.1 Formulation, Appearance, and Packaging

The Sponsor will supply trofinetide oral solution as an aqueous, ready-to-use, strawberry-flavored liquid in 500 mL (16 oz) round high-density polyethylene (HDPE) plastic bottles with a child-resistant closure.

Trofinetide oral solution is a clear, pink to red-colored liquid containing 1 gram of trofinetide in each 5 mL. The trofinetide oral solution also contains purified water, maltitol, strawberry flavor, sucralose, methylparaben sodium, propylparaben sodium, and FD&C Red #40 as inactive ingredients.

Trofinetide is manufactured under current Good Manufacturing Practices.

During the treatment period, study drug will be distributed in a quantity sufficient to ensure the subject has an adequate supply of study drug between study visits.

5.1.2 Product Storage and Stability

Investigational product will be shipped refrigerated at a temperature between 2°C and 8°C (36°F and 46°F) and must be stored at this temperature. Do not freeze.

5.1.3 Dosing and Administration

5.1.3.1 Dosing

Note: Subjects who began treatment with trofinetide under the original protocol dated 08 December 2020 will continue to have trofinetide titration according to the dosing schedule in the original protocol.

Subjects Who Began Treatment Under Original Protocol Dated 08 December 2020

Subjects will begin treatment with trofinetide 2 g (in 10 mL) BID (Table 5–1). The dose will be increased to 3 g (in 15 mL) BID at the Week 2 visit, 4 g (in 20 mL) BID at the Week 4 visit, and 5 g (in 25 mL) BID at the Week 8 visit. In each case, the dose will be increased only if the Investigator judges that the subject is showing acceptable tolerability of the treatment. An ECG must be performed 2 hours (±15 minutes) after the first dose, and a PK sample is to be taken as close as possible to the postdose ECG. A second PK sample will be taken at least 1 hour after the first PK sample.

Table 5–1 Dosing Schedule for Trofinetide for Subjects Who Began Treatment Under Original protocol Dated 08 December 2020

Dose Commences (Visit)	Dose	Total Daily Dose
Day 1 [a]	10 mL (2 g) BID	20 mL (4 g)
Week 2 (Visit 3)	15 mL (3 g) BID	30 mL (6 g)
Week 4 (Visit 4)	20 mL (4 g) BID	40 mL (8 g)
Week 8 (Visit 5)	25 mL (5 g) BID	50 mL (10 g)

[a] The first dose of study drug will be administered after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The day the first dose is taken will be considered Day 1.

Study drug is administered twice a day, once in the morning and once in the afternoon or evening. There should be at least 8 hours between doses.

At any point in the study, if the subject is not able to tolerate administration of the assigned dose, the Investigator may instruct the caregiver to reduce the dose of study drug to a dose as low as 1 g BID. In addition, up to four doses (in total, consecutive or non-consecutive) may be withheld within the first 6 weeks.

After the subject is able to tolerate treatment, the Investigator will increase the dose as tolerated and will continue treatment on the highest dose the subject can tolerate (up to 5 g BID). This final assigned dose, i.e., the highest tolerated dose, must be given BID and the morning and evening doses must be identical.

Study drug will be dispensed at the site during the Baseline visit when the visit is conducted in the clinic. For the remainder of the study, investigational product will be shipped directly to the subject. Study drug shipment, return, and accountability will be performed in accordance with the drug distribution plan.

Subjects Enrolled Under Protocol Amendment 1 Dated 30 June 2021

Subjects will begin treatment with trofinetide 2 g (in 10 mL) BID (Table 5–2). The dose will be increased to 4 g (in 20 mL) BID at the Week 2 visit. At the Week 4 visit, the dose will be increased to 5 g (in 25 mL) BID for subjects who weigh ≥9 to <12 kg, or 6 g (in 30 mL) BID for subjects who weigh 12 to <20 kg. In each case, the dose will be increased only if the Investigator judges that the subject is showing acceptable tolerability of the treatment. An ECG must be performed 2 hours (±15 minutes) after the first dose, and a PK sample is to be taken as close as possible to the postdose ECG. A second PK sample will be taken at least 1 hour after the first PK sample.

Table 5–2 Dosing Schedule for Trofinetide, Based on Weight at Baseline, for Subjects Who Enrolled Under Protocol Amendment 1 Dated 30 June 2021

Dose Commences (Visit)	Weight at Baseline	Dose	Total Daily Dose
Day 1 [a]	All subjects	10 mL (2 g) BID	20 mL (4 g)
Week 2 (Visit 3)	All subjects	20 mL (4 g) BID	40 mL (8 g)
Week 4 (Visit 4)	≥9 to <12 kg	25 mL (5 g) BID	50 mL (10 g)
	12 to <20 kg	30 mL (6 g) BID	60 mL (12 g)

[a] The first dose of study drug will be administered after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The day the first dose is taken will be considered Day 1.

Study drug is administered twice a day, once in the morning and once in the afternoon or evening. There should be at least 8 hours between doses.

At any point in the study, if the subject is not able to tolerate administration of the assigned dose, the Investigator may instruct the caregiver to reduce the dose of study drug to a dose as low as 1 g BID. In addition, up to four doses (in total, consecutive or non-consecutive) may be withheld within the first 6 weeks.

After the subject is able to tolerate treatment, the Investigator will increase the dose as tolerated and will continue treatment on the highest dose the subject can tolerate (up to 5 g BID for subjects who weigh ≥9 to <12 kg, or up to 6 g BID for subjects who weigh 12 to <20 kg). This final assigned dose (i.e., the highest tolerated dose) must be given BID and the morning and evening doses must be identical.

Study drug will be dispensed at the site during the Baseline visit when the visit is conducted in the clinic. For the remainder of the study, investigational product will be shipped directly to the subject. Study drug shipment, return, and accountability will be performed in accordance with the drug distribution plan.

5.1.3.2 Administration of Study Drug

Study drug is supplied in 500 mL (16 oz) bottles with a child-resistant closure. A press-in bottle adapter and syringe for accurately measuring the dose are supplied separately.

Dosing is twice a day, once in the morning and once in the evening. The study drug must not be mixed with any food or liquid, including water. Immediately after administration of the study drug, food or liquid may be consumed if required.

Doses may be taken orally or via gastrostomy tube.

- When study drug is given orally, it may be administered directly from a syringe or transferred into another container to facilitate administration.
- For gastrojejunal tubes, medication should be given via the gastric port. The tube must be flushed with water after study drug administration to clear the tube of study drug. No more than 250 mL of water should be used. Doses should be taken over a 10-minute period.

5.1.4 Method of Assigning Subjects to Treatment Groups

This is an open-label study. All subjects will receive treatment with trofinetide. Details of trofinetide dosing and administration is provided in Section 5.1.3.

5.1.5 Blinding

Not applicable; this is an open-label study.

5.1.6 Study Drug Compliance

If a subject misses one dose of study drug, she must not take an extra dose the next day.

5.1.7 Overdose

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than the maximum recommended dose per protocol. It must be reported, irrespective of outcome, even if toxic effects were not observed (Section 7.4.3). All events of overdose are to be captured as protocol deviations.

5.2 Investigational Product Accountability Procedures

The Investigator or designee will keep current and accurate records of the study drug product dispensed, used, and returned for each subject to assure the health authority and the Sponsor that the study drug is being handled appropriately. Caregivers must be instructed to return all packaging to the Investigator or designee at regularly scheduled clinic or home nursing visits as appropriate.

At appropriate intervals during the study, study drug reconciliation will be performed by the Sponsor (or designee) who may return appropriate unused study drug and used and unused packaging to the Sponsor's designee for destruction.

At the conclusion of the study, final study drug reconciliation will be conducted at the site. Final study drug accountability documentation will be maintained at both the site and by the Sponsor or designee. Any remaining unused study drug and all used and unused packaging will be sent back to the Sponsor's designee for destruction, as allowed by country-specific

regulations. Documentation of study drug destruction will be recorded and maintained by both the Sponsor and the Sponsor's designee.

The Investigator or designee is responsible for taking an inventory of each shipment of study drug received and comparing it with the accompanying material shipping form. The Investigator or designee will verify the accuracy of the information on the form, sign and date it, and provide a copy of it to the Sponsor or designee. Any study drug supplied is for use in this study only and must not be used for any other purpose.

6 STUDY PROCEDURES

Study-specific procedures are detailed below. All assessments will be completed according to the schedule described in Table S–1 and Table S–2. Every effort should be made to complete the required procedures and evaluations at the designated visits and times.

6.1 Screening Assessments

6.1.1 Confirm Diagnosis of RTT and *MECP2* Mutation

The site will confirm that the subject meets criteria for typical/classic RTT or possible RTT (Appendix A) and that there is documentation of disease-causing mutation of the *MECP2* gene. The diagnosis of "possible" RTT is given to those individuals under 3 years old who have not lost any skills but otherwise have clinical features suggestive of RTT (Appendix A).

The genotyping must have been performed at a laboratory certified by the College of American Pathologists (CAP), or under the Clinical Laboratory Improvement Act/Amendment (CLIA), or by an equivalent organization. If documentation of the mutation is not adequate, genomic testing for a mutation in the *MECP2* gene may be conducted as part of Screening.

The documented mutation must be associated with RTT, such as according to the *MECP2* mutation database RettBASE (http://mecp2.chw.edu.au/cgi-bin/mecp2/search/search.cgi?form=combined) (Krishnaraj et al. 2017). If there is any question regarding the association of the mutation with RTT, the Medical Monitor must be consulted. The disease-causing mutation will be documented in the source documentation and eCRF.

6.1.2 Medical History, Including RTT History, and Demographics

A complete medical history, including history of symptoms associated with RTT and history of regression, will be performed at Screening to document all current medical conditions, and previous major medical events and conditions. For subjects who were already receiving care for RTT at the study site, summary documents from the medical record (such as clinician's summaries) should be available as source documentation of major medical conditions or

events (e.g., surgeries). For subjects who were not a part of the clinical site's practice, the study team should make every effort to obtain summary medical records from their other providers in preparation of the screening visit.

6.1.3 The Rett Syndrome Clinical Severity Scale (RTT-CSS)

The RTT-CSS has been evaluated in over 1200 RTT children, adolescents and adults enrolled in the NIH-sponsored Natural History of Rare Diseases Project. This scale has been used as a measure of severity as reported in studies of genotype/phenotype correlations and epilepsy in RTT (Glaze et al. 2010) and was evaluated as an outcome measure in the Neu-2566-RETT-001 study of trofinetide in adolescent and adults with RTT. The scale was derived from that reported in Amir et al. (2000) and Monrós et al. (2001).

The RTT-CSS is a clinician-completed rating scale that measures the severity of core symptoms of RTT. The CSS consists of 13 items, 3 of which measure historical or static characteristics (age of onset of regression, age of onset of stereotypes, head growth) and 10 of which measure current function (somatic growth, independent sitting, ambulation, hand use, scoliosis, language, nonverbal communication, respiratory dysfunction, autonomic symptoms, and epilepsy/seizures) at the time of assessment, i.e., during the study visit.

All items are scored during a clinical interview and examination by the Investigator or qualified designee using either a 5- or 6-point Likert scale. Individual subscale scores and a total score are calculated.

The RTT-CSS will be administered at Screening only.

6.2 Exploratory Efficacy Assessments

All assessments will be administered in a standardized manner. Clinician-completed measures will be completed by trained practitioners. Caregiver-completed assessments will be reviewed by study personnel and caregivers will receive standardized training and guidance on how to complete the measures. To the extent possible, all efforts should be made to maintain the same caregiver (i.e., caregiver rater) and clinician rater (as applicable) across visits for a single subject.

All efficacy assessments designated to be completed at Baseline are to be performed prior to administration of the first dose of study drug.

6.2.1 Clinical Global Impression–Improvement (CGI-I) and Clinical Global Impression–Severity (CGI-S)

The CGI-I will be administered at Visits 3, 4, 5, 6, 7, 8, 9, and 10. Completion of this scale requires the clinician to rate how much the subject's illness has improved or worsened relative to a baseline state. A 7-point scale is used from 1=very much improved, 2=much

improved, 3=minimally improved, 4=no change, 5=minimally worse, 6=much worse, 7=very much worse.

The CGI-S assessment will be made at Screening, Baseline, and Visits 3, 4, 5, 6, 7, 8, 9, and 10. The CGI-S is a 7-point scale that requires the clinician to rate the severity of the subject's illness at the time of assessment, relative to the clinician's experience with subjects who have the same diagnosis. Considering total clinical experience, a subject is assessed on severity of illness at the time of rating: 1, normal, not at all ill; 2, borderline ill; 3, mildly ill; 4, moderately ill; 5, markedly ill; 6, severely ill; or 7, extremely ill.

In this study, the illness being assessed is RTT as a whole.

Following best practice, the CGI-S and CGI-I ratings for the study will be assessed using RTT-specific anchors across major symptom areas in the same manner as in the Phase 2 studies (Neul et al. 2015; Busner and Targum 2007; Glaze et al. 2017; Glaze et al. 2019).

6.2.2 Caregiver Global Impression–Improvement (CaGI-I)

The question of the CaGI-I is identical to the question utilized in the Rett Natural History Study regarding Overall Function and Well-Being and asks "How would you describe your child's overall function?" The CaGI-I will be administered at Visits 6, 7, 8, 9, and 10. Completion of this scale requires the caregiver to rate how much the subject's illness has improved or worsened relative to a baseline state. A 5-point scale is used with the following response options: much improved, improved, unchanged, worse, and much worse. The data will be presented both by text response and also by numeric value in which 1= much improved, 2= improved, 3=unchanged, 4=worse, 5=much worse.

In this study, the illness being assessed is RTT as a whole.

6.2.3 Overall Quality of Life Rating of the Impact of Childhood Neurologic Disability (ICND) Scale

The Impact of Childhood Neurologic Disability (ICND) Scale was developed to evaluate the impact that a child's condition has on the child's and the family's everyday life at the present time and during the previous 3 months (Camfield et al. 2003).

In this study, the caregiver will complete only the Overall Quality of Life rating of the ICND. The caregiver rates overall quality of life of the subject by responding to the following: "Please rate your child's overall 'Quality of Life' on the scale below. Choose the number which you feel is best and circle it". The choices range from 1 ("Poor") to 6 ("Excellent").

The assessment will be administered at Baseline, Visits 6, 7, 8, 9, and 10.

6.3 Safety Assessments

6.3.1 Physical Examination

A general physical examination will be conducted at Screening, Baseline, and Visits 3, 4, 5, 6, 7, 8, 9, and 10. When a study visit takes place off-site, the physical examination will not be required. The physical exam procedures will include the following organ systems:

- Neurological
- Head, ears, eyes, nose, and throat
- Skin
- Cardiovascular
- Respiratory
- Abdomen
- Genitourinary (optional)
- Musculoskeletal

6.3.2 Vital Signs

Vital signs will include body temperature, resting respiration rate, sitting systolic and diastolic blood pressure, and pulse rate. The sitting blood pressure will be measured after the subject has been sitting or supine for ≥3 minutes.

Vital signs to be measured at Screening, Baseline, Visits 3, 4, 5, 6, 7, 8, 9, and 10.

6.3.3 Height, Weight, and Body Mass Index

Height will be measured at Screening and at Visits 6 and 10.

Weight will be measured at Screening, Baseline, and Visits 3, 4, 5, 6, 7, 8, 9, and 10 when the visit takes place in the clinic. When a study visit takes place off-site, weight should be measured whenever possible.

Body mass index will be calculated using the following formula:
Weight (kg) / [height (m)]^2.

6.3.4 Electrocardiograms

All ECGs will be complete, standardized recordings appropriate for the age of the subject, whenever possible. ECGs will be completed in triplicate at Visit 1 (Screening), at Visit 2 (Baseline) predose and at 2 hours (±15 minutes) after dosing, and at Visit 6 (Week 12).
A single ECG will be completed at Visit 3 (Week 2), and Visit 4 (Week 4), Visit 5 (Week 8),

Visit 7 (Week 24), Visit 8 (Week 52), Visit 9 (Week 78), and Visit 10 (Week 104). For visits at which more than one ECG is completed, the average QTcF interval of all legible ECGs will be used to determine the QTcF interval for that visit.

The subject should rest for ≥5 minutes in a supine position before the ECG is obtained. If it is impractical to rest in supine position due to the subject's medical condition, the subject may remain in a partial supine or other position (e.g., upright in wheelchair) for ≥5 minutes before the ECG is obtained and when the ECG is obtained. ECG tracings (paper or electronic) will be reviewed and interpreted by a qualified clinician for prolongation of the QTcF interval and for other cardiac irregularities. ECG tracings and results (ventricular rate, PR interval of ECG, QRS interval of ECG, QT, QTcF, and QTcB intervals) will be included in the subject's study records.

ECGs will also be read by a qualified central reader. The central reading will be the reading that is entered in the database. The results from the reports from the central reader will also be reviewed by the Investigator. At Screening and Baseline, the average QTcF interval of all legible ECGs will be used to determine eligibility.

If the average QTcF value from the set of ECGs done at Screening is prolonged due to an identifiable cause, and it is medically appropriate to address that cause, a repeat set of triplicate ECGs may be performed during the Screening period and before the Baseline visit with the agreement of the Medical Monitor. In this case, the repeat ECGs will be used in determination of subject eligibility.

6.3.4.1 Post-Baseline QTcF Interval Stopping Criteria

In the event that a post-Baseline QTcF duration of ≥500 ms or an increase of ≥60 ms compared to the average QTcF interval at Baseline (before dosing) is observed, study drug administration is to be discontinued. For visits at which more than one ECG is completed, the average QTcF interval of all legible ECGs will be used to determine the QTcF interval for that visit.

6.3.5 Laboratory Evaluations

Laboratory evaluations will be completed according to the schedule presented in Table S–1 and Table S–2, and procedures detailed in the laboratory manual. Additional safety testing may be performed at the discretion of the Investigator or designee. Laboratory testing may be repeated during the Screening period with agreement of the Medical Monitor if the Investigator suspects that a laboratory abnormality is a temporary or reversible finding.

Clinical laboratory sample collection is not required to be completed under fasting conditions. The laboratory evaluations will include the following:

- Clinical chemistry serum tests
    - Sodium (Na), potassium (K), chloride (Cl), phosphorus (P), calcium (Ca), magnesium (Mg), carbon dioxide (CO_2), blood urea nitrogen (BUN), creatinine (CR), uric acid
        - Mg will only be performed at Visit 1 (Screening)
    - Alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), total bilirubin (TBIL), lactate dehydrogenase (LDH)
    - Glucose
    - Albumin (ALB), total protein
    - Thyroid stimulating hormone (TSH), free T3, and free T4
        - Thyroid function tests will be performed at Visit 1 (Screening)
- Hematology tests
    - Complete blood count (CBC) including:
        - White blood cell (WBC) count
        - Complete differential (relative and absolute)
        - Hematocrit (Hct), hemoglobin, red blood cells (RBC), platelets
        - Reticulocyte count
- Urinalysis
    - Blood, RBCs, WBCs, protein, glucose, ketones, specific gravity, pH
    - Reasonable efforts will be made to collect a urine sample from all subjects. When collection of a urine sample proves impractical or impossible (e.g., because the subject is incontinent or unable to cooperate), failure to collect a urine sample will be recorded in the subject's eCRF, and will not be considered a protocol deviation.

6.4 Caregiver Diary

A semi-structured caregiver diary will be completed during screening and treatment in which caregivers will record seizures and seizure-like spells if they are present.

At the Screening visit, the clinician will review the subject's seizure profile (if applicable) and will help the family to characterize the subject's typical seizure types. These are recorded in the caregiver diary.

Study drug dosing and concomitant medication dosing will be recorded in the caregiver diary. Instances of missed or partial doses that occur on any day of the study will also be recorded in the caregiver diary.

The caregiver diary will be completed and collected on an ongoing basis throughout the study beginning at Screening. The clinician will verbally ask about AEs (see Section 7.4.1), review the events recorded in the diary with the caregiver and will make a clinical evaluation, including an evaluation of whether an adverse event will be reported.

6.5 Caregiver Interview

An optional qualitative interview with caregivers of study subjects will be conducted remotely by telephone. Only caregivers of subjects for whom separate informed consent for the caregiver interview is completed will be interviewed. Interviews will be conducted by trained moderators from a 3rd party and are anticipated to be approximately up to 60 minutes in duration. The interviews are planned to elicit a better understanding of the observable signs as well as the impact of study drug and RTT both on the study subject and on the caregiver. The interviews will document changes observed by the caregivers over the course of participation in the study. The interviews will also provide an opportunity to describe the meaningfulness of any improvements that may have occurred from the perspective of the caregiver. Interviews will be audio-recorded and transcribed for analysis. Any personally identifiable health information mentioned during the interview will be redacted by the interviewer prior to transcription of the interview. Furthermore, if any potential adverse events are revealed during the interview, the interviewer will inform the investigator of the potential adverse event. Audio recordings of the interviews will be destroyed following transcription.

Following the analysis of the qualitative data, a summary report that describes the objectives, methods, participants, and results of the qualitative interviews will be prepared and reported separately from the clinical study report.

The caregiver interview will be administered during Treatment Period B, at or before the final study visit (Week 104 [EOT/ET]).

6.6 Pharmacokinetic Assessments

PK blood samples will be collected at each visit during Treatment Period A.

At the Baseline visit, a PK sample is drawn predose. After the first dose of study drug is administered, two PK samples will be collected: the first, 2 hours (±15 minutes) after dosing, as close as possible to the postdose ECG, and the second, at least 1 hour after the first.

In addition to the two PK samples taken at Baseline, two PK samples will be taken at each subsequent visit (or upon early termination [ET]) in Treatment Period A, for a total of ten postdose PK samples over the duration of the study. At Visit 3, Visit 4, Visit 5, and Visit 6, the two PK samples will be collected at one of the following time intervals:

- 1-3 hours after dosing
- 4-7 hours after dosing
- 8-11 hours after dosing

Every subject should provide at least two PK samples from each of the specified time intervals over the course of Visits 3 through 6 (1-3 hours after dosing, 4-7 hours after dosing, and 8-11 hours after dosing). The two PK samples taken within a time interval should be collected at least one hour apart.

Pharmacokinetic samples will also be collected, if possible, at any ET visit during Treatment Period A, or the visit immediately following any SAE, or following any AE leading to discontinuation.

Pharmacokinetic samples will not be collected at the Week 104 visit, or at an ET visit during Treatment Period B.

For all scheduled PK samples (and for unscheduled samples if possible), the dates and times of administration of the study drug over the 2 days prior to and on the morning of the PK sample draw, as well as the date and time of the sample draw, will be recorded. For samples collected from subjects who experience any SAE or experience an AE leading to discontinuation, the date and time of the last dose of study drug prior to the SAE or AE leading to discontinuation will also be recorded.

6.6.1 Blood Sampling

Table 6–1 tabulates the maximum allowable blood collection volumes by weight for affected children and Table 6–2 summarizes the blood volumes to be drawn in the present study in both a 24-hour and a 30-day period. The total amount of blood to be obtained from each subject should not exceed the allowable limits for affected children.

Each PK sample requires collection of 2 mL of venous blood. A total of three 2-mL venous blood samples will be collected for PK sampling from each subject at the Baseline visit for the measurement of trofinetide concentrations in blood (Table 6–2). A total of two 2-mL venous blood samples will be collected from each subject on each subsequent day of PK sampling.

When possible, an additional PK sample will be collected from subjects who experience any SAE or experience an AE leading to discontinuation as soon as possible after the occurrence of that event (see Section 7.4.2).

Additional information for specimen preparation, handling, storage and shipment is provided in Section 6.6.2.

Table 6–1 Blood Collection Volume Limits in Affected Children

Weight	**9 kg**	**10 kg**	**11 kg**	**12 kg**
Maximum allowable blood volume in a 24-hour period	18 mL	20 mL	22 mL	24 mL
Maximum allowable blood volume in a 30-day period	36 mL	40 mL	44 mL	48 mL

Sources: North Shore LIJ Human Subject Protection Program Guidance Document, Maximum Blood Draw Limits, Version 11/24/14

Note: Subjects must weigh ≥9 kg and <20 kg at Screening.

Table 6–2 Blood Collection Volumes for *MECP2* Mutation Testing, Safety Labs and Pharmacokinetic Samples

	Screening Visit	**Baseline Visit[a]**	**Visit 3 (Week 2)**	**Visit 4 (Week 4)**	**Visit 5 (Week 8)**	**Visit 6 (Week 12)**
Testing for *MECP2* Mutation (if necessary)	3.0 mL	- -	- -	- -	- -	- -
Safety Labs						
Chemistry and TSH/free T3/free T4	3.7 mL					
Chemistry	- -	2.2 mL	2.2 mL	2.2 mL	2.2 mL	2.2 mL
Hemoglobin/reticulocyte	1.2 mL	1.2 mL	1.2 mL	1.2 mL	1.2 mL	1.2 mL
Pharmacokinetic Samples						
Predose	- -	2.0 mL	- -	- -	- -	- -
Postdose	- -	4.0 mL	4.0 mL	4.0 mL	4.0 mL	4.0 mL
Total volume in any 24-hour period	**7.9 mL**	**9.4 mL**	**7.4 mL**	**7.4 mL**	**7.4 mL**	**7.4 mL**

Table 6–2 Blood Collection Volumes for *MECP2* Mutation Testing, Safety Labs and Pharmacokinetic Samples (Continued)

Total volume in any 30-day period					
Screening and Baseline	**---------17.3 mL-------**				
Screening, Baseline, and Week 2	**----------24.7 mL----------**				
Baseline, Week 2, and Week 4		**----------24.2 mL----------**			
Week 4 and Week 8				**---------14.8 mL---------**	
Week 8 and Week 12					**--------14.8 mL---------**

Abbreviations: TSH=thyroid stimulating hormone; T3=triiodothyronine; T4=thyroxine

[a] Baseline visit may take place over 2 days (see Section 3.1.2).

6.6.2 Specimen Preparation, Handling, Storage, and Shipment

An indwelling intravenous cannula may be used for collection of serial PK blood samples (saline is to be used to flush the indwelling cannula); otherwise, PK blood samples will be obtained via standard venipuncture procedures. Pre-prepared PK sampling tubes will be provided to each site within the lab visit kits for collection and storage of PK samples. Blood samples will be processed for determination of trofinetide whole blood concentrations (and of concentrations of possible metabolites identified).

At each time point, blood will be collected, processed as appropriate, and samples will be shipped to the central laboratory for storage and to the bioanalytical laboratory for analysis. The date and actual time of PK blood collection must be collected and recorded in the subject's eCRF. Each label will state the study number, subject number, analyte (whole blood), study day, and scheduled sample time.

A laboratory manual will be provided for sample processing, storage, and shipping procedures.

7 ADVERSE EVENTS

7.1 Specification of Safety Parameters

7.1.1 Definition of Adverse Event

An AE is defined as "any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study drug, whether or not considered related to study drug".

An AE can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality or seriousness. An AE can arise from any use of the drug (e.g.,

off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE.

AEs do not include the following:

- Stable or intermittent chronic conditions (such as myopia requiring eyeglasses) that are present prior to Baseline and do not worsen during the study
- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is an AE if not present at Baseline.
- Overdose of concomitant medication without any signs or symptoms unless the subject is hospitalized for observation; if an overdose occurs, it will be reported on an overdose form.
- Hospitalization for elective surgery planned prior to study (situation where an untoward medical occurrence has not occurred)

7.1.2 Definition of Serious Adverse Event

In addition to the severity rating, each AE will be classified by the Investigator as "serious" or "not serious." The seriousness of an event will be defined according to the applicable regulations and generally refers to the outcome of an event. An SAE is one that meets one or more of the following:

- Is fatal
- Is immediately life threatening
- Results in disability or permanent damage
- Requires hospitalization
- Prolongs existing hospitalization
- Is a congenital anomaly or birth defect (in an offspring)
- Is medically significant

Definition of Life Threatening

A life-threatening event places the subject at immediate risk of death from the event as it occurred. This does not include an AE, which, had it occurred in a more severe form, might have caused death.

Definition of Hospitalization

Hospitalization is defined by the Sponsor as a full admission to the hospital for diagnosis and treatment. This includes prolongation of an existing inpatient hospitalization.

Examples of visits to a hospital facility that do **not** meet the serious criteria for hospitalization include:

- Emergency room visits (that do not result in a full hospital admission)
- Outpatient surgery
- Preplanned or elective procedures
- Protocol procedures
- Social hospitalization, defined as admission to the hospital as a result of inadequate family support or care at the subject's primary residence

Definition of Disability or Permanent Damage

Disability is defined as a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

Definition of Medically Significant

Important medical events (medically significant events) that may not result in death, be life threatening, or require hospitalization may be considered to be an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.

An SAE may also include any other event that the Investigator or Medical Monitor judges to be serious or that suggests a significant hazard, contraindication, side effect, or precaution.

7.2 Classification of an Adverse Event

7.2.1 Severity of Event

The severity of each AE will be graded on a 3-point scale and reported in detail as indicated on the eCRF:

- **Mild**: awareness of sign or symptom but easily tolerated, causing minimal discomfort, and not interfering with normal everyday activities
- **Moderate**: sufficiently discomforting to interfere with normal everyday activities

- **Severe**: incapacitating and/or preventing normal everyday activities

7.2.2 Relationship to Study Drug

The causality of each AE should be assessed and classified by the Investigator as "related" or "not related." An event is considered related if there is a reasonable possibility that the event may have been caused by the product under investigation (i.e., there are facts, evidence, or arguments to suggest possible causation).

Consider the following when assessing causality:

- Temporal associations between the agent and the event
- Response to cessation (de-challenge) or re-challenge
- Compatibility with known class effect
- Known effects of concomitant medications
- Pre-existing risk factors
- A plausible mechanism
- Concurrent illnesses

7.2.3 Duration

The start and stop dates for AEs will be recorded using the following criteria:

- **Start:** Date of the first episode of the AE or date of significant sustained worsening in severity
- **Stop:** Date when AE either ceased permanently or worsened in severity

For AEs of diarrhea, the start date and stop date of a decrease in severity will also be recorded.

7.2.4 Frequency

The frequency of the AE should be indicated according to the following definitions:

- **Single:** Experienced once, without recurrence
- **Recurrent:** More than one discrete episode with the same severity

7.2.5 Action Taken with Study Drug

- **Dose not changed:** No change in study drug
- **Dose decreased**: Study drug dose decreased
- **Drug interrupted:** Study drug temporarily stopped

- **Drug withdrawn:** Study drug discontinued permanently
- **Not applicable**
- **Unknown**

7.2.6 Therapy

- **None:** No new treatment instituted
- **Medication:** New treatment initiated as a direct result of AE
- **Other:** Other action required

7.2.7 Outcome

- **Recovered/resolved:** Recovered or resolved
- **Recovered/resolved with sequelae:** Recovered or resolved with sequelae
- **Not recovered/not resolved:** Not recovered or not resolved
- **Fatal:** Death due to an AE
- **Unknown:** Unknown

7.2.8 Seriousness

- **Not serious**
- **Serious** (see Section 7.1.2)

7.2.9 Definition of Unexpectedness

An AE, the nature or severity of which is not consistent with the information provided in the Reference Safety Information section of the current trofinetide Investigator's Brochure.

7.3 Time Period and Frequency for Event Assessment and Follow-up

Adverse events will be recorded from the time informed consent is obtained through the study safety follow-up period. If an AE is ongoing at the end of the study safety follow-up period, every reasonable attempt should be made to follow and appropriately treat the subject until the AE resolves or until the Investigator deems the AE to be chronic or stable.

In the event that a subject discontinues and has an ongoing AE at the time of discontinuation (Section 4.4.2) or is withdrawn from the study because of an AE, a blood sample should be collected and the subject should be followed and appropriately treated until the AE resolves or until the Investigator deems the AE to be chronic or stable.

7.4 Reporting Procedures

7.4.1 Adverse Event Reporting

The Investigator must record all observed AEs and all reported AEs. At each visit, the Investigator should ask the subject and/or caregiver a nonspecific question (e.g., "Have you noticed anything different since your last visit?") to assess whether any AEs have been experienced since the last report or visit.

Note that any use of medication (and specifically any newly prescribed medication) during the course of a study may indicate the occurrence of an AE that may need to be recorded on both the AE and the concomitant medication page.

All AEs, serious and not serious, will be recorded on the AE eCRF page using appropriate medical terminology. Severity and relationship to study drug will be assessed by the Investigator.

When possible, clinical AEs should be described by diagnosis and not by symptoms (e.g., "cold" or "seasonal allergies" instead of "runny nose").

All AEs, *whether or not related to the study drug*, must be fully and completely documented on the AE eCRF and in the subject's notes.

7.4.2 Serious Adverse Event Reporting

The reporting of SAEs by the Sponsor or designee to the regulatory authorities is a regulatory requirement. Each regulatory authority has established a timetable for reporting SAEs based upon established criteria.

Serious AEs must be reported within 24 hours of discovery to the Sponsor or its designee; use the appropriate form for initial and/or follow-up reporting.

In the event an SAE or an AE leading to withdrawal is reported, a blood sample for determination of whole blood concentrations of trofinetide will be collected.

At a minimum, events identified by the Sponsor to require expedited reporting as serious, unexpected, and related to study drug must be brought to the attention of the responsible institutional review board/ethics committee (IRB/EC), as per applicable regulations. These will be provided by the Sponsor after their assessment. For European Union member states, the Sponsor or its designee will provide reports of suspected unexpected serious adverse reactions (SUSARs) directly to the ECs, as required by local legislation. In all other countries, it is the Investigator's responsibility to provide these expedited reports to the responsible IRB/EC. It is also the Investigator's responsibility to notify the responsible IRB/EC regarding any new and significant safety information.

When an SAE occurs, Investigators will review all documentation related to the event and will complete the paper SAE form with all required information (for initial and/or follow-up information) and fax or email (within 24 hours of discovery) to the contact information provided on the SAE form.

Subjects will be followed through the safety follow-up period (i.e., 30[+4] days after last dose of study drug) for any SAEs and/or other reportable information until such events have resolved or the Investigator deems them to be chronic or stable.

In the event of any SAE (other than death), the study subject's caregiver will be instructed to contact the Investigator (or designee) using the telephone number provided in the ICF. All subjects experiencing an SAE will be seen by the Investigator or designee as soon as is feasible following the report of the SAE.

Serious AEs occurring after the study follow-up period (i.e., 30[+4] days after last dose of study drug) should be reported if in the judgment of the Investigator there is "a reasonable possibility" that the event may have been caused by the product.

SAEs should also be reported to the IRB/EC according to local regulations.

7.4.3 Reporting of Overdose

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than the maximum recommended dose per protocol. It must be reported to the Sponsor or designee on the Overdose form within 24 hours of discovery. In addition, all events of overdose are to be captured as protocol deviations.

8 CLINICAL MONITORING

Routine monitoring of study sites is described in Section 11.2.

Clinical site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported study data are accurate, complete, and verifiable, and that the conduct of the study is in compliance with the currently approved protocol and amendment(s) as applicable, with GCP, and with applicable regulatory requirements. Details of the study site monitoring process are described in a separate clinical monitoring plan document.

9 STATISTICAL METHODS AND DATA ANALYSIS

9.1 Statistical and Analytical Plans

Statistical methods will be documented in detail in a statistical analysis plan (SAP) to be approved by the Sponsor prior to database lock. Deviations from the approved SAP will be described and justified in the final clinical study report.

9.2 Statistical Hypotheses

This is an open-label and single arm study. All statistical analyses will be descriptive. No statistical hypothesis will be tested.

9.3 Sample Size Determination

Approximately ten (10) to fifteen (15) subjects will be enrolled in this study, at least 1 subject who weighs ≥9 to <11 kg, and at least 4 subjects who are less than 4 years of age at Screening, including at least one subject who is 2 years of age. The sample size is not based on statistical considerations and is believed to be adequate to characterize the PK of trofinetide in this population.

9.4 Subject Populations for Analysis

The following populations will be defined and used in the analysis:

The **Safety Analysis Set** will consist of all enrolled subjects who received at least one dose of study medication. This analysis set will be used for all safety as well as any descriptive efficacy analyses.

The **PK Analysis Set** will consist of all subjects who receive at least 1 dose of study drug and have sufficient blood concentration data to calculate at least one PK parameter.

9.5 Statistical Analyses

9.5.1 General Approach

Continuous measurement results will be reported using the number of subjects with data values, mean, standard error of the mean, median, standard deviation, minimum, and maximum. For each categorical outcome, the number and percentage of subjects in each category will be reported.

9.5.2 Safety Analyses

Safety results will be summarized using descriptive statistics. Adverse events will be coded into standard terminology using the Medical Dictionary for Regulatory Activities (MedDRA). Treatment-emergent adverse events (TEAEs), TEAEs leading to discontinuation, TEAEs considered related to study drug, TEAEs by maximum severity, and serious adverse events (SAEs) reported after study medication start, will all be summarized.

Descriptive statistics for ECG, vital signs and weight, and clinical laboratory parameters, including changes from Baseline, will be tabulated by timepoint. Additionally, categorical analyses will be conducted on the incidence of subjects with prolonged QTc (QT interval of ECG corrected for heart rate) intervals and changes in QTc intervals in accordance with International Council for Harmonisation (ICH) guidelines.

Additional safety analysis details will be specified in the SAP.

9.5.3 Pharmacokinetic Analyses

Pharmacokinetic (PK) and exploratory efficacy measures will be collected from all subjects at the Baseline visit before dosing, at the Baseline visit after dosing, and after dosing at Weeks 2, 4, 8, and 12.

Whole blood concentration data for trofinetide will be listed and summarized using descriptive statistics. Population PK analyses will be performed to characterize the PK in children 2 to 5 years of age. The details of the PK analysis will be presented in a separate population PK report in accordance with a separate data analysis plan (DAP).

9.5.4 Efficacy Analyses

Descriptive summary statistics for the CGI-I and CaGI-I (observed value only), and for the CGI-S and ICND QoL score (observed values and change from Baseline) will be summarized by visit using the Safety Analysis Set.

9.6 Interim Analyses

Interim analyses will be conducted after completion of Treatment Period A (including safety follow-up period if applicable), and during Treatment Period B if required for regulatory purposes.

9.7 Measures to Minimize Bias

Not applicable; this is an open-label study.

9.8 Breaking the Study Blind/Subject Code

Not applicable; this is an open-label study.

10 STUDY MANAGEMENT AND DATA COLLECTION

10.1 Data Collection and Management Responsibilities

All documents required for the conduct of the study as specified in the ICH GCP guidelines will be maintained by the Investigator in an orderly manner and made available for monitoring and/or auditing by the Sponsor and regulatory authorities.

The Investigator and institution must permit authorized representatives of the Sponsor or designees (including monitors and auditors), regulatory authorities (including inspectors), and the IRB/EC direct access to source documents (such as original medical records) as allowed by local regulations. Direct access includes permission to examine, analyze, verify, and reproduce any records and reports that are needed for the evaluation of the study. The

Investigator must ensure the reliability and availability of source documents from which the information on the eCRF was derived.

10.2 Source Documents

All study specific information obtained at each study visit must be recorded in the subject's record (source documentation), and then entered into a validated electronic data capture (EDC) database by trained site personnel. The source documentation may consist of source notes captured by site personnel as well as laboratory reports, ECG reports, and electronic source data.

10.3 Case Report Forms

Subject data required by this protocol are to be recorded in an EDC system on eCRFs. The Investigator and his or her site personnel will be responsible for completing the eCRFs. The Investigator is responsible for the accuracy and reliability of all the information recorded on the eCRFs. All information requested on the eCRFs needs to be supplied, including subject identification data, visit date(s), assessment values, etc., and any omission or discrepancy will require explanation. All information on eCRFs must be traceable to source documentation (unless eCRF is considered the source) at the site.

10.4 Confidentiality

The Investigator must ensure that each subject's anonymity is maintained as described below. On the eCRFs, medical records, or other documents submitted to the Sponsor or designees, subjects must be identified by a subject identification number only. Subject identifiers uniquely identify subjects within the study and do not identify any person specifically. Documents that are not for submission to the Sponsor or designees (e.g., signed ICFs) should be kept in strict confidence by the Investigator in compliance with Federal regulations or other applicable laws or ICH guidance on GCP.

10.5 Study Records Retention

Investigators are required to maintain all essential study documentation as per ICH GCP guidelines. This includes, but is not limited to, copies of signed, dated and completed eCRFs, documentation of eCRF corrections, signed ICFs, audio recordings, subject-related source documentation, and adequate records for the receipt and disposition of all study drug. Investigators should maintain all essential study documentation, for a period of at least 2 years following the last approval of marketing application in an ICH region (US, Europe, and Japan), or until at least 2 years after the drug investigational program is discontinued, unless a longer period is required by applicable law or regulation. Only the Sponsor can

notify an Investigator or vendor when any records may be discarded. Investigators should contact the Sponsor before destroying any files.

10.6 Protocol Exceptions and Deviations

No prospective entry criteria protocol deviations are allowed; all subjects must meet all eligibility criteria in order to participate in the study.

Protocol waivers for eligibility will not be granted by the Sponsor under any circumstances. If, during the course of a subject's post-enrollment participation in the trial it is discovered that the subject did not meet all eligibility criteria, this will be reported as a major protocol deviation and not a waiver. In this situation, the subject will be discontinued, unless the discontinuation presents an unacceptable medical risk. The justification to allow the subject to continue in the trial will be made by the Sponsor, with medical input from the Investigator, and will be documented. All follow-up safety assessments must be completed and documented as outlined in the protocol (Section 3.1.4). The Investigator must report any protocol deviation to the Sponsor and, if required, to the IRB/EC in accordance with local regulations, within reasonable time.

10.7 Protocol Amendments

Changes to the protocol may be made only by the Sponsor (with or without consultation with the Investigator). All protocol modifications must be submitted to the site IRB/EC in accordance with local requirements and, if required, to regulatory authorities, as either an amendment or a notification. Approval for amendments must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the changes involve only logistical or administrative aspects of the trial. No approval is required for notifications.

11 QUALITY MANAGEMENT

11.1 Risk Management

The Sponsor utilizes the ICH E6 (GCP) Revision 2 risk management approach that includes methods to assure and control the quality of the trial proportionate to the risks inherent in the trial and the importance of the information collected. The intent is that all aspects of this trial are operationally feasible and that any unnecessary complexity, procedures, and data collection are avoided. The Sponsor's risk management approach includes the following documented activities:

- Critical Process and Data Identification: during protocol development, risks of processes and data that are critical to ensure human subject protection and the reliability of trial results are identified and assessed.

- Risk Identification: risks to critical trial processes, governing systems, investigational product, trial design, data collection, and recording are identified.
- Risk Evaluation: identified risks are evaluated by considering the following factors: (a) likelihood of occurrence, (b) impact on human subject protection and data integrity, and (c) detectability of errors.
- Risk Control: risks that can be avoided, reduced (i.e., mitigated), or accepted are differentiated. Risk mitigation activities are incorporated in protocol design and implementation, study plans, training, processes, and other documents governing the oversight and execution of study activities. Where possible, predefined quality tolerance limits are defined to identify systematic issues that can impact subject safety or data integrity and deviations from the predefined quality tolerance limits will trigger an evaluation and possibly an action. Contingency plans are developed for issues with a high risk factor that cannot be avoided.
- Periodic risk review, communication, and escalation of risk management activities during trial execution and risk outcome reporting in the clinical study report (CSR).

11.2 Quality Control and Quality Assurance

The Sponsor or designees and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., eCRFs and other pertinent data) provided that subject confidentiality is respected.

The Sponsor's or designee's monitor is responsible for inspecting the eCRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the eCRFs.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

In accordance with ICH guidance on GCP and the Sponsor's audit plans, sites participating in this study may be audited. These audits may include a review of site facilities (e.g., pharmacy, drug storage areas, and laboratories) and review of study-related records may occur in order to evaluate the trial conduct and compliance with the protocol, ICH guidance on GCP, and applicable regulatory requirements.

The Sponsor's or designee's representatives, regulatory authority inspectors and IRB/EC representatives who obtain direct access to source documents should also respect subject

confidentiality, taking all reasonable precautions in accordance with applicable regulatory requirements to maintain the confidentiality of subjects' identities.

12 ETHICAL CONSIDERATIONS

12.1 Ethical Standard

The study will be conducted in compliance with the protocol, the Declaration of Helsinki, ICH GCP, and other applicable regulatory requirements (e.g., Serious Breach reporting, urgent safety measures, and European Union General Data Protection Regulation [EU GDPR]).

The study will be performed in accordance with current US Health Insurance Portability and Accountability Act (HIPAA) regulations, US FDA GCP Regulations (US CFR 21 parts 50, 54, 56, and 312), and ICH guidance on GCP (E6) and clinical safety data management (E2A).

In accordance with Directive 75/318/EEC, as amended by Directive 91/507/EEC, the final clinical study report will be signed by an Investigator and/or Coordinating Investigator who will be designated prior to the writing of the clinical study report.

12.2 Institutional Review Board/Ethics Committee

The Investigator or designee will provide the IRB/EC with all requisite material, including a copy of the protocol, informed consent, and any subject information or advertising materials. The study will not be initiated until the IRB/EC provides written approval of the protocol and the informed consent and until approved documents have been obtained by the Investigator and copies received by the Sponsor. All amendments will be sent to the IRB/EC for information (minor amendment) or for submission (major amendment) before implementation. The Investigator will supply the IRB/EC and the Sponsor with appropriate reports on the progress of this study, including any necessary safety updates, in accordance with the applicable government regulations and in agreement with policy established by the Sponsor.

12.3 Informed Consent Process

In accord with the provisions of the US CFR 21 part 50, and since this study involves greater than minimal risk but presents the prospect of direct benefit to all subjects enrolled, consent shall be obtained from an LAR, typically a guardian, or at least one parent, in accordance with local IRB requirements.

Properly executed, informed consent (and assent, if applicable) must be obtained from each LAR/subject prior to any screening procedures. The LAR is defined as "An individual or judicial or other body authorized under applicable law to consent on behalf of a prospective

subject to the subject's participation in the procedures involved in the research" (US CFR 21 part 50).

Written informed consent will be obtained from the LAR.

The subject's caregiver must also provide informed consent regarding their participation in the study prior to participating in any study procedures.

The informed consent must, at a minimum, include the elements of consent described in the ICH guidance on GCP and the US CFR 21 part 50.25. A copy of the ICF planned for use will be reviewed by the Sponsor or designee for acceptability and must be submitted by the Investigator or designee together with the protocol, to the appropriate IRB/EC for review and approval prior to the start of the study at that investigational site. Consent forms must be in a language fully comprehensible to the prospective subject's LAR. The Investigator must provide the Sponsor or designee with a copy of the IRB/EC letter approving the protocol and the ICF before the study drug supplies will be shipped and the study can be initiated.

The consent form must be revised if new information becomes available during the study that may be relevant to the LAR's willingness for the subject to continue participation. Any revision must be submitted to the appropriate IRB/EC for review and approval in advance of use.

12.3.1 Consent and Other Informational Documents Provided to Subjects

The LAR must be given a copy of the signed informed consent and the original maintained in the designated location at the site.

12.3.2 Consent Procedures and Other Informational Documents Provided to Subjects

It is the Investigator or designee's responsibility to obtain written informed consent from the LAR after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. The LAR must be given ample time to decide about study participation and opportunity to inquire about details of the study. The IRB/EC-approved consent form must be personally signed and dated by the LAR and by the person who conducted the informed consent discussion. The Investigator or appropriate site personnel must document the details of obtaining informed consent in the subject's study documents.

The subject's caregiver must also indicate their understanding of the study and their role as a caregiver to the subject during the study. The subject's caregiver must provide written consent prior to any screening visit procedures being performed indicating their agreement to participate in the study in the caregiver role.

Participation in the caregiver interview is optional. Informed consent must be obtained by the Investigator (or designee), as appropriate, prior to the interviewer contacting the caregiver and conducting the optional caregiver interview.

Where applicable and permitted, informed consent forms may be administered remotely in accordance with ICH E6 and applicable regulations.

Records related to a study subject's participation will be maintained and processed according to local laws, and where applicable, the European Union General Data Protection Regulation (EU GDPR). The consent and study information documentation will include statements describing local and regional requirements concerning data privacy, and who to contact for questions.

13 PUBLICATION PLAN

All publication rights are delineated in the Clinical Study Agreement and/or other separate agreements with the Investigator and/or Institution, as applicable.

14 CONFLICT OF INTEREST POLICY

14.1 Finance, Insurance, and Indemnity

Arrangements for finance, insurance, and indemnity are delineated in the Clinical Study Agreement and/or other separate agreements with the Investigator and/or Institution, as applicable.

15 LITERATURE REFERENCES

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16 APPENDICES

Appendix A Rett Syndrome Diagnostic Criteria

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[a]
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[a]
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[b]
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[c]
2. Grossly abnormal psychomotor development in first 6 months of life[d]

Supportive Criteria for atypical RTT:[e]

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”

Table footnotes provided on next page

Source: Neul et al. 2010

[a] 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.

[b] 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.

[c] There should be clear evidence (neurological or ophthalmological examination and MRI/CT) that the presumed insult directly resulted in neurological dysfunction.

[d] 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 during the first six months of life is common in RTT and do not constitute an exclusionary criterion.

[e] 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 fulfil 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.