Official 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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STATISTICAL ANALYSIS PLAN

Protocol No.:	ACP-2566-009
Protocol Title:	An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age who Have Rett Syndrome
Drug:	Trofinetide oral solution
Sponsor:	Acadia Pharmaceuticals Inc.
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ABBREVIATIONS

AE	adverse event
ATC	Anatomical/Therapeutic/Chemical
BID	bis in die; twice daily
BLQ	below the limit of quantification
BMI	body mass index
CaGI-I	Caregiver Global Impression – Improvement
CGI-I	Clinical Global Impression – Improvement
CGI-S	Clinical Global Impression – Severity
COVID-19	coronavirus disease 2019
CSR	clinical study report
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
ECG	electrocardiogram
EDC	electronic data capture
EOT	end of treatment
ET	early termination
ICND	Impact of Childhood Neurologic Disability Scale
MECP2	gene encoding methyl-CpG binding protein 2 (in humans)
MedDRA	Medical Dictionary for Regulatory Activities
PCI	potentially clinically important
PD	pharmacodynamic(s)
PHE	public health emergency
PK	pharmacokinetic(s)
PT	preferred term
QT interval	QT interval for heart rate of ECG
QTc	QT interval of ECG corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
RTT	Rett Syndrome

RTT-CSS	Rett Syndrome Clinical Severity Scale
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SE	standard error
SOC	system organ class
TEAE	treatment-emergent adverse event

1. INTRODUCTION

This statistical analysis plan (SAP) provides a technical and detailed elaboration of the statistical analyses of efficacy and safety data for Study ACP-2566-009. This plan should be read in conjunction with the study protocol and the electronic case report forms (eCRFs).

Specifications for tables, figures, and listings are contained in separate documents. Statistical analyses for population pharmacokinetic (PK) will be presented in a separate report and therefore will not be included in this SAP.

The SAP version 1.0 is based on protocol Amendment 1, dated 30 June 2021. The SAP version 2.0 is based on protocol Amendment 2, dated 19 December 2022. Main changes include:

- added summary for number of caregivers consented and participated for optional exit interview
- added summary for number of subjects who took commercially marketed trofinetide within 30 days after study completion
- updated definition for treatment-emergent adverse events with Day 1 onset.

Note that for the caregiver optional exit interviews, 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.

2. OBJECTIVES

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

2.2 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

3. STUDY DESIGN

3.1 General Study Design

This is a multicenter, open-label study of trofinetide for the treatment of girls 2 to 5 years of age with Rett syndrome. 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 <12kg can also be enrolled.

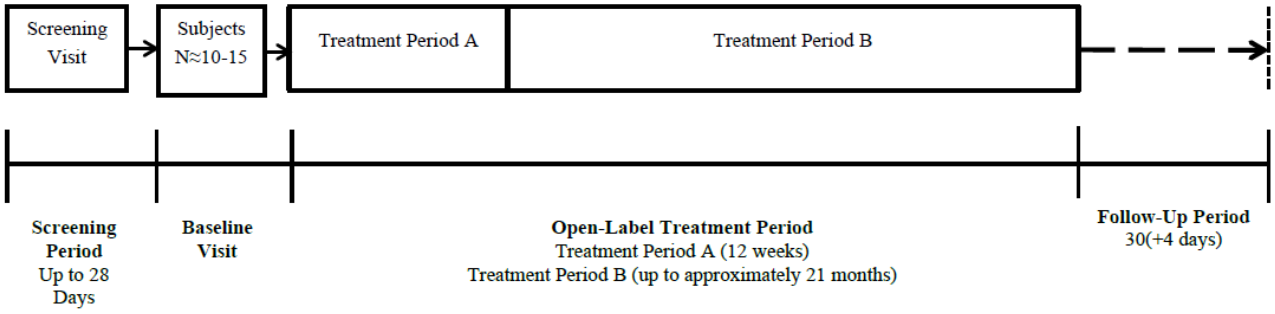
Approximately 10 to 15 subjects are expected to be enrolled, at least 1 subject who weighs ≥9 to <11 kg, and at least 4 subjects who are less than 4 years of age at Screening, including at least one subject who is 2 years of age. Approximately 10 sites will participate in this study.

The duration of the study will be 26 months and will have three main periods (Figure 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 study design is summarized in Figure 1.

Figure 1 Schematic of Study Design



The schedule of events and assessments is provided in appendices (21.1 and 21.2).

All subjects are dosed under protocol amendment 1 dated 30 June 2021 and protocol amendment 2 dated 19 December 2022, and will have trofinetide titration according to the dosing schedule in Table 1. 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 4 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.

Table 1 Dosing Schedule 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.

3.2 Randomization

Not Applicable. This is not a randomized study.

3.3 Blinding

Not Applicable. This is an open-label study.

3.4 Determination of Sample Size

Approximately 10 to 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 1 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.

4. ANALYSIS SETS

Safety Analysis Set

The Safety Analysis Set consists 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.

Pharmacokinetics (PK) Analysis Set

The PK Analysis Set consists of all subjects in the Safety Analysis Set with at least 1 quantifiable PK concentration.

5. DATA HANDLING CONVENTIONS

All data collected in the study will be listed.

5.1 General Data Reporting Conventions

Continuous variables will be summarized using the following descriptive statistics: number of subjects, mean, standard error (SE), standard deviation (SD), median, minimum, and maximum. Unless specified otherwise, means and medians will be presented to one more decimal place than the raw data, and the SDs and SEs will be presented to two more decimal places than the raw data. In general, the maximum number of decimal places is 4 and values will be truncated to 4 decimal places in situations where there are more than 4 decimal places. Wherever possible data will be decimal aligned.

Height, weight and BMI will be presented with a maximum of one decimal place.

Categorical variables will be summarized by the number of subjects and the percentage of subjects in each category; the number of subjects and the percentage of subjects with missing data will be summarized for demographic and baseline characteristics (if applicable). Categories with zero counts will be displayed as "- -". Percentages will be presented with one decimal place.

Duration in months will be calculated as ([number of days / 365.25] *12).

Laboratory values that are collected with "<" or ">" signs will generally be analysed as the numerical value without the sign in tables and figures. In listings, these data will be reported as collected with the sign.

For tables, all subjects will be pooled together as a "Total" group for summary.

5.2 Derived Variables

In general, assessment of total scores will be derived within the analysis datasets. In the event that total scores are also collected on the electronic case report form (eCRF), the derived values will be used for all analyses. Both the raw and derived scores will be presented in listings.

5.2.1 Rett Syndrome Clinical Severity Scale (RTT-CSS)

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, and head growth; 10 of which measure current function at the time of assessment: somatic growth, independent sitting, ambulation, hand use, scoliosis, language, nonverbal communication, respiratory dysfunction, autonomic symptoms, and epilepsy/seizures. 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. The total score ranging from 0 to 58 will be calculated as the sum of scores for all 13 items. Higher total scores indicate greater severity of symptoms. If there are any missing item scores, the total score will be considered missing.

5.2.2 Clinical Global Impression–Improvement (CGI-I)

The CGI-I scale is used by 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.

Higher CGI-I scores denote less improvement in the illness.

5.2.3 Clinical Global Impression–Severity (CGI-S)

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.

Higher CGI-S scores denote more severe illness.

5.2.4 Caregiver Global Impression–Improvement (CaGI-I)

The CaGI-I scale is a variation of the CGI scale used to ask caregivers for a global assessment of the subject's illness. The 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 from 1=much improved, 2=improved, 3=unchanged, 4=worse, 5=much worse.

Higher CaGI-I scores denote less improvement in the illness.

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

The overall quality of life score rating of the ICND will be evaluated. The numeric score of the child's overall quality of life ranges from 1 ("Poor") to 6 ("Excellent"); lower overall quality of life scores indicate lower quality of life.

5.3 Data Presentation Conventions

- 1 year = 365.25 days. Year is calculated as (days/365.25) and will be rounded to 1 digit after the decimal point (tenths) for presentation purposes.

- 1 month = 30.4375 days. Month is calculated as (days/30.4375) and will be rounded to 1 digit after the decimal point (tenths) for presentation purposes.
- Body mass index (BMI) is calculated as [weight (kg)/height $(m)^2$]

5.4 Study Day

If the date of assessment occurs on or after the first dose date, then study day will be calculated as (date of assessment – date of first dose) + 1. If the date of assessment occurs prior to the first dose date, then study day will be calculated as (date of assessment – date of first dose). There is no study day 0.

5.5 Baseline Definition

Baseline will be defined as the last non-missing assessment, including those from repeated and unscheduled measurements, before the first trofinetide dose is administered.

5.6 Analysis Visit Windows

Efficacy, safety, and PK assessments will be summarized by analysis visit as presented in Table 2 below.

Table 2 Analysis Visit Windows

Analysis Visit	Study Visit	Target Study Day	Study Day Interval
Baseline (Day 1)	2	1	≤ 1
Week 2	3	15	2 – 21
Week 4	4	29	22 – 42
Week 8	5	57	43 – 70
Week 12	6	85	71 – 126
Week 24	7	169	127 – 266
Week 52	8	365	267 – 455
Week 78	9	547	456 – 637
Week 104	10	729	638 – 759

5.6.1 Unscheduled Assessments

Both Scheduled and Unscheduled assessments, including the assessments at early termination visits, will be included for planned timepoint analyses based on the above analysis visit windowing rules. All assessments will be presented in data listings.

5.6.2 Multiple Measurements within Visit Windows

In the event that more than one assessment falls within a given window, the assessment closest to the target study day will be selected for the by-visit analysis. If two assessments are equidistant from the target study day, then the chronologically last assessment will be used. Exceptions are

made for incomplete assessments, in which case, more complete assessments will be given priority.

For safety analyses where the extreme values should be selected (e.g., overall post-Baseline minimum, overall post-Baseline maximum, and potentially clinically important values), all non-missing post-Baseline values should be considered, regardless of whether the value is selected for the by-visit summaries. All assessments will be presented in data listings.

5.7 Missing or Incomplete Date for Last Dose of Study Drug

For subjects with completely missing last dose date, the last dose date will be imputed by the last expected dosing date, defined as the earliest of the following three dates:

- the non-missing drug return date of the last dispensed drug kits,
- the last drug dispensed date plus the number of days that the dosing would continue if given to the subject per protocol,
- EOT/ET date.

For subjects with partial missing last dose date, the imputation will be compared against the last expected dosing date as defined above. Detailed imputation algorithms will be documented in a separate programming specifications document. The missing or incomplete dates will be displayed in the data listings as reported on the eCRF rather than the imputed dates.

5.8 Missing or Incomplete Date for Prior or Concomitant Medications

Missing or incomplete medication start or stop dates will be imputed for the purpose of determining whether the medication is taken concomitantly or not (see Section 12 for definition). When the chronological order of medication use relative to the study drug treatment period is unclear due to missing or incomplete date(s), the medication will be considered as concomitant. The imputation algorithms will be detailed in the analysis dataset specification document. The missing or incomplete dates as captured on the eCRF will be displayed in the data listings.

5.9 Missing or Incomplete Date for Adverse Events

Missing or incomplete adverse event (AE) start dates will be imputed for the purpose of determining whether the AEs are treatment-emergent or not (see Section 15.2 for definition). When the chronological order of an AE onset relative to the study drug treatment period is unclear due to missing or incomplete date(s), the AE will be considered as treatment-emergent. The imputation algorithms will be detailed in the analysis dataset specification document. The missing or incomplete dates captured on the eCRF will be displayed in the data listings.

5.10 Missing Severity Assessment for Adverse Events

If the severity is missing for an AE starting on or after the date of the first dose of study drug, then a severity of "Severe" will be assigned. The imputed values for severity assessment will be used for incidence summaries, while the actual values will be used in data listings.

5.11 Missing Relationship to Study Drug for Adverse Events

If the relationship to study drug is missing for an AE starting on or after the date of the first dose of study drug, a causality of "Related" will be assigned. The imputed values for relationship to study drug will be used for incidence summaries, while the actual values will be presented in data listings.

5.12 Character Values of Clinical Laboratory Variables

If the reported value of a clinical laboratory variable cannot be used in a statistical analysis due to, for example, a character string reported for a numeric variable, an appropriately determined coded value may be used in the statistical analysis. The coding algorithms will be detailed in the analysis dataset specification document. The actual values as reported in the database will be presented in data listings.

6. STUDY ENROLLMENT

The number of subjects screened, subjects screen failed, and subjects dosed will be summarized. Data listings of subject enrollment and subject eligibility (i.e. inclusion/exclusion criteria) will be provided.

7. SUBJECT DISPOSITION

The number and percentage of subjects who completed the study or discontinued early and the reason for study discontinuation will be summarized using the Safety Analysis Set.

The number of caregivers who consented and participated in the optional exit interview, and the number of subjects who took commercially marketed trofinetide within 30 days after study completion will also be summarized using the Safety Analysis Set.

8. PROTOCOL DEVIATIONS

Protocol deviations will be reviewed periodically over the course of the study. The review process, definition of the deviation categories, and the classification of a deviation as major or minor are detailed in the Protocol Deviation Management Plan. Protocol deviations will also be assessed with respect to relationship to the COVID-19 PHE. A summary of the number and percentage of subjects with major protocol deviations for each deviation category will be presented for the Safety Analysis Set. Three data listings of all protocol deviations, COVID-19-PHE related protocol deviations, and non-COVID-19-PHE related protocol deviations will be provided.

9. DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline characteristics will be summarized for the Safety Analysis Set using descriptive statistics. Variables include, but are not limited to, age at screening, sex, race, race subgroup (white vs non-white), ethnicity, height, weight, BMI, RTT-CSS total score at Screening, Baseline Overall Quality of Life score, and Baseline CGI-S score.

10. MEDICAL HISTORY

Medical history reported terms will be coded with Medical Dictionary for Regulatory Activities (MedDRA) version 24.0. The subject incidence will be summarized for each system organ class (SOC) and preferred term (PT) for the Safety Analysis Set. A subject will be counted only once per SOC or per PT for the summary.

A listing of the SOC, PT, body system, verbatim for the medical history condition/event, start and stop dates (when available), and an indicator for whether or not the condition is ongoing will be provided.

Rett Syndrome History

The age at Rett syndrome diagnosis, the age at symptoms first noticed, the number and percentage of subjects by *MECP2* gene mutation, and the number and percentage of subjects with more than one *MECP2* mutation (Yes, No) collected on the Rett History eCRF page will be summarized.

Rett syndrome history will be listed.

10.1 *MECP2* MUTATION GROUPINGS

The number and percentage of subjects will be examined in the following MECP2 mutation groupings.

Mutations	Code in EDC	Category	Severity
R106W	1	R106W	Severe
R133C	2	R133C	Mild
T158M	3	T158M	Moderate
R168X	4	R168X	Severe
R255X	5	R255X	Severe
R270X	6	R270X	Severe
R294X	7	R294X	Mild
R306C	8	R306C	Mild
C298G (L100V)	9	Other mutations	Mild
G317A (R106Q	10	Other mutations	Mild
C421G (Y141X)	11	Other mutations	Unknown
C455G (P152R)	12	Other mutations	Severe
C302G (P101R)	13	Other mutations	Severe
C401G (S134C)	14	Other mutations	Severe
C423G (Y141X)	15	Other mutations	Unknown
C468G (D156E)	16	Other mutations	Mild
C674G (P225R)	17	Other mutations	Mild
C965T (P322L)	18	Other mutations	Mild
710del1	19	Other mutations	Severe
806del1	20	Other mutations	Severe
807del1	21	Other mutations	Severe
1157del41	22	C-terminal Truncations	Mild
1157del44	23	C-terminal Truncations	Mild

Mutations	Code in EDC	Category	Severity
1163del26	24	C-terminal Truncations	Mild
1163del35	25	C-terminal Truncations	Mild
1164del44	26	C-terminal Truncations	Mild
1168del6	27	C-terminal Truncations	Mild
Exon 1+2	28	Large Deletions	Severe
Exon 3	29	Large Deletions	Severe
Exon 3+4	30	Large Deletions	Severe
Exon 4	31	Large Deletions	Severe
Exons 1-4	32	Large Deletions	Severe
All Others	33	Other mutations	Derived from CRF

Missing and unknown severity will not be imputed.

11. EXTENT OF EXPOSURE AND TREATMENT COMPLIANCE

Extent of exposure and treatment compliance will be summarized as both continuous variables and categorical variables for the Safety Analysis Set.

11.1 Exposure to Study drug

Duration of exposure to study drug will be calculated for each subject as (last dose date – first dose date + 1). The number and percentage of subjects within each of the following exposure levels in terms of duration of exposure will also be tabulated: <2 weeks (1 to 13 days), 2 to <4 weeks (14 to 27 days), 4 to <8 weeks (28 to 55 days), 8 to <12 weeks (56 to 83 days), 12 to <24 weeks (84 to 167 days), 24 to <52 weeks (168 to 363 days), 52 to <78 weeks (364 to 545 days), 78 to <104 weeks (546 to 727 days), and >=104 weeks (728 days or longer).

11.2 Measurement of Treatment Compliance

The study drug is provided in liquid form supplied in a 500 mL bottle. Study drug compliance will be calculated based on the drug accountability and dose modification data as collected on the eCRF. The study drug compliance will be calculated as (the total volume of drug actually taken (in mL) divided by the total volume of drug expected to be taken)*100.

The total volume of drug expected to be taken will be based on the duration of exposure and dosing schedule (see Table 1). However, if there is any dose modification prescribed by the investigator due to intolerance, the total drug expected to be taken will be adjusted accordingly to account for the modified prescribed dose schedule as recorded in the EDC.

The total volume of drug actually taken will be calculated as (total drug dispensed – total drug returned) (height in cm of drug remaining converted into volume in mL based on the following conversion Table 3).

Treatment compliance will be summarized as a categorical variable. The number and percentage of subjects within each of the following compliance levels will be tabulated: <80%, 80 to 120% and >120%.

Details of treatment compliance calculation are provided in a separate programming specifications document.

Table 3 Conversion of Height of Remaining Liquid (cm) into Volume (mL)

Height of Remaining Liquid (cm)	Estimated Volume (ml)	Height of Remaining Liquid (cm)	Estimated Volume (ml)
0.5	18	7.0	248
1.0	35	7.5	266
1.5	53	8.0	283
2.0	71	8.5	301
2.5	89	9.0	319
3.0	106	9.5	336
3.5	124	10.0	354
4.0	142	10.5	372
4.5	159	11.0	389
5.0	177	11.5	407
5.5	195	12.0	425
6.0	212	12.5	443
6.5	230	13.0	460

12. PRIOR, CONCOMITANT, AND POST-TREATMENT MEDICATION

Prior medication is defined as any medication with stop dates prior to the date of the first dose of study drug. Concomitant medication is defined as any medications that are ongoing at the first dose of study drug or with a start date between the dates of the first and last doses of study drug, inclusive. Post-treatment medication is defined as any medication with a start date after the date of the last dose of study drug. Medications will be coded using WHO Drug Global Dictionary March 2021.

The number and percentage of subjects taking concomitant and post-treatment medications will be tabulated separately by each drug class (ATC Level 3) and medication PT for the Safety Analysis Set. Multiple medication usage by a subject in the same category will be counted only once. Listing of prior, concomitant and post-treatment medications will also be provided.

COVID-19 Infection Related Medications

Concomitant medications related to COVID-19 Infection will also be listed.

13. EFFICACY ANALYSES

Unless otherwise specified, all efficacy analyses will be performed using the Safety Analysis Set. No hypothesis testing is planned. Descriptive summaries of all efficacy endpoints will be presented.

13.1 Exploratory Efficacy Endpoints

The exploratory efficacy endpoints are:

- CGI-I score at Weeks 2, 4, 8, 12, 24, 52, 78, and 104
- Change from Baseline to Weeks 2, 4, 8, 12, 24, 52, 78, and 104 in CGI-S score
- CaGI-I score at Weeks 12, 24, 52, 78, and 104
- Change from Baseline to Weeks 12, 24, 52, 78, and 104 in Overall Quality of Life Rating of the ICND Scale

13.2 Adjustment for Covariates

Not applicable.

13.3 Handling of Missing Data

Missing data imputation rules are described in Section 5.2.

13.4 Multiple Comparisons / Multiplicity

No hypothesis testing is planned.

14. METHODS OF EFFICACY ANALYSES

14.1 Analysis of Continuous Efficacy Endpoints

Descriptive statistics for all efficacy endpoints listed in Section 13.1 will be tabulated by analysis visit (see Section 5.6). The summaries of observed values and change from baseline results will be presented.

15. SAFETY ANALYSES

All safety analyses will be performed using the Safety Analysis Set. Safety summaries will be presented using descriptive statistics. For each continuous measure in clinical laboratory variables, vital signs, and electrocardiogram, change from baseline results will be displayed by analysis visit.

15.1 Primary Safety Endpoints

The primary safety endpoints are:

- Treatment-emergent adverse events (TEAEs)
- Serious adverse events (SAEs)
- Withdrawals due to adverse events (AEs)
- Potentially clinically important changes in other safety assessments

15.2 Adverse Events

Adverse events will be coded using MedDRA dictionary version 24.0.

An AE (classified by preferred term) will be considered a treatment-emergent AE (TEAE) if started after first dose administration and no later than last dose date + 30 days.

The event counts, the number, and percentage of subjects reporting TEAEs will be tabulated by system organ class (SOC) and PT; and by SOC, PT, and maximum severity. A subject is counted at most once per SOC and PT even if the subject has more than one TEAE. A subject is counted at most once per SOC and PT at the maximum severity for the summary by maximum severity. The display in these tables will be sorted alphabetically by SOC and then by descending subject frequency for the PTs within each SOC.

The event counts, the number and percentage of subjects with any TEAEs will also be tabulated by PT without SOCs. This table will be sorted by descending subject frequency.

Treatment-emergent SAEs, TEAEs leading to study drug withdrawn, and TEAEs related to study drug will be summarized by SOC and PT. The tables will be sorted alphabetically by SOC and then by descending subject frequency for the PTs within each SOC. In addition, the incidence of fatal treatment-emergent AEs (i.e., events that cause death) will be summarized separately by SOC and PT.

An AE listing by subject will display all events, including those which are not treatment-emergent, and will include the verbatim term in addition to the MedDRA SOC and PT. This listing will also include all relevant eCRF data associated with the event: date of onset, date

resolved, date of last dose, severity, frequency, outcome, relationship to study drug, and action taken with study drug. Separate listings will be presented for treatment-emergent SAEs, TEAEs leading to study drug withdrawn, fatal TEAEs (if any), and TEAEs related to COVID-19 infection.

15.3 Clinical Laboratory Variables

Due to COVID-19 PHE related disruptions, it is possible that some test results may be collected from a local laboratory. Local laboratory results and the associated normal ranges will be converted to SI units; local laboratory results, in SI units, will then be normalized to central lab ranges to be included in summary data analysis together with the central laboratory results. The normalization will be performed using the following scale transformation equation:

$$s = L_s + (x - L_x)\frac{U_s - L_s}{U_x - L_x}$$

where s is the normalized individual laboratory value to be used for summary; x is the original value from the local lab; L_x and U_x are the lower and upper limits from the local lab; L_s and U_s are the lower and upper limits from the central lab.

For labs with only a single upper (or lower) limit, the following scale transformation equation will be used:

$$s = x\frac{U_s}{U_x}$$

where s is the normalized individual laboratory value to be used for summary; x is the original value from the local lab; U_s is the upper (or lower) limit from the central lab; U_x is the upper (or lower) limit from the local laboratory. Local laboratory results and normalized results will be included in data listings. Only central lab and normalized local lab results will be used for summary of change from baseline, shift, and potentially clinically important (PCI) analyses.

Clinical laboratory assessments are performed according to the schedule presented in appendices (21.1 and 21.2).

- Clinical chemistry serum tests include the following:
    - 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 include the following:
  - 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 tests include the following:
  - Blood, RBCs, WBCs, protein, glucose, ketones, specific gravity, pH

Clinical laboratory values (in Système International [SI] units) and the change from Baseline values will be summarized at each post-Baseline visit using descriptive statistics. The overall minimum, maximum as well as the last post-Baseline observed and change from Baseline values will also be summarized. For urinalysis with categorical results, the number and percentage of subjects will be tabulated by category at Baseline and each post-Baseline visit, and the denominator is the number of subjects with non-missing values for the given parameter and visit.

Laboratory values will also be summarized in shift tables to determine the number and percentage of subjects with values classified as below, within, and above normal ranges at each post-Baseline visit relative to the same classification at the Baseline visit. For the by-visit shift summary, the denominator is the number of subjects with non-missing values at Baseline and the given visit for the given parameter. For the shift to the overall post-Baseline minimum or maximum, all post-Baseline values will be considered, including unscheduled and out of window values and the denominator is the number of subjects with non-missing Baseline value and at least 1 post-Baseline value for the given parameter.

Clinical laboratory values are potentially clinically important (PCI) if they meet either the low or high PCI criteria listed in Table 4 and Table 5. The number and percentage of subjects with post-Baseline PCI values for each of the categories in Table 4 and Table 5 will be summarized

for selected parameters. For the overall post-Baseline summaries of PCI values, all post-Baseline values will be considered, including unscheduled and out of window values. Subjects with multiple PCI values for a given parameter will be counted only once for that parameter. For the overall post-Baseline summary, the numerator of the percentage is the number of subjects with at least 1 post-Baseline PCI laboratory value for the given parameter, and the denominator is the number of subjects with at least 1 post-Baseline laboratory value for the given parameter.

Table 4 Criteria for Potentially Clinically Important Laboratory Values – Hematology and Chemistry

Analyte	Conventional Unit	Low PCI Criteria	High PCI Criteria	SI Unit	Low PCI Criteria	High PCI Criteria
Hematology (whole blood)						
Hemoglobin (female)	g/dL	<10	>17	g/L	<100	>170
Hematocrit (female)	%	<30	>50	L/L	<0.3	>0.5
Leukocyte (White Blood Cell Count)	x 10^3/uL	≤2.8	≥15	x 10^9/L	≤2.8	≥15
Neutrophils	x 10^3/uL	≤1.5	No upper limit	x 10^9/L	≤1.5	No upper limit
Platelet Count	x 10^3/uL	≤75	≥700	x 10^9/L	≤75	≥700
Chemistry (serum or plasma)						
ALT (SGPT)	U/L	No lower limit	≥3 X ULN	U/L	No lower limit	≥3 X ULN
AST (SGOT)	U/L	No lower limit	≥3 X ULN	U/L	No lower limit	≥3 X ULN
Total Bilirubin	mg/dL	No lower limit	≥1.5 ULN	umol/L	No lower limit	≥1.5 ULN
BUN	mg/dL	No lower limit	≥30.0	mmol/L	No lower limit	≥10.71
Sodium	mEq/L	≤125	≥155	mmol/L	≤125	≥155
Potassium	mEq/L	≤3.0	≥5.5	mmol/L	≤3.0	≥5.5
Calcium, total	mg/dL	<8.0	>11.0	mmol/L	<2.0	>2.75
Lactate Dehydrogenase (LDH)	U/L	No lower limit	≥3 X ULN	U/L	No lower limit	≥3 X ULN
Alkaline Phosphatase	U/L	No lower limit	≥3 X ULN	U/L	No lower limit	≥3 X ULN
Uric acid (female)	mg/dL	No lower limit	≥8.5	umol/L	No lower limit	≥505.75
Albumin	g/dL	≤2.6	≥6.0	g/L	≤26	≥60
Total Protein	g/dL	≤5.0	≥10.0	g/L	≤50	≥100
Chloride	mEq/L	≤85	≥120	mmol/L	≤85	≥120
Glucose (random)	mg/dL	≤45.1	≥200.0	mmol/L	≤2.48	≥11
Serum Creatinine	mg/dL	Not Applicable	>1.5 ULN	umol/L	Not Applicable	>1.5 ULN
Gamma-Glutamyl Transferase (GGT)	U/L	Not Applicable	≥3 ULN	U/L	Not Applicable	≥3 ULN

Table 5 Criteria for Potentially Clinically Important Laboratory Values - Urinalysis

Urinalysis (qualitative dipstick)	Low PCI Criteria	High PCI Criteria
Blood (occult blood)	Not Applicable	≥ Moderate
Protein	Not Applicable	≥ 100 mg/dL
Glucose	Not Applicable	≥ 500 mg/dL

Clinical laboratory data will be displayed in data listings with date and study day of collection. All units will be displayed according to SI conventions for units. Out of range values will be flagged in the data listings (i.e., 'L' or 'H'). A separate listing will be provided for a subset of the chemistry, hematology, and urinalysis analytes with values classified as PCI.

15.4 Vital Signs

Vital signs and weight will be collected throughout the study; height will be measured at Screening, Week 12, and Week 104/EOT/ET. Observed vital signs including weight and BMI and the changes from Baseline at each post-Baseline visit will be summarized using descriptive statistics.

Vital sign values will be considered PCI if they meet the criteria listed in Table 6. The number and percentage of subjects with post-Baseline vital signs that are PCI will be summarized at each post-Baseline visit and for overall post-Baseline. For the overall post-Baseline summaries, all post-Baseline values will be considered, including unscheduled and out of window values. Subjects with multiple PCI values for a given parameter will be counted only once for that parameter. For the by-visit summary, the numerator for the percentage is the number of subjects with a post-Baseline PCI vital sign for the given parameter and visit, and the denominator is the number of subjects with non-missing values for the given parameter and visit. For the overall post-Baseline summary, the numerator for the percentage is the number of subjects with at least 1 post-Baseline PCI vital sign for the given parameter, and the denominator is the number of subjects with non-missing Baseline and at least 1 post-Baseline vital sign for the given parameter. A listing of subjects with any PCI vital sign values will be provided.

Table 6 Criteria for Potentially Clinically Important Vital Signs

Vital Sign Parameter	Unit	Criteria			
		Observed Value	And/Or	Change Relative to Baseline	Change from Supine to Standing
Systolic blood pressure (supine or sitting)	mmHg	≥140	And	Increase of ≥20	-
		≤70	And	Decrease of ≥20	-
Diastolic blood pressure (supine or sitting)	mmHg	≥90	And	Increase of ≥15	-
		≤50	And	Decrease of ≥15	-
Pulse (supine or sitting)	bpm	≥131	And	Increase of ≥15	-
		≤50	And	Decrease of ≥15	-
Weight	kg	Not Applicable		Increase of ≥7%	-
				Decrease of ≥7%	-

15.5 Electrocardiogram (ECG)

ECGs are collected throughout the study at every visit. Observed values of ECG variables (e.g., heart rate, PR interval, QRS interval, QT interval, and QTc interval) and the changes from Baseline at each assessment time point will be summarized.

QTcB and QTcF will also be categorized into the following categories (msec), and the number and percentage of subjects in each category will be summarized at each visit and for the overall post-Baseline maximum:

- Observed: ≤450, 451 - ≤480, 481 - ≤500, and >500; >450; >480.
- Change from Baseline: ≤10, 11 – 30, 31 – 60, and >60; >30.

For cardiologist's interpretations, the number and percentage of subjects with ECG results that are determined as normal or abnormal will be summarized at scheduled visits. The overall post-baseline worst interpretation will also be summarized (i.e. if a subject has one or more post-baseline ECG results that is/are considered as abnormal, this subject will be counted in the abnormal category). Cardiologist's interpretations will also be summarized in a shift table. The shifts from Baseline to overall post-Baseline worst interpretation will also be presented. For the by-visit shift summary, the denominator is the number of subjects with non-missing cardiologist's interpretation at Baseline and the given visit. For the summaries of shift from Baseline to the overall post-Baseline worst interpretation, the denominator is the number of subjects with non-missing Baseline and at least 1 post-Baseline cardiologist's interpretation.

Electrocardiogram variable values will be considered PCI if they meet the criteria listed in Table 7. The number and percentage of subjects with post-baseline PCI values will be presented at each post-Baseline visit and for overall post-Baseline. For the by-visit summary, the numerator for the percentage is the number of subjects with a post-Baseline PCI ECG for the given parameter and visit, and the denominator is the number of subjects with non-missing values for the given parameter and visit. For the overall post-Baseline summary, the numerator for the percentage is the number of subjects with at least 1 post-Baseline PCI ECG for the given parameter, and the denominator is the number of subjects with at least 1 post-Baseline ECG value for the given parameter. A listing of all subjects with any PCI ECG values will be provided.

Table 7 Criteria for Potentially Clinically Important ECG Values

ECG Parameter	Unit	High PCI Criteria
QRS Interval	msec	≥120
PR Interval	msec	≥220
QTcB	msec	>500
QTcF	msec	>500
QTcB: change from baseline	>60 msec	
QTcF: change from baseline	>60 msec	

15.6 Physical Examination

Physical examinations are performed throughout the study at every visit in the clinic. When a study visit takes place off-site, the physical examination is not required. Physical examination results (normal, abnormal, and not done) will be summarized in a frequency table by body system and analysis visit. A listing of abnormal physical examination data will be provided.

15.7 Other Safety Endpoints

There are no other safety endpoints in this study.

16. CLINICAL PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

For trofinetide-treated subjects, whole blood concentration for trofinetide will be listed. Whole blood concentration data for trofinetide will be summarized for the PK Analysis Set at each analysis visit using descriptive statistics. Concentrations that are below the limit of quantification (BLQ) will be displayed as "BLQ" in the data listings and imputed as 0 for computing summary statistics.

If data allow, population PK and PK/PD analyses will be performed to further characterize the PK profile and exposure response relationship of trofinetide using measures of safety and efficacy parameters. The results of population PK and PK/PD modeling will be presented in a separate report.

17. INTERIM ANALYSIS

All available data up to and including an assigned data cut date will be cleaned and included for an interim analysis to serve the purpose of submission of data as part of a New Drug Application, (NDA), including the writing of an interim synoptic clinical study report (CSR). An interim PK analysis including all available PK data by the date of interim analysis cut will be presented in a separate report.

All pre-specified analyses (Sections 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and 16) will be performed.

17.1 Examination of Subject Disposition for Treatment Period A

In addition to the analysis specified in Section 7, the number and percentage of subjects who are ongoing in Treatment Period A, who completed Treatment Period A (i.e. completed Week 12 visit), who discontinued the study in Period A and the reason for study discontinuation will be summarized using the Safety Analysis Set.

17.2 Examination of Subgroups for Adverse Events

In addition to the analysis specified in Section 15.2, the following summaries will be performed for adverse events with onset during Treatment Period A:

- TEAEs with onset during Treatment Period A: by SOC and PT; by SOC, PT and maximum severity; by PT only
- Treatment-emergent SAEs with onset during Treatment Period A: by SOC and PT
- TEAEs with onset during Treatment Period A that lead to study drug withdrawn during Treatment Period A: by SOC and PT
- TEAEs with onset during Treatment Period A that are related to study drug: by SOC and PT
- Fatal TEAEs with onset during Treatment Period A: by SOC and PT

18. DATA MONITORING/REVIEW COMMITTEE

Data from this study will be reviewed by the Data and Safety Monitoring Board (DSMB) that reviews all ongoing clinical studies of trofinetide. Details on the data review are provided in a separate DSMB charter.

19. COMPUTER METHODS

Statistical analyses will be performed using Version 9.4 (or newer) of SAS® on a qualified and validated environment.

Validation and quality control of the tables, listings and figures containing the results of the statistical analyses will follow appropriate standard operating procedures.

20. CHANGES TO ANALYSES SPECIFIED IN PROTOCOL

No changes are made to the analyses specified in the protocol.

21. APPENDICES

21.1 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

21.1 Schedule of Events and Assessments for Study ACP-2566-009 (Screening, Baseline, and Treatment Period A) (Continued)

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
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.

21.2 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).

21.3 Summary of Version Changes

Version No:	Document History Description of Update	Author(s)	Version Date
1.0	Original version	[redacted]	15 February 2022
2.0	Amended version	[redacted]	26 June 2023