Official Title: A 40-Week, Open-label Extension Study of Trofinetide for the Treatment of Girls and Women with Rett Syndrome

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STATISTICAL ANALYSIS PLAN

Protocol No.:	ACP-2566-004
Protocol Title:	A 40-Week, Open-label Extension Study of Trofinetide for the Treatment of Girls and Women with Rett Syndrome
Drug:	trofinetide oral solution
Sponsor:	Acadia Pharmaceuticals Inc.
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ABBREVIATIONS

AE	adverse event
ATC	Anatomical/Therapeutic/Chemical
BMI	body mass index
CGI-I	Clinical Global Impression – Improvement
CGI-S	Clinical Global Impression – Severity
COVID-19	coronavirus disease 2019
DSMB	Data and Safety Monitoring Board
eCRF	electronic case report form
ECG	Electrocardiogram
EDC	electronic data capture
FDA	Food and Drug Administration
GJ	Gastrojejunal
GSD	Guidance for Site Documentation and Data Management Querying of Data Impacted by COVID-19
ICND	Impact of Childhood Neurologic Disability Scale
MedDRA	Medical Dictionary for Regulatory Activities
NDA	new drug application
PCI	potentially clinically important
PD	pharmacodynamic(s)
PHE	public health emergency
PK	pharmacokinetic(s)
QTcB	QT Interval Corrected for Heart Rate using Bazett's Formula
QTcF	QT Interval Corrected for Heart Rate using Fridericia's Formula
RSBQ	Rett Syndrome Behaviour Questionnaire
RTT	Rett Syndrome
RTT-AMB	Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills
RTT-CBI	Rett Syndrome Caregiver Burden Inventory
RTT-COMC	Rett Syndrome Clinician Rating of Ability to Communicate Choices
RTT-CSS	Rett Syndrome Clinical Severity Scale

RTT-HF	Rett Syndrome Clinician Rating of Hand Function
RTT-VCOM	Rett Syndrome Clinician Rating of Verbal Communication
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 as described in the protocol amendment 2, dated 07 August 2020.

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

This plan should be read in conjunction with the study protocol and the electronic case report forms (eCRFs).

2. OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to investigate the safety and tolerability of long-term treatment with oral trofinetide in girls and women with Rett syndrome.

2.2 Secondary Objectives

The secondary objectives for this study are:

- To investigate the efficacy of long-term treatment with oral trofinetide in girls and women with Rett syndrome
- To investigate the efficacy of long-term treatment with oral trofinetide in girls and women with Rett syndrome on overall ability to communicate
- To investigate the benefit of long-term treatment with oral trofinetide on overall quality of life for girls and women with Rett syndrome
- To investigate the efficacy of long-term treatment with oral trofinetide in girls and women with Rett syndrome on:
    - Hand function
    - Ambulation and other gross motor skills
    - Ability to communicate choices and preferences
    - Ability to communicate verbally
- To investigate the efficacy of long-term treatment with oral trofinetide on a global assessment of the severity of illness in girls and women with Rett syndrome
- To investigate the benefit of long-term treatment with oral trofinetide on the burden on caregivers of girls and women with Rett syndrome
- To investigate the benefit of long-term treatment with oral trofinetide on the impact of the disability on the child's and family's everyday life

2.3 Pharmacokinetic Objectives

The pharmacokinetic objectives of this study are:

- To characterize the pharmacokinetics (PK) of trofinetide in girls and women with Rett syndrome following long-term treatment with oral trofinetide

- To assess the pharmacokinetic/pharmacodynamic (PK/PD) relationship using safety and efficacy endpoints in girls and women with Rett syndrome following long-term treatment with oral trofinetide

3. STUDY DESIGN

3.1 General Study Design

This is a 40-week, multicenter, open-label extension (OLE) study to evaluate long-term safety and tolerability of trofinetide in girls and women with Rett syndrome. Subjects who complete the preceding double-blind study (ACP-2566-003) will be eligible to enroll in the OLE.

The Week 12/EOT visit of the antecedent study will serve as the Baseline visit of the present study. The data gathered at the Week 12/EOT visit of the antecedent study serves as the baseline data of the present study.

Because of the special circumstances surrounding the Coronavirus Disease 2019 (COVID-19) public health emergency in the spring of 2020, subjects who were discontinued from Study ACP-2566-003 before completion because of the COVID-19 public health emergency and subjects who completed Study ACP-2566-003, but were prevented from entering the present study because of the COVID-19 public health emergency, may be re-evaluated for eligibility at the Baseline visit of the present study.

However, subjects who are discontinued from Study ACP-2566-003 for other reasons other than COVID-19 pandemic, or who are not able to rollover to this study at the Week 12/EOT visit of the antecedent study, will not be able to enter the present study.

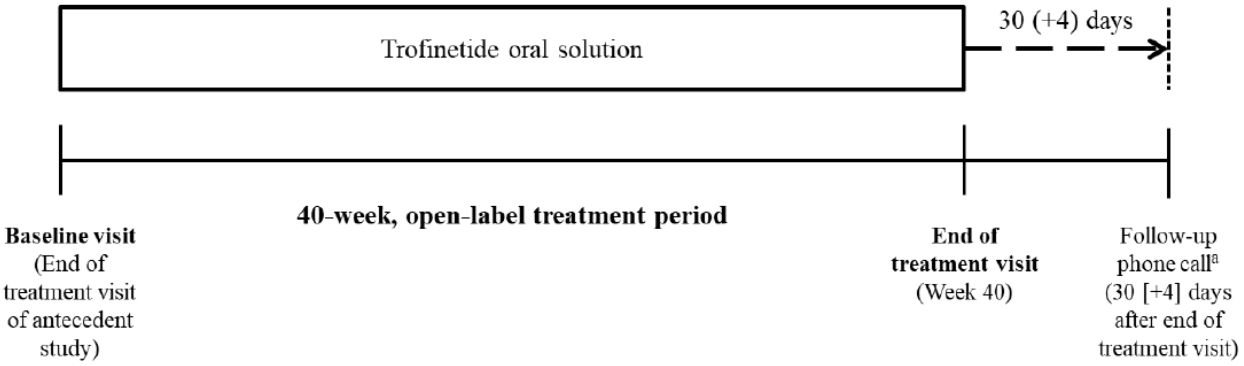
The duration of participation for individual study subjects will be approximately 44 weeks. Approximately 28 sites will participate in this study.

The study will have two periods (Figure 1):

- Treatment period: 40 weeks
- Safety follow-up period: 30 days

The study design is summarized in Figure 1.

Figure 1 Schematic of Study Design



[a] Subjects who roll over into the subsequent open-label study (ACP-2566-005) will not have the safety follow-up telephone call or telemedicine visit.

The schedule of events and assessments is provided in the appendix (Section 21.1). As shown in the schedule of events, study visits may be done off-site rather than in the clinic with the prior approval of the Sponsor or Medical Monitor.

For subjects who remained on the originally assigned dose in the antecedent study, the assigned dose in this study will be based on the subject's weight at the Baseline visit of the present study, as outlined below in Table 1. Thus, for a particular subject whose weight at the Baseline of this study moves them into a different weight category according to Table 1, the assigned dose given in this study may be different from the originally assigned dose given in the antecedent study. After Baseline the dose will not be changed based on a change in weight that places the subject into a different weight category.

Subjects whose originally assigned dose in the antecedent study was decreased for tolerability reasons will remain on that same dose in this study. The dose should be increased at some point during the study, if tolerated, to the dose that would have been assigned based on the weight category at Baseline of the present study.

Table 1 Dosing Schedule Based on Weight at Baseline of the Present Study

Weight	Dose	Total Daily Dose
12-20 kg	30 mL (6 g) BID	60 mL (12 g)
>20-35 kg	40 mL (8 g) BID	80 mL (16 g)
>35-50 kg	50 mL (10 g) BID	100 mL (20 g)
>50 kg	60 mL (12 g) BID	120 mL (24 g)

Abbreviations: BID=twice daily

3.2 Schedule of Assessments

The schedule of assessments is in the Appendix (Section 21.1).

3.3 Randomization

Not Applicable. This is not a randomized study.

3.4 Blinding

This is an open-label extension study in which all patients will receive trofinetide. However, the investigators will not be unblinded of the antecedent study's, ACP-2566-003, treatment assignment during the conduct of study ACP-2566-004.

3.5 Determination of Sample Size

Up to approximately 180 subjects will be enrolled. The sample size for this study is not based on statistical considerations, but will depend on the number of subjects who transition into this open-label extension study from the antecedent study.

3.6 Coronavirus Disease 2019

In March 2020, the emerging coronavirus disease 2019 (COVID-19) pandemic resulted in implementation of urgent safety measures designed to ensure subject safety. Mechanisms to record information on the potential impact of the COVID-19 pandemic on data itself, as well as data collection and integrity, were implemented (as detailed in the appendix of ACP-2566-004's Data Management Plan titled "Guidance for Site Documentation and Data Management Querying of Data Impacted by COVID-19" [GSD] included in eTMF).

Relationship to the public health emergency caused by the COVID-19 pandemic will be assessed for early terminations, protocol deviations, selected medications, and selected adverse events as detailed in the GSD.

4. ANALYSIS SETS

Safety Analysis Set

The Safety Analysis Set consists of all subjects who received at least one dose of study drug in this study.

Pharmacokinetics (PK) Analysis Set

The PK Analysis Set consists of the subjects in Safety Analysis Set with at least one measurable trofinetide whole blood concentration. Subjects will be classified according to the actual treatment received.

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. For descriptive summaries of efficacy, the 95% confidence interval (CI), calculated as mean ± 1.96*SE, will be presented. Unless specified otherwise, means, medians, and CIs 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 not have zero percentages displayed. For demographic summaries, percentages will be calculated by using the total number of subjects in the given treatment group of the antecedent study as the denominator. Percentages will be presented with one decimal place.

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

Lab values, not including PK concentration data, that are collected with "<" or ">" signs will generally be analyzed as the numerical value without the sign in tables and figures. In listings, these data will be reported as collected with the sign.

For each continuous measure in safety and efficacy analyses, change from baseline results will be presented by treatment group of the antecedent study and overall in two ways:

1. Main analysis: using the baseline of this study, as defined in Section 5.5, to report the changes across the timepoints of this open-label Study ACP-2566-004.
2. Exploratory analysis: using the baseline from the antecedent study, ACP-2566-003, to report the changes across the timepoints of this open-label Study ACP-2566-004.

5.2 Derived Efficacy Variables

In general, assessment total scores and subscores will be derived within the analysis datasets. In the event that total scores and/or subscores are also collected on the electronic case report from

(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 Behaviour Questionnaire (RSBQ)

The RSBQ is a 45-item caregiver-completed rating scale in which the caregiver rates items as "0" (Not True), "1" (somewhat or sometimes true) or "2" (very true). The RSBQ includes 8 subscales:1) general mood, 2) breathing problems, 3) hand behavior, 4) face movements, 5) body rocking/expressionless face, 6) night-time behaviors, 7) fear/anxiety, and 8) walking/standing. The total score ranging from 0 to 90 will be calculated as the sum of the scores for all 45 items. Score for item 31 (Uses eye gaze to convey feelings, needs and wishes.) will be reversed (2-observed item score) in the calculation of total score and subscores. Higher total scores indicate greater frequency of symptoms. If there are 9 or less missing item scores, the total score will be calculated by the arithmetic mean of non-missing items multiplied by 45, rounded to the nearest integer. The RSBQ total score will be considered missing if there are missing scores for 10 or more items.

For the calculation of RSBQ subscores, the items under each RSBQ subscore will be summed. If an item is missing it will not be imputed and the subscore will be considered missing.

RSBQ Subscores

1) General mood consists of:
    a. Abrupt changes in mood (Item 14)
    b. Spells of screaming for no apparent reason during the day (Item 2)
    c. There are times when she appears miserable for no apparent reason (Item 16)
    d. Spells of inconsolable crying for no apparent reason during the day (Item 30)
    e. There are times when she is irritable for no apparent reason (Item 29)
    f. Screams hysterically for long periods of time and cannot be consoled (Item 22)
    g. There are certain days/periods where she performs worse than usual (Item 15)
    h. Vocalises for no apparent reason (Item 36)
2) Breathing problems consists of:
    a. There are times when breath is held (Item 5)
    b. Swallows air (Item 19)
    c. Abdomen fills with air and sometimes feels hard (Item 25)
    d. There are times when breathing is deep and fast (hyperventilation) (Item 1)
    e. Air or saliva expelled from mouth with force (Item 6)
3) Hand behaviors consists of:
    a. Does not use hands for purposeful grasping (Item 18)
    b. Restricted repertoire of hand movement (Item 24)

c. Hand movements uniform and monotonous (Item 20)
d. The amount of time spent looking at an object is longer than time spent holding or manipulating them (Item 43)
e. Has difficulty in breaking/stopping hand stereotypies (Item 35)
f. Has frequent naps during the day (Item 21)

4) Repetitive face movements consists of:
   a. Makes mouth grimaces (Item 28)
   b. Makes repetitive tongue movements (Item 32)
   c. Makes grimacing expressions with face (Item 34)
   d. Makes repetitive movements involving fingers around tongue (Item 4)
5) Body rocking and expressionless face consists of:
   a. Rocks body repeatedly (Item 41)
   b. Expressionless face (Item 12)
   c. Rocks self when hands are prevented from moving (Item 33)
   d. Seems to look through people in to the distance (Item 17)
   e. Uses eye gaze to convey feelings, needs and wishes (reversed) (Item 31)
   f. Tendency to bring hands together in front of chin or chest (Item 40)
6) Night-time behaviours consists of:
   a. Spells of screaming for no apparent reason during the night (Item 13)
   b. Spells of inconsolable crying for no apparent reason during the night (Item 42)
   c. Spells of laughter for no apparent reason during the night (Item 37)
7) Fear/Anxiety consists of:
   a. Spells of apparent panic (Item 38)
   b. Spells of apparent anxiety/fear in unfamiliar situations (Item 7)
   c. Seems frightened when sudden changes in own body position (Item 9)
   d. There are times when parts of the body are held rigid (Item 10)
8) Walking/standing consists of:
   a. Walks with stiff legs (Item 39)
   b. Although can stand independently tends to lean on objects or people (Item 23)

Items not included in Subscores

a. Makes repetitive hand movements with hands apart (Item 3)
b. Grinds teeth (Item 8)
c. Shifts gaze with a slow horizontal turn of head (Item 11)
d. Spells of laughter for no apparent reason during the day (Item 26)
e. Has wounds on hands as a result of repetitive hand movements (Item 27)
f. Appears isolated (Item 44)
g. Vacant ‘staring’ spells (Item 45)

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 more severe illness and less improvement in the illness respectively.

5.2.3 Communication and Symbolic Behavior Scales Developmental Profile™ Infant-Toddler Checklist – Social Composite Score (CSBS-DP-IT Social)

The Checklist consists of 24 questions that range from 0 to 4 points within each of 7 Clusters. Credit of 0 points is given for items checked "Not Yet", 1 point for items checked "Sometimes", or 2 points for items checked "Often". For items that describe a series of numbers or ranges, credit of 0 points is given for items checked "None" and 1 to 4 points for items containing numbered choices.

Three composite scores assessing 7 skill areas can be calculated. The Social Composite score is comprised of 13 items in the skill areas "Emotion and Eye Gaze" (items 1 to 4), "Communication" (items 5 to 8), and "Gestures" (items 9 to 13). The Speech Composite score, including the skill areas "Sounds" and "Words" is based on items 14 to 18. The Symbolic Composite score, including the skill areas "Understanding" and "Object Use", is based on items 19 to 24.

The Social Composite raw score (comprised of items 1 to 13) ranging from 0 to 26 will be calculated as the sum of the scores for all 13 items. Higher Social Composite raw scores indicate better social communication development. If there are 2 or less missing item scores, the Social Composite raw score will be calculated by the arithmetic mean of the non-missing item scores multiplied by 13, rounded to the nearest integer. The Social Composite raw score will be considered as missing if there are missing scores for 3 or more items.

5.2.4 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 analyzed as a separate endpoint. The numeric scale 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.

5.2.5 Rett Syndrome Clinician Rating of Hand Function (RTT-HF)

The RTT-HF is a clinician completed clinical assessment of the subject's ability to use her hands for functional purposes. The assessment is made on an 8-point Likert scale (0-7) with 0 denoting normal functioning and 7 the most severe impairment.

5.2.6 Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills (RTT-AMB)

The RTT-AMB is a clinician completed clinical assessment of the subject's ability to sit, stand, and ambulate. The assessment is made on an 8-point Likert scale (0-7) with 0 denoting normal functioning and 7 the most severe impairment.

5.2.7 Rett Syndrome Clinician Rating of Ability to Communicate Choices (RTT-COMC)

The RTT-COMC is a clinician completed clinical assessment of the subject's ability to communicate her choices or preferences, which can include the use of nonverbal means such as eye contact or gestures. The assessment is made on an 8-point Likert scale (0-7) with 0 denoting normal functioning and 7 the most severe impairment.

5.2.8 Rett Syndrome Clinician Rating of Verbal Communication (RTT-VCOM)

The RTT-VCOM is a clinician completed clinical assessment of the subject's ability to communicate verbally. The assessment is made on an 8-point Likert scale (0-7) with 0 denoting normal functioning and 7 the most severe impairment.

5.2.9 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 and less improvement in the illness respectively.

5.2.10 Rett Syndrome Caregiver Burden Inventory (RTT-CBI)

The RTT-CBI consists of 24 negatively worded items (Items 1 through 24). Frequency ratings are on a 5-point Likert scale including: 0-never; 1-rarely; 2-sometimes; 3-frequently and 4-nearly always. The RTT-CBI also includes 2 positively worded items (items 25 and 26) that comprise the Optimism Index; this index will not be used for analysis. The total score ranging from 0 to 96

will be calculated as the sum of the scores for Items 1-24. If there are 4 or fewer missing item scores, the RTT-CBI total score will be calculated by the arithmetic mean of the non-missing item scores multiplied by 24, rounded to the nearest integer. The RTT-CBI total score will be considered as missing if there are missing scores for 5 or more items.

5.2.11 Impact of Childhood Neurologic Disability (ICND) Scale Total Score

The ICND scale evaluates the effect of four health problems on 11 aspects of the child's or the family's life which are scored 0 ("Not at all"), 1 ("A little"), 2 ("Some"), 3 ("A lot"), or "Does not apply". The four health problems are 1) inattentiveness, impulsivity, or mood, 2) ability to think and remember, 3) neurologic or physical limitations, and 4) epilepsy. The ICND total score ranging from 0 to 132 will be calculated as follows:

1. For each health problem, the score will be calculated as the sum of the 11 item scores. The health problem score will be considered missing
    a. if more than 5 item scores are either missing or "Does not apply"
    b. or if more than 3 item scores are missing for reasons other than the response of "Does not apply".
2. The ICND total score will be calculated as the sum of the average of each problem score multiplied by 11.

$$ICND\ Total\ Score = \sum_{k=1}^{4} problem\_score\_average_k * 11$$

where k indexes the four health problems.

The ICND total score will be considered missing if at least 1 health problem score is missing. Imputed ICND total scores will be rounded to the nearest integer.

Higher ICND total scores indicate worse health problems. The ICND total score does not include the Overall Quality of Life Rating.

5.3 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.

5.4 Baseline Definition

Data from the latest predose value of (Study ACP-2566-003 Week 12/EOT visit, unscheduled visit within Week 12 window, visit within 2 weeks of last double-blind dose, or Study ACP-2566-004 Baseline Visit) will be carried over as Baseline information in the present study, as applicable (e.g., not for the CGI-I for which this is not applicable).

Subjects for whom the Baseline visit of this study is not on the same day as the Week 12/EOT visit of the antecedent study (e.g., subjects in the antecedent study who were prevented from entering this study by the COVID-19 public health emergency) will have blood drawn for Baseline values three to fourteen days before the Baseline visit.

5.5 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	Target Study Day	Study Day Interval
Baseline (Day 1)	1	≤ 1
Day 1 Post First Dose*	1	1
Week 2	15	2 – 49
Week 12	85	50 – 133
Week 26	183	134 – 231
Week 40	281	232 – 310

*For ECG and PK assessments only; other assessments post-first dose on Day 1 will go to the Week 2 analysis visit window.

5.5.1 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.

5.5.2 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 may be made for incomplete assessments, in which case, more complete assessments may be given priority. Details are provided in a separate programming specifications document.

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.

5.6 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 dates: last drug kit dispense date + scheduled dosing interval per protocol, EOT/ET date and the return date of the last dispensed drug kit.

For subjects with partial missing last dose date, the imputation will be compared against the last expected dosing date as defined above. Detailed 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.

5.7 Missing or Incomplete Dates 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 11 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.

5.8 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 12.2 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.

5.9 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.

5.10 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.

5.11 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. SUBJECT DISPOSITION

The number of sites that screened at least 1 subject, number of sites that enrolled at least 1 subject, and number of subjects enrolled will be tabulated. In addition, the number of subjects enrolled at each site will also be tabulated by Analysis Set.

The number and percentage of subjects who completed the study, discontinued early (all discontinued and by discontinuation reasons including reason due to the COVID-19 public health emergency (PHE), and the reason for discontinuation will be summarized using the Safety Analysis Sets. All subjects excluded from the Safety Analysis Set and the reason(s) for exclusion will be listed.

7. 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 in three ways: all protocol deviations, COVID-19-PHE related protocol deviations, and non-COVID-19-PHE related protocol deviations.

Three data listings of all protocol deviations, COVID-19-PHE related protocol deviations, and non-COVID-19-PHE related protocol deviations will be provided.

8. 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, age group (5-10 years old, 11-15 years old, and 16-20 years old), race, ethnicity, height, weight, BMI, Baseline RSBQ total score, Baseline RSBQ severity (< 35 total score and ≥ 35 total score), and Baseline CGI-S score.

Race will also be categorized by White vs. Non-White. The reported age reflects a subject's age at the informed consent date. Baseline characteristics for subjects in ACP-2566-004 will also be summarized using the baseline of the antecedent study separately.

9. MEDICAL HISTORY

Medical history reported terms will be coded with Medical Dictionary for Regulatory Activities (MedDRA), version 23.0 or newer. The subject incidence will be summarized for each system organ class (SOC) and preferred term for the Safety Analysis Set. A subject will be counted only once per SOC or per preferred term for the summary.

A listing of the SOC, preferred term, 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 first symptoms 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. EXTENT OF EXPOSURE AND TREATMENT COMPLIANCE

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

10.1 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 <12 weeks (14 to 83 days), 12 to <26 weeks (84 to 181 days), 26 to <40 weeks (182 to 279 days), and >=40 weeks (280 days or longer).

10.2 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 as in Table 3. 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 4).

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 Dosing Schedule and Total Expected Volume Based on Weight at Baseline of the Present Study

Weight	Dose	Total Daily Dose	Total Expected Volume
12-20 kg	30 mL (6 g) BID	60 mL (12 g)	60 mL x Number of Days Expected to be Dosed
>20-35 kg	40 mL (8 g) BID	80 mL (16 g)	80 mL x Number of Days Expected to be Dosed
>35-50 kg	50 mL (10 g) BID	100 mL (20 g)	100 mL x Number of Days Expected to be Dosed
>50 kg	60 mL (12 g) BID	120 mL (24 g)	120 mL x Number of Days Expected to be Dosed

BID = twice daily

Table 4 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

11. 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 double-blind study drug from study ACP-2566-003. 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 2020 or newer version.

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

COVID-19 Infection Related Medications

Concomitant medication analyses described above will also be tabulated and listed by relationship to COVID-19 Infection (Not related to COVID-19 Infection vs. Related to COVID-19 Infection).

12. 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.

12.1 Efficacy Variables

Secondary Efficacy Endpoints

- RSBQ total score – change from Baseline to Weeks 2, 12, 26, and 40
- CGI-I score at Weeks 2, 12, 26, and 40 (assessing improvement from the Baseline of the present study)

Change from Baseline to Weeks 2, 12, 26, and 40 in:

- CSBS-DP-IT Social
- Overall Quality of Life Rating of the ICND
- RTT-HF score
- RTT-AMB score
- RTT-COMC score
- RTT-VCOM score
- CGI-S score
- RTT-CBI total score (items 1-24)
- ICND total score

Response rate based on CGI-I at Weeks 2, 12, 26, and 40

12.2 Adjustment for Covariates

Not applicable.

12.3 Handling of Missing Data

Missing data imputation rules are described in Section 5.2.

12.4 Multiple Comparisons / Multiplicity

No hypothesis testing is planned.

12.5 Examination of Subgroups

Subgroup analysis by *MECP2* mutation groupings may be performed for RSBQ and CGI-I.

12.5.1 *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
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.

13. METHODS OF EFFICACY ANALYSES

13.1 Analysis of Continuous Efficacy Endpoints

Descriptive statistics for all efficacy endpoints listed in Section 12.1 will be tabulated by treatment group of the antecedent study. The summaries of the change from baseline results will be presented in two ways (main analysis and exploratory analysis) as specified in Section 5.1.

14. SAFETY ANALYSES

All safety analyses will be performed using the Safety Analysis Set. Safety summaries will be summarized using descriptive statistics by treatment group of the antecedent study and overall. For each continuous measure in clinical laboratory variables, vital signs, and electrocardiogram, change from baseline results will be presented in two ways as specified in Section 5.1.

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

14.1 Adverse Events

Adverse events will be coded using MedDRA dictionary, Version 23.0 or newer.

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. AEs reported on Day 1 based on Baseline (pre-dose) findings (e.g., clinically significantly abnormal vital signs, laboratory test results, or electrocardiogram parameters) will not be considered as TEAEs.

The event counts, the number, and percentage of subjects reporting TEAEs in the treatment group of the antecedent study and overall will be tabulated by system organ class (SOC) and preferred term; by SOC, preferred term, and maximum severity; and by SOC, preferred term, and relationship to study drug. If more than one TEAE occurs with the same preferred term for the same subject, then the subject will be counted only once for that preferred term using the most severe and most related occurrence for the summarization by severity and by relationship to study drug. The display in these tables will be sorted alphabetically by SOC and then by descending subject frequency for the preferred terms (combined counts from overall group) within each SOC.

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

The incidence of most frequently reported (preferred terms reported by ≥ 5% of subjects overall) TEAEs, SAEs reported after treatment start, TEAEs leading to drug withdrawn, and TEAEs related to study drug will be summarized by SOC and preferred term. The tables will be sorted alphabetically by SOC and then by descending frequency within each SOC in overall group. In addition, the incidence of fatal treatment-emergent AEs (i.e., events that cause death) will be summarized separately by SOC and preferred term.

These summary tables except for the most frequently reported TEAEs tables will also be presented by relationship to COVID-19 Infection (Not related to COVID-19 Infection vs. Related to COVID-19 Infection).

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 preferred term. 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 subjects with treatment-emergent SAEs, related TEAEs, TEAEs leading to drug withdrawn, fatal TEAEs (if any), and TEAEs Related to COVID-19 Infection.

14.2 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 at Baseline, Week 2, Week 12, Week 26 and Week 40/EOT.

- Clinical chemistry serum tests include the following:

  - Sodium (Na), potassium (K), chloride (Cl), phosphorus (P), calcium (Ca), carbon dioxide (CO_2), blood urea nitrogen (BUN), creatinine (CR), uric acid
  - 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 (Baseline) and Visit 5

- Pregnancy test
  - A serum pregnancy test will be performed at all designated visits for subjects of childbearing potential
- 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 Tables 5 and 6. The number and percentage of subjects with post-Baseline PCI values for each of the categories in Table 5 and 6 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 5 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	U/L	No lower limit	≥3 X ULN	U/L	No lower limit	≥3 X ULN
AST	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 6 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.

At the initiation of the study, the main reference ranges (the reference ranges provided by the central laboratory validated for use for the general population) were used for the purpose of flagging abnormal laboratory values for the investigator to assess clinical significance. During the study, at the request of a leading investigator an alternative set of reference ranges, more specific to patients with Rett syndrome rather than to the general population, was used for the purpose of flagging abnormal lab values for the investigator to assess clinical significance.

All analysis will be done using the main reference ranges, not the alternative ranges. During the course of the study the main reference ranges were updated by the central laboratory. The latest version of the reference ranges provided by the central laboratory will be used for all analysis; PCI status and categorical labs (Low, Normal, High) and shifts will be derived from the latest version of the main reference range. Both of the reference ranges (main and alternative) and the corresponding categorical assessment will be listed.

The pregnancy results (positive or negative) will be presented in a listing.

14.3 Vital Signs

Vital signs will be collected throughout the study; height will be measured at Baseline and Week 40/EOT; weight will be measured at Baseline, Week 2, Week 12, Week 26 and Week 40/EOT. 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 7. 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 at least 1 post-Baseline vital sign for the given parameter. A listing of overall and of subjects with any PCI vital sign values will be provided.

Table 7 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	≥180	And	Increase of ≥20	-
		≤90	And	Decrease of ≥20	-
Diastolic blood pressure (supine or sitting)	mmHg	≥105	And	Increase of ≥15	-
		≤50	And	Decrease of ≥15	-
Pulse (supine or sitting)	bpm	≥120	And	Increase of ≥15	-
		≤50	And	Decrease of ≥15	-
Weight	kg	Not Applicable		Increase of ≥7%	-
				Decrease of ≥7%	-

14.4 Electrocardiogram (ECG)

12-lead ECGs are collected throughout the study at every visit.

When ECG is collected multiple times (e.g. at Screening visit, Baseline visit both before dosing and 2-3 hours after dosing, and Week 40/EOT/ET visit), the average of all available values on the same day will be considered as one assessment for the summaries, except for Day 1 where averages will be by pre-dose and post-dose averages. The averages will be rounded to integer values.

Observed (average) values of ECG variables (e.g., heart rate, PR interval, QRS interval, QT interval, QTcB and QTcF interval) and the changes from Baseline at each assessment time point will be summarized by treatment group.

Observed (average) values of 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 by treatment group 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 given treatment group. 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 for the given treatment group.

Electrocardiogram variable average values will be considered PCI if they meet the criteria listed in Table 8. The number and percentage of subjects with post-baseline PCI values will be summarized by treatment group 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, visit and treatment group, and the denominator is the number of subjects with non-missing values for the given parameter, visit and treatment group. 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 treatment group, and the denominator is the number of subjects with at least 1 post-Baseline ECG value for the given parameter and treatment group. A listing of overall and of all subjects with any PCI ECG values will be provided.

Table 8 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	

14.5 Physical Examination

Physical examinations are performed throughout the study at every visit in the clinic. Physical examination results (normal, abnormal, and not done) will be summarized in a frequency table by body system and visit. A listing of physical examination data will be listed.

14.6 Other Safety Endpoints

There are no other safety endpoints in this study.

15. 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 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. Trofinetide whole blood concentration data will remain blinded until the unblinding of the clinical database at the end of the study.

16. INTERIM ANALYSIS

No interim analysis is planned in this study, If a new drug application (NDA) submission happens before the completion of this study, an interim data cut will be scheduled for that purpose.

17. DATA MONITORING/REVIEW COMMITTEE

Data from this study will be reviewed by the Data and Safety Monitoring Board (DSMB) for study ACP-2566-003.

18. 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.

19. CHANGES TO ANALYSES SPECIFIED IN PROTOCOL

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

20. REFERENCES

EMA (2009). Guideline on Missing Data in Confirmatory Clinical Trials, European Medicines Agency, London, UK.

FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic Guidance for Industry, Investigators, and Institutional Review Boards March 2020

Points to consider on implications of Coronavirus disease 4 (COVID-19) on methodological aspects of ongoing clinical 5 trials, March 25 2020.

21. APPENDICES

21.1 Schedule of Assessments

Period	Baseline	Open-label Treatment Period				Safety Follow-up[k]
Visit Week	0	2	12	26	40/EOT/ET	EOT/ET+ 30 days
Visit Number	1	2	3	4	5	
Visit window (days)	N/A	±3	±7	±7	+7	+4
Type of Visit[l]	Clinic or Off-site					Telephone or Telemedicine
Informed consent	X[a]				X[a]	
Inclusion/exclusion criteria	X					
Medical history update	X					
Physical examination[l]	X	X	X	X	X	
Vital signs[b]	X	X	X	X	X	
Height	X				X	
Weight[l]	X[l]	X	X	X	X	
12-lead electrocardiogram (ECG)[e]	X[c]	X	X	X	X[c]	
Clinical laboratory tests (hematology, chemistry)[m]	X	X	X	X	X	
Urinalysis	X	X	X	X	X	
TSH, Free T3, Free T4	X				X	
Serum pregnancy test[d]	X		X	X	X	
Blood samples for pharmacokinetics	X[e]	X[f]	X[f]	X[f]	X[f]	
Blood sample for optional analysis for biomarkers[g]	X[h]		X	X	X	
Rett Syndrome Behaviour Questionnaire (RSBQ)	X	X	X	X	X	
Clinical Global Impression–Improvement (CGI-I)[i]		X	X	X	X	
Clinical Global Impression–Severity (CGI-S)	X	X	X	X	X	
Communication and Symbolic Behavior Scales-Developmental Profile™ Infant-Toddler (CSBS-DP-IT) Checklist	X	X	X	X	X	

21.1 Schedule of Assessments (Continued)

Period	Baseline	Open-label Treatment Period				Safety Follow-up[k]
Visit Week	0	2	12	26	40/EOT/ET	EOT/ET+ 30 days
Visit Number	1	2	3	4	5	
Visit window (days)	N/A	±3	±7	±7	+7	+4
Type of Visit[l]	Clinic or Off-site					Telephone or Telemedicine
Impact of Childhood Neurologic Disability Scale (ICND)	X				X	
Rett Syndrome Clinician Rating of Hand Function (RTT-HF)	X	X	X	X	X	
Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills (RTT-AMB)	X	X	X	X	X	
Rett Syndrome Clinician Rating of Ability to Communicate Choices (RTT-COMC)[l]	X	X	X	X	X	
Rett Syndrome Clinician Rating of Verbal Communication (RTT-VCOM)	X	X	X	X	X	
Rett Syndrome Caregiver Burden Inventory (RTT-CBI)	X		X		X	
Dispensing and review of semi-structured caregiver diary	X	X	X	X	X	
Concomitant medications	X	X	X	X	X	X
Assessment of adverse events	X	X	X	X	X	X
Study drug dispensed[j]	X					
Authorization of study drug dispensation[j]	X----------X					
Study drug return[j]		X----------X				
Study drug accountability[j]		X	X	X	X	

Abbreviations: EOT=end of treatment; ET=early termination

a Unless subjects are enrolling in the present study under special circumstances due to the COVID-19 public health emergency (detailed in the inclusion/exclusion criteria), consent for the present study must be obtained prior to performance of the EOT procedures at the Week 12/EOT visit in the antecedent study. Data from the Week 12/EOT visit procedures of the antecedent study will be carried over as Baseline information in the present study, as applicable. Subjects who enter the present study under special circumstances due to the COVID-19 public health emergency will have consent obtained for the present study before any procedures are performed (e.g., the blood draw before the

Baseline visit). For subjects who decide to continue into subsequent open-label extension (OLE) study (ACP-2566-005), informed consent for ACP-2566-005 must be obtained prior to performing the Week 40/EOT procedures.

b 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 for ≥3 minutes.

c ECGs will be completed in triplicate at Visit 1 (Baseline) both before dosing and 2-3 hours after dosing and at Visit 5 (Week 40/EOT/ET). For those subjects who completed the antecedent study, the ECG taken at the Week 12/EOT visit in the antecedent study is the predose Baseline ECG of the present study. A single ECG will be completed at Visit 2 (Week 2), Visit 3 (Week 12), and Visit 4 (Week 26).

d For subjects who have reached menarche and have not had surgical sterilization

e The PK sample taken at the Week 12/EOT visit in the antecedent study is the predose PK blood sample for the present study. A postdose PK blood sample will be collected at the end of ECG assessment 2-3 hours after the first dose of study drug in the present study.

f PK samples at Visits 2, 3, 4, and 5 will be collected at one of the following time intervals: 1) 2-3 hours after dosing OR 2) 4-6 hours after dosing OR 3) 7-11 hours after dosing. Every effort should be made to collect the PK samples at discrete time intervals during Visits 2, 3, 4, and 5. However, if the interval is the same across these visits, then the collection time should vary within that interval.

g Participation in the effort to identify biomarkers is an optional component of the study requiring a separate informed consent.

h The biomarker sample taken at the Week 12/EOT visit in the antecedent study is the Baseline sample for the present study.

i The CGI-I assessments at Weeks 2, 12, 26, and 40/EOT will be a comparison with the baseline state of the illness in the present study (i.e., Visit 1 of ACP-2566-004).

j Investigational product will be shipped directly to the subject. Confirmation of delivery to the subject will be made by a visiting nurse. Study drug shipment, return, and accountability will be performed in accordance with the drug distribution plan. In addition, study drug will be dispensed at the site during the Baseline visit when the visit is conducted in the clinic.

k Subjects who roll over into the subsequent open-label study (ACP-2566-005) will not have the safety follow-up telephone call or telemedicine visit.

l Study visits may be done off-site rather than in the clinic 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. Measurement of weight is necessary at Baseline in order to determine the starting dose. The RTT-COMC should be completed if possible, but it is not required.

m Subjects for whom the Baseline visit of this study is not on the same day as the Week 12/EOT visit of the antecedent study will have blood drawn for Baseline values 3 to 14 days before the Baseline visit.

21.2 Summary of Version Changes

Version No:	Document History Description of Update	Author(s)	Version Date
1.0	Original version	[redacted]	09 December 2020
2.0	Added *MECP2* Groupings; added definitions of RSBQ subscores; clarified analysis visit windowing	[redacted]	21 April 2021
3.0	Clarified text throughout.	[redacted]	20 October 2021