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Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome

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Abstract

Objective

To determine safety, tolerability, and pharmacokinetics of trofinetide and evaluate its efficacy in female children/adolescents with Rett syndrome (RTT), a debilitating neurodevelopmental condition for which no pharmacotherapies directed at core features are available.

Methods

This was a phase 2, multicenter, double-blind, placebo-controlled, parallel-group study, in which safety/tolerability, pharmacokinetics, and clinical response to trofinetide were characterized in 82 children/adolescents with RTT, aged 5 to 15 years. Sixty-two participants were randomized 1:1:1:1 to receive placebo twice a day (bid) for 14 days, followed by placebo, 50, 100, or 200 mg/kg bid of trofinetide for 42 days. Following blinded safety data review, 20 additional participants were randomized 1:1 to the 200 mg/kg or placebo bid groups. Safety assessments included adverse events, clinical laboratory tests, physical examinations, and concomitant medications. Clinician- and caregiver-based efficacy measurements assessed clinically relevant, phenotypic dimensions of impairment of RTT.

Results

All dose levels were well tolerated and generally safe. Trofinetide at 200 mg/kg bid showed statistically significant and clinically relevant improvements relative to placebo on the Rett Syndrome Behaviour Questionnaire, RTT-Clinician Domain Specific Concerns–Visual Analog Scale, and Clinical Global Impression Scale–Improvement. Exploratory analyses suggested that observed changes correlated with trofinetide exposure.

Conclusion

These results, together with those from a previous adolescent/adult trial, indicate trofinetide's potential for treating core RTT symptoms and support further trials.

Classification of evidence

This study provides Class I evidence that for children/adolescents with RTT, trofinetide was safe, well-tolerated, and demonstrated improvement over placebo at 200 mg/kg bid in functionally important dimensions of RTT.

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*These authors made equal contributions to the conduct and/or analysis of the study described in this manuscript.

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Glossary

AE = adverse event; **AUC** = area under the concentration vs time curve; **bid** = twice a day; **CGI-I** = Clinical Global Impression Scale–Improvement; **IGF1** = insulinlike growth factor 1; **IRB** = institutional review board; **ITT** = intention to treat; **MBA** = Motor Behavioral Assessment; **mITT** = modified intention to treat; **PK** = pharmacokinetic; **PKPD** = pharmacokinetic-pharmacodynamic; **RSBQ** = Rett Syndrome Behaviour Questionnaire; **RTT** = Rett syndrome; **RTT-DSC** = Rett syndrome–Clinician Domain Specific Concerns; **SAE** = serious adverse event; **VAS** = Visual Analog Scale.

Rett syndrome (RTT) is a neurodevelopmental disorder affecting 1 in 10,000–15,000 females.[1–4] Most cases are caused by loss of function mutations in the X-linked gene, methyl-CpG-binding protein 2 (*MECP2*),[5,6] encoding a protein that binds to DNA and regulates transcription.[6–8]

RTT is characterized by developmental regression including loss of expressive language and purposeful hand use, impaired or absent ambulation, and onset of stereotypical hand movements.[9] Individuals with RTT have severe motor deficits and autonomic, gastrointestinal, and other systemic symptoms.[10] Neurobehavioral impairments are prevalent, including anxiety-like behaviors, disruptive behavior, and mood dysregulation.[11–14] Disease burden is severe for individuals and their families, the effects of which are lifelong.[15,16] No approved or effective treatment is available for the core or other prevalent symptoms of RTT.

Trofinetide (glycyl-L-2-methylprolyl-L-glutamic acid) is an analog of the amino-terminal tripeptide of insulinlike growth factor 1 (IGF1 [1–3]). RTT mouse models demonstrated that IGF1 (1–3) treatment improves disease symptoms.[17] Trofinetide is believed to have potential in treating RTT by normalizing aberrant neuronal and glial function secondary to anti-inflammatory and trophic effects, which inhibit astrogliosis and pathologic microglial activation; normalizing synaptic protein synthesis, dendritic morphology, and neuronal signaling; and enhancing antioxidant response[18–20]. A phase 2 study in adolescent/adult females with RTT demonstrated excellent safety/tolerability and preliminary evidence of efficacy at 70 mg/kg twice a day (bid) for 28 days.[21] Herein, we report a phase 2 study in a larger pediatric cohort, evaluating higher doses and longer treatment duration, and assessing pharmacokinetic-pharmacodynamic (PKPD) correlations to support the dose rationale for future studies.

Methods

Classification of evidence

The primary research questions were, (1) Is treatment with trofinetide safe and well-tolerated in the pediatric RTT population? and (2) What is the pharmacokinetic (PK) profile of trofinetide in this population? The secondary question was, (3) Does trofinetide treatment demonstrate improvement of RTT symptoms over placebo? This study provides Class I evidence showing that trofinetide at doses of 50, 100, and 200 mg/kg bid was well tolerated and generally safe in children/adolescents with RTT, in whom increasing doses were associated with proportional increase in systemic exposure (linear PKs), and at 200 mg/kg bid demonstrated improvement over placebo across functionally important dimensions of RTT. The study also provides insight into potential efficacy measures for future studies.

Study design

Study design (dose, length of treatment, and safety and efficacy outcome measures) was informed by the previous adolescent/adult trial (Rett-001).[21] The present study (hereafter Rett-002) was an exploratory, phase 2, multicenter, double-blind, placebo-controlled, parallel-group trial. The first 62 participants were randomized 1:1:1:1 to placebo or 1 of 3 dose levels of trofinetide: 50, 100, or 200 mg/kg bid, stratified by age. Following review of blinded safety and tolerability data, the study design was modified to enable enrollment of 20 additional participants randomized 1:1 to 200 mg/kg bid or placebo bid. The aim was to enrich both the high-dose and placebo groups to maximize the likelihood of detecting clinical benefit in this population for whom there are no alternative pharmacotherapies. Total duration of treatment was 56 days, with participants receiving single-blind placebo treatment for 14 days followed by 42 days of double-blind treatment with either drug or placebo. Participants had a posttreatment visit approximately 10 days after the end of treatment. Study design and subject disposition are shown in figure 1.

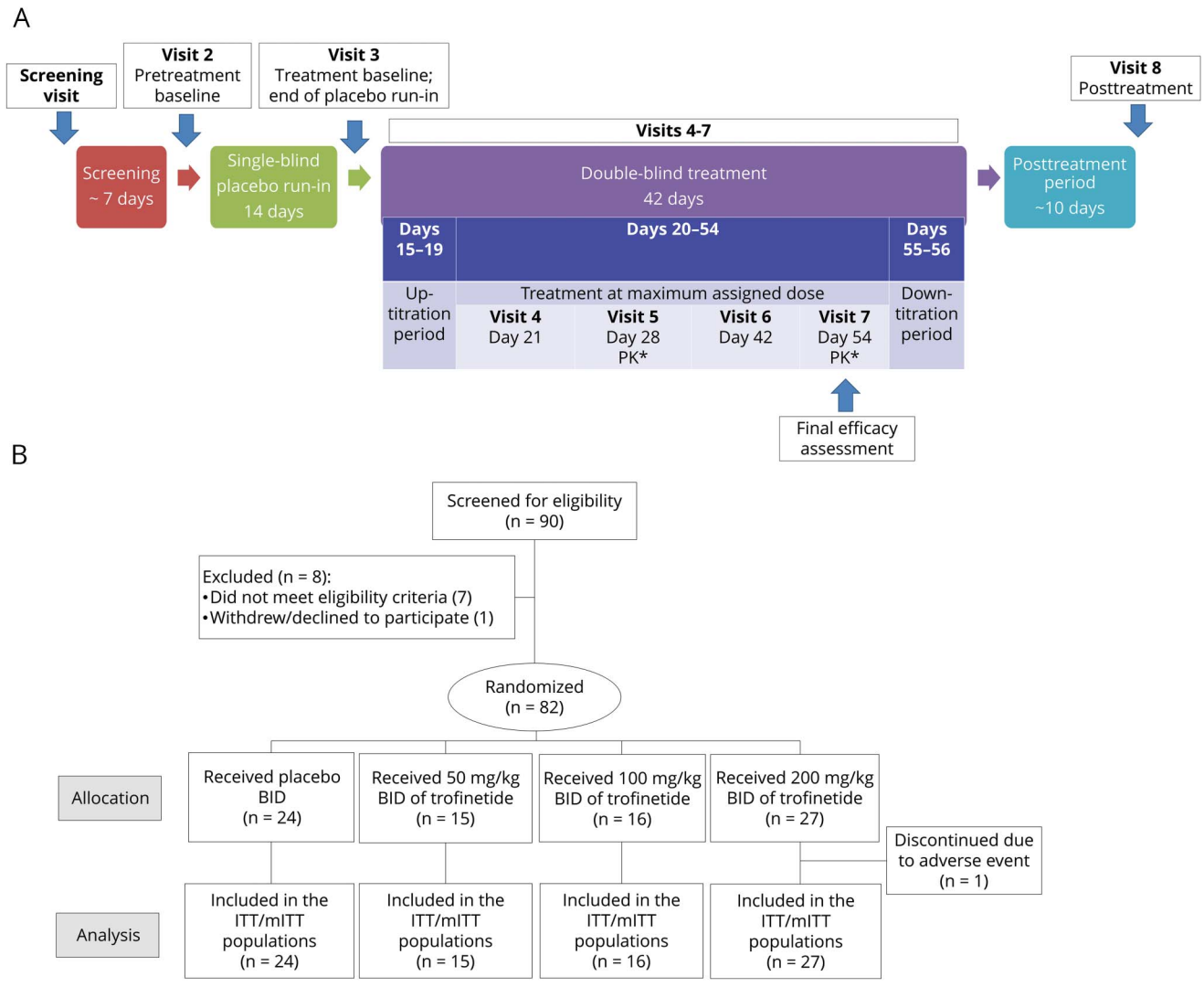
Randomization and treatment allocation

The randomization scheme used 2 age strata (ages 5–10 and 11–15 years) and then assignment to treatment blocks. At the end of the 1-week screening period, the participant was randomized if she met all eligibility criteria, and written informed consent was provided by a legally authorized representative. Randomization was via a web-based randomization system operating 24 hours per day, 7 days per week. Participants were assigned a unique randomization number. Treatment assignments were not disclosed to the sponsor, participants, caregivers, investigators, or research site personnel.

Dosing/dose schedule

Trofinetide (also known as NNZ-2566) and placebo were administered as a volume-matched strawberry-flavored liquid either orally or via gastrostomy tube. Participants were blindly up-titrated to their assigned dose based on a predefined dosing schedule. The 50 mg/kg group was up-titrated over 2 days (8.5 mg/kg bid, 35 mg/kg bid, then 50 mg/kg bid), the 100 mg/kg over 3 days (17.5 mg/kg bid, 35 mg/kg bid, 50 mg/kg bid, then 100 mg/kg bid), and the 200 mg/kg group over 5 days (17.5 mg/kg bid, 35 mg/kg bid, 50 mg/kg bid, 100 mg/kg

Figure 1 Study design (A) and participant disposition (B)



BID = twice daily dosing; ITT = intention to treat; mITT = modified intention to treat; PK = pharmacokinetic.

bid, 150 mg/kg bid, then 200 mg/kg bid). The 100 and 200 mg/kg groups were also down-titrated from their maximum dose after day 54 (100 mg/kg bid on day 55 and 50 mg/kg bid on day 56). As such, day 54 was the last day when all participants were treated on their maximally assigned dose level.

Participants

Eighty-two girls between 5 and 15 years of age participated in the study. All participants met the 2010 diagnostic criteria for classic RTT,[9] had molecular documentation of a pathogenic *MECP2* variant, were in the postregression stage, and were stable on current pharmacologic and behavioral treatments for at least 4 weeks (seizure profile and antiepileptic drugs had to be stable for 8 weeks). Caregivers recorded seizure frequency, medications, and behavioral treatments in a paper diary during the 1-week screening period to confirm stability through the start of the study medication. Data were collected at 12 RTT research centers in the United States.

Standard protocol approvals, registrations, and patient consents

The study was registered on Clinicaltrials.gov (NCT02715115). Enrollment commenced in March 2016 and the study was completed in January 2017 after target enrollment was reached and participants completed the study. The study was approved at each study site by its institutional review board (IRB) or by the designated centralized IRB for the study. Written informed consent was obtained by the parent or legal guardian for all participants. Oral or written assent was obtained from participants deemed able by the recruiting physician and per local IRB regulations.

Safety assessments

Safety evaluations included monitoring of adverse events (AEs), clinical laboratory tests (urinalysis, hematology, chemistry [including hemoglobin A_{1c}, electrolytes, minerals, protein, lipids, and tests of thyroid, renal, and liver function]),

vital signs, ECGs, physical examinations, funduscopy and tonsil size, and concomitant medications. Seizures were monitored in the caregiver diary.

PK sampling

PK samples were collected on day 28 (predose and 2–4 hours post dose) and day 54 (predose, 2–3 hours, and 4–6 hours post dose). Samples of whole blood were collected into 2-mL lithium heparin Vacutainer tubes from a cannula port or via venipuncture and stored until shipment at −70°C (or colder) no more than 2 hours after sample collection. The date and times of collection/storage in the freezer were recorded in a log. The date and time of the most recent dose of study medication and most recent meal relative to that dose were recorded in the caregiver diary. Samples were shipped at designated times on dry ice and using temperature monitoring to the laboratory for storage and analysis.[22]

Efficacy outcome measures

Efficacy assessments were performed during the treatment and posttreatment visits. Baseline assessments occurred before the first dose of study medication. All clinician raters completed a standardized training and completed regular calibration sessions during the study. Study staff reviewed instructions for completing the caregiver measures with the caregiver rater at each session and reviewed the forms for completeness.

Efficacy measurements were categorized into 4 domains: (1) clinician-completed syndrome-specific measures (RTT Motor Behavioral Assessment [MBA], RTT Domain Specific Concerns–Visual Analog Scale); (2) clinician-completed syndrome-specific global measures (Clinical Global Impression Scales, Improvement and Severity); (3) caregiver-completed syndrome-specific measures (Rett Syndrome Behaviour Questionnaire, Caregiver Top 3 Concerns–Visual Analog Scale, RTT Caregiver Burden Inventory); and (4) physiologic measures (heart and respiratory rate).

For statistical analysis, the end points were further characterized into priority levels of core, secondary, and exploratory. The main efficacy analysis was conducted on core end points, which were identified in the statistical analysis plan prior to unblinding of treatment codes. Core efficacy end points included 5 measures (both clinician- and caregiver-completed) as described below.

Core efficacy end points

The RTT MBA is a clinician-completed rating scale that has been used as part of the Rett Syndrome Natural History Study.[23–25] The MBA has 34 items, captured on a 4-point Likert scale, grouped in 3 subscales—Behavior/Social, Orofacial/Respiratory, and Motor/Physical Signs, and a modified scoring rubric used as an outcome variable in Rett-001.[21]

The RTT-Clinician Domain Specific Concerns–Visual Analog Scale (RTT-DSC-VAS) is a clinician-completed VAS assessing the severity of concerns in: (1) hand use; (2) ambulation; (3) seizures; (4) autonomic features; (5) behavior; (6) attentiveness; (7) social interaction; and (8) language/communication. Concerns are identified on an individual basis at baseline. Severity is scored at baseline and follow-up visits for each concern by measuring the number of centimeters on a 10-cm VAS line and reported as a percentage of the line. A total VAS score for each participant is calculated as the sum of the scores for the 8 concerns. If a subject had no symptoms/concerns in an area, that domain was not rated, and the score was null for that concern.

The Clinical Global Impression Scale–Improvement (CGI-I) is a clinician-completed assessment of how much the individual's illness has improved/worsened relative to a baseline state, scored using a standardized rubric that is specific to the clinical features of RTT.[26] A 7-point scale is used: 1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; 7 = very much worse. Day 14 assessments (treatment baseline, end of placebo run-in) were made relative to the pretreatment baseline visit. For all subsequent visits, assessments were made relative to the day-14 visit.

The Rett Syndrome Behaviour Questionnaire (RSBQ) is a validated caregiver-completed rating scale assessing a wide range of neurobehavioral symptoms known to be impaired in RTT.[27–31] The RSBQ has been correlated with functioning and quality of life and characterized across a range of ages and genetic variations in RTT.[13,14,30,32] The scale includes 45 items rated on a Likert scale from 0 to 2, 39 of them grouped into 8 subscales (General Mood, Breathing Problems, Hand Behaviors, Repetitive Face Movements, Body Rocking and Expressionless Face, Night-time Behaviors, Fear/Anxiety, and Walking/Standing) whose ratings reflect severity and frequency of symptoms. A total, representing the sum of the 45 items (maximum score 90), and 8 subscale scores are obtained.

The Caregiver Top 3 Concerns VAS is a syndrome-specific measure of 3 signs or symptoms identified by caregivers at baseline on an individual, per-participant basis, as being priority concerns they would like to see improved as a result of treatment.[33] Caregivers were required to choose 3 concerns from any symptom domain related to the participant's RTT. The severity of each concern is scored using a 10-cm VAS using the aforementioned methodology for the RTT-DSC.

Sample size

This was an exploratory study with primary outcomes relating to assessment of safety and PK, and secondary outcomes relating to efficacy. The study design with approximately 82 participants enabled randomization of approximately 15 per group to the 50 and 100 mg/kg groups and approximately 24 per group for the 200 mg/kg and placebo groups, which was deemed to be sufficient to detect treatment differences.

Statistical analyses

Safety analyses were conducted for all participants randomized, according to actual treatment received (intention-to-

treat [ITT] population). AEs and other safety data were summarized as frequencies and percentages by treatment groups. AEs were also summarized by system organ class and preferred term using number and percentages. AEs and serious AEs (SAEs) were listed by severity and by relationship to study treatment. Discontinuations caused by AEs were also listed.

Percent and number of participants with abnormal clinical laboratory or ECG findings were summarized by time point and treatment group. Vital signs, ECG, thyroid, funduscopy, and tonsillar findings were summarized by time point and treatment group. Participants taking concomitant medications were summarized by preferred term and treatment group using numbers and percentages.

An integrated population PK model was developed to describe trofinetide's PK in pediatric patients with RTT using sparse samples.[22] In addition to clearance, volume of distribution, and absorption rate constant, secondary PK parameters included T_{max} (time to maximum concentration), C_{max} (maximum concentration), C_{min} (trough concentration), C_{ss} (steady-state concentration), $t_{1/2}$ (terminal elimination half-life), and AUC (area under the concentration vs time curve). Derived PK parameters were used to assess the PKPD correlations between 12-hour exposure ($AUC_{0\text{-}12}$) and cumulative exposure over the course of treatment ($AUC_{0\text{-}x}$) and change in core measures. The PK population included participants who received study drug to at least the morning day-28 dose and had a PK sample collection at the specified postdose time point(s).

The efficacy analysis was conducted for the modified ITT (mITT) population, which included all participants randomized to receive at least one dose of double-blind study medication, and differences between each active treatment group and the placebo group were assessed. Comparisons of mean change on each core efficacy measure were assessed from treatment baseline (day 14, end of placebo run-in) to day 54 (end of treatment assessment, the last visit before down-titration). For the CGI-I, actual values at the end of treatment were compared as there are no baseline values for CGI-I.

Analyses were performed for each core end point using a generalized linear model. Treatment baseline and placebo response (change from pretreatment baseline to treatment baseline or observed value at treatment baseline for CGI-I) were included as covariates in the model, but dropped from the final model if not significant at the $p \leq 0.1$ two-sided level. Effect sizes were determined using Cohen *d*. For efficacy analyses of the mITT population, missing data were imputed with the median value for the participant's assigned dose group at that visit. The imputation was performed for individual instrument items; any subscale subtotals and totals for a given instrument were calculated based on the imputed individual items. No multiplicity adjustments were performed. If normality and homogeneity of variance assumptions were substantially violated, an appropriate nonparametric test would be performed instead. Conclusions about the study's overall evidence of clinical improvement were based on the totality of efficacy data for the prespecified core end points rather than for a single primary end point.

Data availability

The data reported are part of an ongoing sponsor-led clinical development program. As such, complete datasets for the study will not be made available with the report.

Results

Demographics

A total of 82 participants from 12 study sites were randomized in this study: 24 in the placebo bid group, 15 in the 50 mg/kg bid trofinetide group, 16 in 100 mg/kg bid trofinetide group, and 27 in the 200 mg/kg bid trofinetide group. All 82 participants were included in the ITT population (safety analysis) and in the mITT population (efficacy analysis). The PK population included all 58 participants who received treatment with trofinetide. The mean age of the cohort was 9.7 years (range 5.1–15.9 years), 94% were white, and mean weight was 26.1 kg. Overall demographic characteristics for participants were balanced across the treatment groups; information for the mITT population is shown in table 1.

Safety

Safety and tolerability of trofinetide was very good at all 3 dose levels. No deaths occurred in the study. Only one participant (200 mg/kg bid group) was withdrawn from the study at the request of her parents because of increased mild gastroesophageal reflux, moderate diarrhea, and mild vomiting, which resolved uneventfully after discontinuation. Four SAEs occurred in 3 participants: 1 participant receiving placebo, 1 participant receiving 100 mg/kg bid, and 1 participant receiving 200 mg/kg bid. All the SAEs were deemed not related to study medication and resolved by the end of the study.

A summary of AEs during the double-blind treatment period occurring in at least 2 participants is shown in table 2. The most common AEs reported during the double-blind period across all treatment groups were diarrhea (27%), vomiting (15%), upper respiratory tract infection (12%), and pyrexia (10%). Diarrhea was reported in 27% in the 50 mg/kg bid group, 13% for the 100 mg/kg bid group, and 56% in the 200 mg/kg bid group. Most AEs were mild or moderate in intensity and most events were considered not related to study drug.

No systematic evidence of withdrawal effects was observed when the study drug was discontinued. Clinical laboratory tests, ECGs, vital signs, and physical examinations (including funduscopy and tonsil size) indicated no time- or dose-dependent patterns.

Efficacy

Trofinetide demonstrated statistically significant evidence of clinical improvement ($p < 0.05$) for the 200 mg/kg bid dose over placebo in 3 core measures: RSBQ (total score, core

Table 1 Baseline demographics by treatment group (modified intention to treat)

	Placebo (n = 24)	50 mg/kg (n = 15)	100 mg/kg (n = 16)	200 mg/kg (n = 27)	All participants (n = 82)
Age, y					
Mean (SD)	9.38 (3.26)	10.06 (3.18)	10.81 (3.10)	9.23 (3.88)	9.73 (3.43)
Median	9.64	9.54	9.66	7.49	9.41
Height,[a] cm					
Mean (SD)	122.69 (12.67)	124.12 (11.70)	129.55 (12.76)	121.55 (15.19)	123.86 (13.50)
Median	119.70	122.10	130.30	117.00	120.90
Weight, kg					
Mean (SD)	24.20 (6.87)	26.13 (9.78)	30.43 (12.16)	25.22 (11.51)	26.10 (10.24)
Median	23.75	22.60	28.65	21.30	23.05
BMI,[a] kg/cm^2					
Mean (SD)	16.00 (2.85)	16.50 (3.61)	17.70 (5.06)	16.31 (3.57)	16.52 (3.70)
Median	15.97	15.04	16.96	15.70	15.81
Age category, n (%)					
≤10 y	15 (63)	10 (67)	10 (63)	17 (63)	52 (63)
>10 y	9 (38)	5 (33)	6 (38)	10 (37)	30 (37)
Ethnicity, n (%)					
Hispanic	0 (0)	1 (7)	1 (6)	6 (22)	8 (10)
Not Hispanic	24 (100)	14 (93)	14 (88)	21 (78)	73 (89)
Not reported	0 (0)	0 (0)	1 (6)	0 (0)	1 (1)
Race, n (%)					
American Indian or Alaskan Native	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Asian	1 (4)	0 (0)	0 (0)	2 (7)	3 (4)
Black or African American	0 (0)	0 (0)	1 (6)	0 (0)	1 (1)
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
White	22 (92)	15 (100)	15 (94)	25 (93)	77 (94)
Other	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)

Abbreviation: BMI = body mass index.
[a] For height and BMI for the placebo group, n = 23.

neurobehavioral RTT symptoms, $p = 0.042$), CGI-I (overall clinical status, $p = 0.029$), and RTT-DSC (most concerning aspects of RTT identified by clinicians, $p = 0.025$). Results for the 5 core efficacy measures across the treatment groups are shown in table 3.

Among the 3 outcome measures with $p < 0.05$, the magnitude of effect (% change of the median score) for RTT-DSC was 15% (vs 5% for placebo) and the magnitude of effect (% change of the mean score) for RSBQ was 16% (vs 6% for placebo). For CGI-I, the mean was 3.0 for the 200 mg/kg bid group and was 3.5 for placebo, with more than 20% of participants scoring a 2, "much improved," compared with less than 5% of those on placebo. Cohen *d* effect sizes were −0.645 for CGI-I, −0.247 for the RTT-DSC, and −0.487 for the RSBQ total.

Pharmacokinetics and pharmacodynamics

Trofinetide showed linear PKs across the dose range tested. These results are consistent with previous data in adult and adolescent patients (Rett-001). From a drug metabolism perspective, no accumulation, metabolic inhibition, or induction was observed during treatment. Geometric mean estimates for the predicted area under the concentration vs time curve (AUC_{0-12}, μg/mL·h) were 136.5 (50 mg/kg bid), 321.2 (100 mg/kg bid), and 530.6 (200 mg/kg bid). Geometric mean estimates of peak concentration (C_{max}, μg/mL) were 18.7 (50

Table 2 Incidence of treatment-emergent adverse events during the double-blind period (days 15–56, intention-to-treat population)

System organ class preferred term[a]	Placebo (n = 24)	50 mg/kg (n = 15)	100 mg/kg (n = 16)	200 mg/kg (n = 27)	Total (n = 82)
Reported at least 1 event	14 (58)	8 (53)	11 (69)	19 (70)	52 (63)
Gastrointestinal disorders					
Diarrhea	1 (4)	4 (27)	2 (13)	15 (56)	22 (27)
Vomiting	3 (13)	1 (7)	2 (13)	6 (22)	12 (15)
Constipation	0 (0)	0 (0)	0 (0)	2 (7)	2 (2)
General disorders and administration-site conditions					
Pyrexia	2 (8)	0 (0)	3 (19)	3 (11)	8 (10)
Infections and infestations					
Upper respiratory tract infection	3 (13)	1 (7)	0 (0)	5 (19)	9 (11)
Nasopharyngitis	2 (8)	1 (7)	0 (0)	1 (4)	4 (5)
Ear infection	2 (8)	0 (0)	0 (0)	1 (4)	3 (4)
Gastroenteritis	0 (0)	0 (0)	1 (6)	1 (4)	2 (2)
Pneumonia	1 (4)	0 (0)	0 (0)	1 (4)	2 (2)
Metabolism and nutrition disorders					
Decreased appetite	1 (4)	1 (7)	0 (0)	0 (0)	2 (2)
Nervous system disorders					
Somnolence	1 (4)	1 (7)	0 (0)	0 (0)	2 (2)
Seizure	1 (4)	0 (0)	0 (0)	0 (0)	1 (1)
Seizure cluster	0 (0)	0 (0)	0 (0)	1 (4)	1 (1)
Tonic convulsion	0 (0)	0 (0)	1 (6)	0 (0)	1 (1)
Psychiatric disorders					
Irritability	0 (0)	0 (0)	1 (6)	1 (4)	2 (2)
Respiratory, thoracic, and mediastinal disorders					
Cough	2 (8)	0 (0)	1 (6)	1 (4)	4 (5)
Sinus congestion	0 (0)	0 (0)	1 (6)	2 (7)	3 (4)
Rhinorrhea	1 (4)	1 (7)	0 (0)	0 (0)	2 (2)
Skin and subcutaneous tissue disorders					
Dermatitis diaper	0 (0)	1 (7)	0 (0)	1 (4)	2 (2)
Rash maculopapular	1 (4)	0 (0)	0 (0)	1 (4)	2 (2)

Data represent n (%) of participants.
[a] Events occurring in at least 2 participants. Seizure adverse events are an adverse event of interest in this population, so all seizure-related adverse events are listed.

mg/kg bid), 48.7 (100 mg/kg bid), and 83.7 (200 mg/kg bid). The geometric mean of the apparent terminal elimination half-life ($T_{1/2}$) varied from 5.3 to 6.1 hours across the 3 dosing groups.

As observed previously in adults and adolescent patients, body weight had a significant effect on clearance and volume of distribution and consequently on overall systemic exposure to trofinetide. Participants with lower body weight had lower overall systemic exposure to trofinetide, despite the use of a dosing regimen based on body weight (i.e., mg/kg). Differences in bioavailability were also observed between morning and evening doses, which may be explained by different factors, including circadian variation in absorption or metabolism or eventually food effect,[22] which also appears to occur in preclinical studies (unpublished data, BioAnalytical Systems, Inc, 2011).

Table 3 Change from treatment baseline (day 14) to end of treatment (day 54) in core efficacy outcomes (modified intention to treat)

Outcome measure	Prespecified covariates, $p \leq 0.1$[a]	Placebo (n = 24)	50 mg/kg (n = 15)	100 mg/kg (n = 16)	200 mg/kg (n = 27)
RSBQ total					
Treatment baseline, mean (SD)		39.5 (11.83)	44.7 (13.57)	40.3 (11.26)	42.2 (10.99)
Change D14–D54, LSmean (SE)	PR	−2.3 (1.54)	−3.0 (1.95)	−1.5 (1.96)	−6.7 (1.46)
***p* Value vs placebo**		NA	0.768	0.749	0.042
RTT-DSC total					
Exact median test[b]					
Treatment baseline, median		473.3	450.0	445.35	516.6
Change D14–D54, median	UnAdj	−25.85	−32.50	−12.10	−76.00
***p* Value vs placebo**		NA	0.999	0.748	0.025
CGI-I					
Treatment baseline		—[c]	—[c]	—[c]	—[c]
Change D14–D54, LSmean (SE)	UnAdj	3.5 (0.14)	3.3 (0.17)	3.4 (0.17)	3.0 (0.13)
***p* Value vs placebo**		NA	0.391	0.703	0.029
Top 3 caregiver concerns					
Treatment baseline, mean (SD)		223.87 (54.51)	237.69 (63.97)	211.55 (42.60)	245.90 (49.12)
Change D14–D54, LSmean (SE)	UnAdj	−12.52 (8.78)	−16.56 (11.10)	−2.09 (10.75)	−18.54 (8.28)
***p* Value vs placebo**		NA	0.776	0.455	0.619
MBA total					
Treatment baseline, mean (SD)		48.8 (7.99)	46.6 (8.77)	48.6 (8.82)	46.6 (13.10)
Change D14–D54, LSmean (SE)	TBL, PR	−2.6 (0.99)	−2.8 (1.25)	−2.4 (1.23)	−2.9 (0.94)
***p* Value vs placebo**		NA	0.872	0.925	0.840

Abbreviations: CGI-I = Clinical Global Impression Scale–Improvement; D = day; LSmean = least-squares mean; MBA = Motor Behavioral Assessment; NA = not applicable; PR = placebo response; RSBQ = Rett Syndrome Behaviour Questionnaire; RTT-DSC = Rett syndrome–Clinician Domain Specific Concerns; TBL = treatment baseline; UnAdj = unadjusted.
[a] Prespecified model covariates if $p \leq 0.1$: TBL and/or PR. UnAdj is noted if neither covariate was included in the final model. No multiplicity adjustments were performed.
[b] The distribution of data in the RTT-DSC was nonnormal. Consequently, group medians were used in the analysis of this end point and statistical significance was determined by the exact median test.
[c] CGI-I has no pretreatment baseline value. The CGI-I values at day 14 are ratings of change from day 0 (the pre-treatment baseline) to day 14 (end of placebo run-in). CGI-I assessments done after day 14 are referenced to participant's status at day 14. Mean of actual scores was assessed at day 54 (end of treatment).

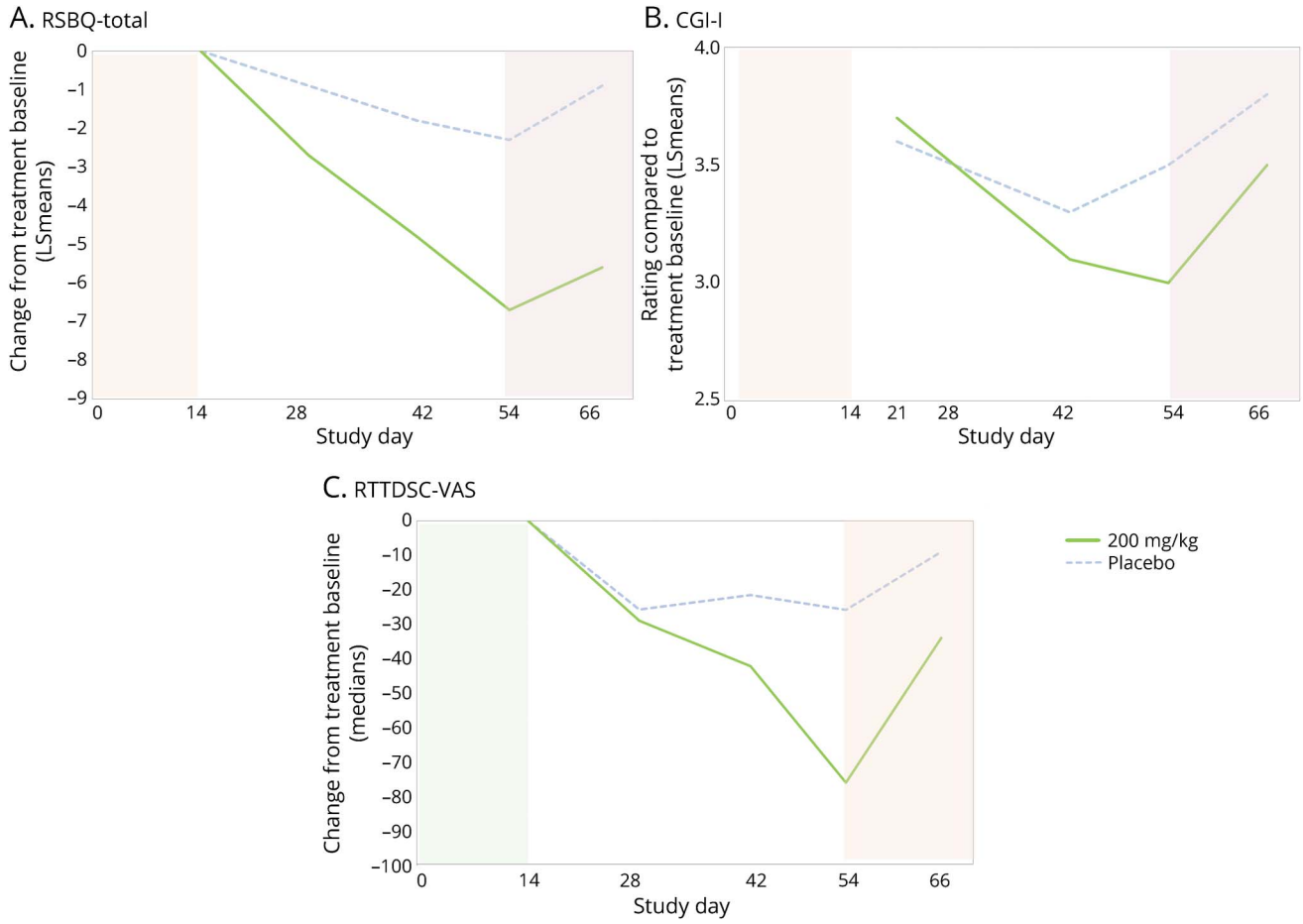
At the end of the dosing period, data indicate that clinical benefit was continuing to accrue, as the difference between the placebo and 200 mg/kg bid groups appeared to continue to increase (figure 2). Furthermore, there is evidence of a diminution of effect following cessation of dosing. Moreover, as shown in figure 3, an exploratory PKPD analysis suggests a correlation between trofinetide exposure (expressed as $AUC_{0–12}$ [dosing interval] and cumulative AUC [over 42 days]) and the magnitude of response on RSBQ, RTT-DSC, and CGI-I.

Additional prespecified analyses

Across the core efficacy measures, improvement was seen in clinically important symptom areas core to RTT: breathing problems, repetitive movements (including hand function), mood dysfunction (including nighttime behaviors), ambulation, and seizures. On the RSBQ, based on comparison with placebo and Cohen *d* effect sizes (figure 4A), all of the subscales except one were directionally in favor of the 200 mg/kg bid treatment group with notable improvement in mood dysfunction and disruptive behavior (General Mood subscale, $p = 0.007$), breathing problems (Breathing subscale $p = 0.095$), and repetitive movements (Repetitive Face Movement subscale, $p = 0.047$).

On RTT-DSC, improvements were notable in 2 domains, ambulation ($p = 0.040$) and seizures ($p = 0.057$), but were also observed to be directionally in favor of 200 mg/kg bid treatment for attentiveness and social interaction based on Cohen *d* effect sizes (figure 4B).

Figure 2 Core efficacy measures demonstrating improvement in the 200 mg/kg group compared to placebo



(A) Change from treatment baseline of the 200 mg/kg group compared to placebo in the mITT population for the RSBQ total score. EOT measured at day 54. Posttreatment follow-up at day 66. Improvement is a decrease in score. LSmeans: placebo response included as covariate in the model. (B) Absolute values of the 200 mg/kg group compared to placebo in the mITT population for the CGI-I. EOT measured at day 54. Posttreatment follow-up at day 66. Improvement is lower score. LSmeans. (C) Change from treatment baseline of the 200 mg/kg group compared to placebo in the mITT population for the RTT-DSC. EOT measured at day 54. Posttreatment follow-up at day 66. Improvement is a decrease in score. Exact median test. Treatment baseline = day 14. CGI-I = Clinical Global Impression Scale–Improvement; EOT = end of treatment; LSmean = least-squares mean; mITT = modified intention to treat; RSBQ = Rett Syndrome Behaviour Questionnaire; RTT-DSC = Rett syndrome–Clinician Domain Specific Concerns; VAS = Visual Analog Scale.

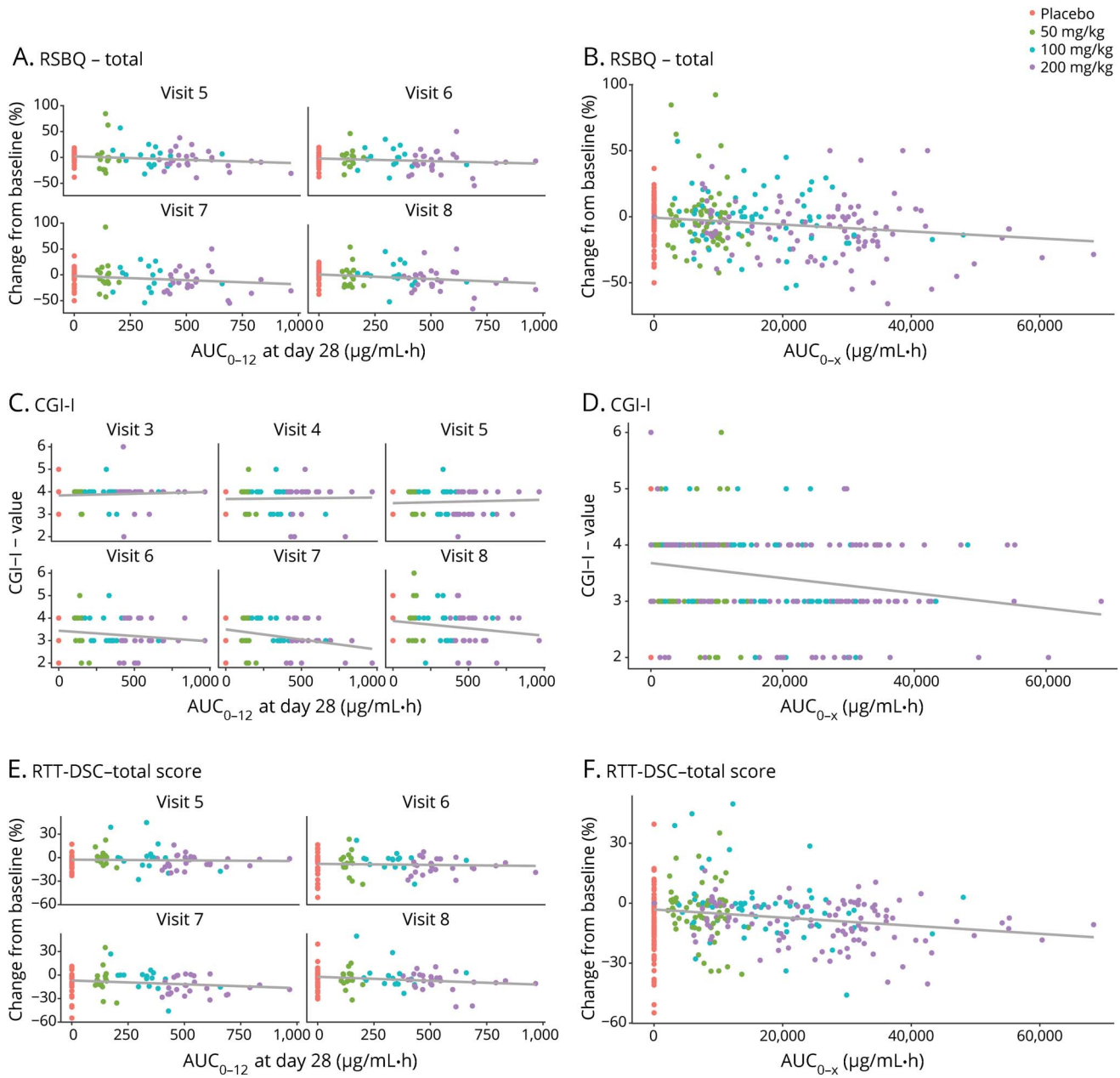
On RTT-DSC, concerns are identified on an individual, per-participant basis. The types of symptoms that improved for ambulation included being able to walk unassisted or improving on a certain motor milestone (e.g., walking a certain distance, walking on stairs, improved stability). For seizures, concerns included both frequency and severity but symptoms that improved appear to be primarily the frequency of seizures.

Discussion

This pediatric phase 2 study confirmed previous findings and expanded the safety, tolerability, and efficacy profile observed in the initial adolescent/adult phase 2 trofinetide trial in RTT.[21] All dose levels of trofinetide were generally safe and well-tolerated in individuals with RTT aged 5 to 15 years. No sentinel safety events occurred. Few SAEs and only one discontinuation because of AEs occurred, and no overt time-dependent issues related to tolerability were noted. Diarrhea occurred more frequently in the active treatment groups but did not affect overall tolerability. It is important to note that chronic constipation and corresponding pharmacologic treatment are highly prevalent in RTT, which may be a confounding factor. Objective assessments (e.g., laboratory assessments, vital signs) did not reveal any systematic pattern of clinical detriment.

Efficacy measures indicate clinically relevant improvements in a range of core symptoms of RTT at the highest dose of 200 mg/kg bid as shown by statistically significant improvement in 3 of the 5 core efficacy end points. The magnitude of this positive response was at a clinically meaningful level, as evidenced by the effect size of the differences in the RSBQ and RTT-DSC total and subscale scores and a face validity assessment of the range of symptoms. The findings on the 2 rating scales are supported by improvements on overall functioning as

Figure 3 Relationship between change from treatment baseline in RBSQ, CGI-I, and RTT-DSC and trofinetide exposure

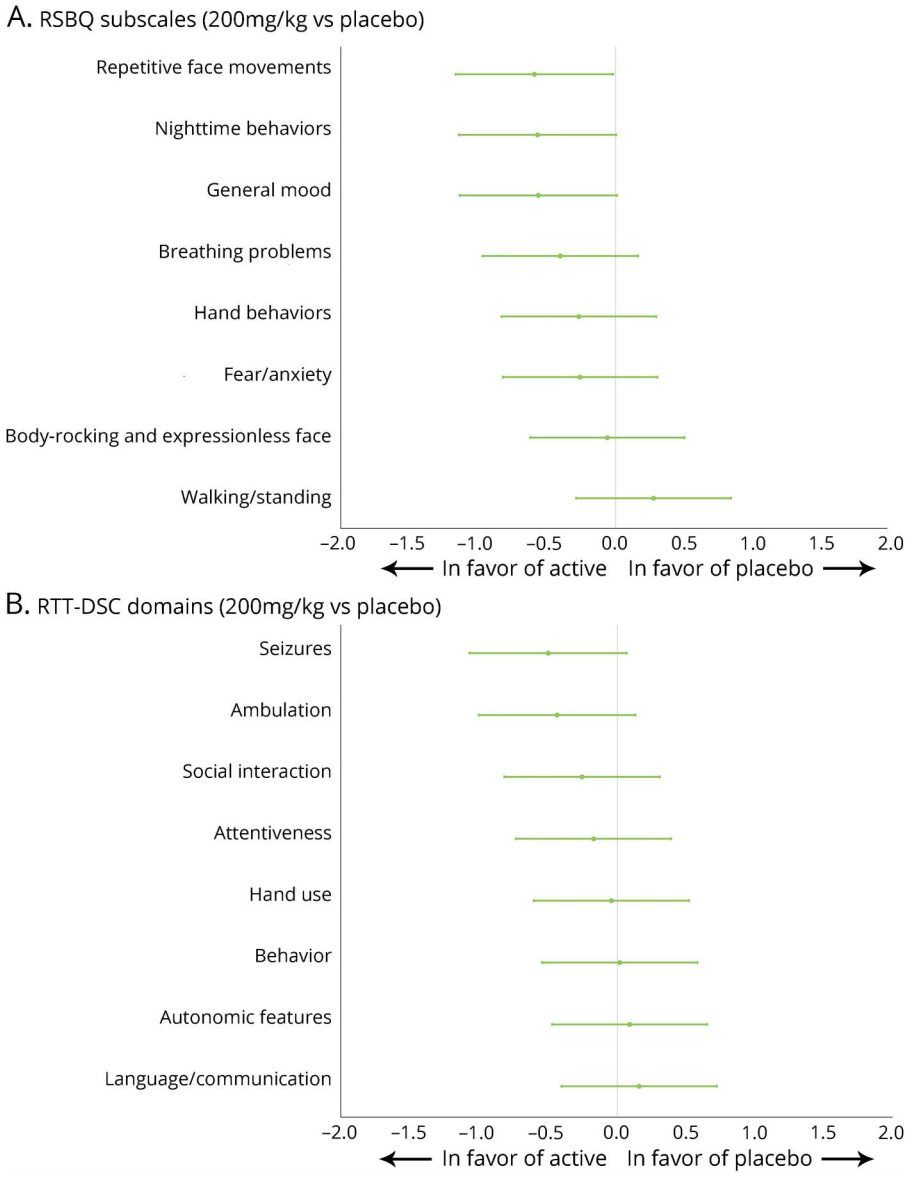


Relationship between change from treatment baseline in RBSQ, CGI-I, and RTT-DSC and AUC_{0-12} at different visits and over the active treatment period. (A) Relationship between percentage change from treatment baseline in RSBQ-total score and AUC_{0-12} at day 28. (B) Relationship between percentage change from treatment baseline in RSBQ-total score and cumulative AUC during the active dosing period. (C) Relationship between CGI-I scores and AUC_{0-12} at day 28. (D) Relationship between CGI-I score and cumulative AUC during the active dosing period. (E) Relationship between percentage change from treatment baseline in RTT-DSC score and AUC_{0-12} at day 28. (F) Relationship between percentage change from treatment baseline in RTT-DSC score and cumulative AUC during the active dosing period. Visit 3 (day 14, end of placebo run-in), visit 4 (day 21), visit 5 (day 28), visit 6 (day 42), visit 7 (day 54, end of treatment), visit 8 (day 66, posttreatment). Solid lines are obtained by linear regression. Where applicable, placebo data (AUC = 0) from different visits are pooled together. AUC = area under the concentration vs time curve; CGI-I = Clinical Global Impression Scale–Improvement; RSBQ = Rett Syndrome Behaviour Questionnaire; RTT-DSC = Rett syndrome–Clinician Domain Specific Concerns.

measured by the CGI-I. Both the CGI-I and RSBQ total had medium effect sizes, and 4 of the RSBQ subscales and 2 of the RTT-DSC subscales showed Cohen *d* effect sizes ≥0.3 (small to medium). Effect sizes of this range are reported in trials of other CNS disorders using behavioral-based rating scales and are comparable to those reported for Food and Drug Administration–approved treatments for other disorders, such as major depressive disorder (mean 0.3, range 0.17–0.42).[34]

Of note, the RSBQ and RTT-DSC data suggest improvements across a range of symptom domains and individual symptoms, which included repetitive behaviors, breathing problems, mood abnormalities/disruptive behavior, ambulation

Figure 4 Cohen *d* effect sizes for the RSBQ and RTT-DSC-VAS (200 mg/kg vs placebo at day 54)



Cohen *d* effect sizes for the RSBQ (A) and RTT-DSC-VAS (B). Cohen *d* values are unadjusted, that is, do not incorporate covariate information from treatment baseline or placebo response. bid = twice a day; RSBQ = Rett Syndrome Behaviour Questionnaire; RTT-DSC = Rett syndrome–Clinician Domain Specific Concerns; VAS = Visual Analog Scale.

impairment, and seizures. This multiple-domain effect is consistent with the adolescent/adult Rett-001 trial,[21] which showed improvement in measures covering a wide range of manifestations of the disease (e.g., MBA, CGI-I), and consistent with the generalized mechanisms of action of trofinetide.[17,35] As in the present study, in Rett-001 apparent clinical benefits were also exhibited in both clinician and caregiver assessments and in a number of core RTT symptoms.[21] In addition, as in the present study, the clinical improvement was still increasing at the end of treatment and declined after cessation of treatment. The relative consistency of effects (albeit captured by a partially different set of measures and in an older population with shorter exposure to trofinetide) gives additional confidence that the results of the previous study were not simply attributable to chance.

There are no validated biomarkers available to assess drug-induced physiologic, cognitive, and behavioral effects, which can ultimately be linked to clinical improvement and efficacy. Such a limitation may be partly overcome by the evaluation of exposure-response relationships.[36,37] In this pediatric study, despite the variability of the relatively small cohort, a correlation was found between drug exposure and magnitude of changes in response to treatment. While an integrated analysis of all available data needs to be performed (including an evaluation of the response over a longer treatment period to establish the dose-exposure-response relationship), the efficacy data from this study suggest that the group separation during the dosing period was due to beneficial treatment effect and not to other differences between the 2 arms that occurred simply by chance. Taken together with the observation of sustained improvement at the end of treatment, and

the diminution of effect post treatment, these observations also suggest that clinical benefit is likely to increase further with a longer treatment period than was studied in this trial. Overall, the observed clinical improvement in the present pediatric trial was more manifest than in the previous trial, with younger age (i.e., greater neuroplasticity), higher doses (i.e., higher drug exposure), and longer drug treatment duration (i.e., 28 days in Rett-001 vs 42 days in Rett-002) as potential contributors.

The PK findings replicate findings from the adult trial showing that weight is an influential moderator of systemic exposure, a feature that is common to many drugs used in children.[38,39] While mg/kg dosing was used, dose levels were not banded by weight in this protocol, as such systemic exposure was not comparable across all participants receiving a given dose of trofinetide. Consequently, dose per se was not as important as drug exposure as a determinant factor for the magnitude of the response to trofinetide. In addition, as exposure will vary not only with the dose, but also with body weight, identifying dosing regimens that provide optimal exposure will likely reduce variability in response. Therefore, weight-banded dosing or similar algorithms should be considered in future trials to adjust dosing in this population.[39,40]

For this phase 2 study in a pediatric population, the RSBQ was added to the assessment battery. The RSBQ is the most widely used behavioral instrument in RTT, in part because of its disorder-specificity and its reliability and validity in particular for the RTT pediatric population.[12–14,32] More recently, the RSBQ has also shown sensitivity to interventions and correlations with functioning and quality of life in RTT.[14,28,31] The RSBQ could more properly be labeled as a "neurobehavioral" measure since it includes RTT features that are modulated rather than triggered by behavior (e.g., breathing problems). Thus, the RSBQ is an instrument suitable for assessing multiple core RTT features, similar to the MBA. The RSBQ subscale analyses in this study also suggest that, despite its generalized effect, trofinetide may have greater effects on some neurobehavioral symptoms (e.g., mood abnormalities/disruptive behavior). Future studies aiming at replicating the results of this pediatric trial would benefit from including the RSBQ as a primary end point and should also consider evaluating RSBQ subscales such as the General Mood.

While supportive of trofinetide's potential as a treatment for multiple RTT core symptoms, the present study had several limitations. As a phase 2 study, the 2 most obvious are small cohort size and short duration. In addition, no biomarker or clinical characteristic allowed identification of potential responders to trofinetide. Also, as noted above, since weight was a factor moderating exposure, some participants may not have experienced the maximal potential exposure for the dose level they received. Lastly, as this was an exploratory study, we have deemed it appropriate not to include adjustment for multiplicity for the statistical comparisons. Consideration of the above-mentioned factors and the known shortcomings of available outcome measures for RTT[29,41] in the design of future trials will be important for their success. RTT is a debilitating and life-threatening neurodevelopmental disorder for which no therapies are available that address its core features. The results presented here provide evidence that trofinetide is a potentially viable treatment for the core signs and symptoms of RTT and support further trials in this population.

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Disclosure

D. Glaze was an investigator in the Neuren trofinetide trial reported in this article and the Rett-001 trial of trofinetide in adolescents and adults. He is a consultant to and participates in trials sponsored by Newron Pharmaceuticals SpA. J. Neul was an investigator in the Neuren trofinetide trial reported in this article and the Rett-001 trial of trofinetide in adolescents and adults. He is a consultant to Ovid, AveXis, Eloxx Pharmaceuticals, Biohaven, Teva, and Takeda Pharmaceuticals. He participates in clinical trials sponsored by Newron Pharmaceuticals SpA. W. Kaufmann was part of the study personnel for the Neuren trofinetide trial reported in this article. He is a consultant to Anavex, AveXis, Biohaven, Cydan, Echo, Edison, EryDel, GW Pharmaceuticals, Marinus, Newron Pharmaceuticals SpA, Ovid Therapeutics, Stalicla, and Zynerba. He has been a consultant and participating principal investigator (PI) in sponsor-led trials for Roche, Novartis, and Seaside Therapeutics. Walter Kaufmann was also PI of 2 previous IGF1 (mecasermin) trials in Rett syndrome. He also received research support from Ipsen Pharmaceuticals and Eloxx Pharmaceuticals. E. Berry-Kravis was an investigator in the Neuren trofinetide trial reported in this article and a PI on a Neuren-sponsored trial in fragile X. She is a consultant to Cydan, Fulcrum, GW, Marinus, BioMarin, Zynerba, Yamo, and Neurotrope Pharmaceuticals. She is a consultant and has been a participating PI in sponsor-led trials for Ovid Therapeutics, Alcobra, Roche, Novartis, and Seaside Therapeutics. She receives research support and has been a co-PI of a registration trial for VTS-270 in Niemann-Pick type C 1 for Vtesse/Sucampo/Mallinckrodt. She receives research support from Asuragen Inc. for work on validation of FXS testing methods. S. Condon is a biostatistician for Vital Systems, Inc., a contract research organization utilized by Neuren for the trial described in this article. G. Stoms is president of Vital Systems, Inc., a contract research organization utilized by Neuren for the conduct and analysis of this clinical trial, the Rett-001 trial of trofinetide in adolescents and adults, and trials in fragile X and traumatic brain injury sponsored by Neuren. S. Oosterholt is a clinical pharmacologist at UCL

supporting PKPD data analysis for this trial, the Rett-001 trial of trofinetide in adolescents and adults, and trials in fragile X and traumatic brain injury, sponsored by Neuren Pharmaceuticals Ltd. O. Della Pasqua is chair of Clinical Pharmacology & Therapeutics Group at the University College London. He is also senior director of Clinical Pharmacology Life Cycle Management at GlaxoSmithKline R&D, which was not involved with the study. L. Glass is an executive of Neuren Pharmaceuticals. N. Jones is an executive of Neuren Pharmaceuticals. A. Percy was an investigator in the Neuren trofinetide clinical trial reported in this article and Rett-001 trial of trofinetide in adolescents and adults. He is a consultant to Anavex, AveXis, and Teva and participates in trials sponsored by Newron Pharmaceuticals SpA. Go to Neurology.org/N for full disclosures.

Publication history

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Appendix 1 Authors

Name	Location	Role	Contribution
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Jeffrey L. Neul, MD, PhD	University of California, San Diego, Department of Neurosciences. Current affiliation: Vanderbilt University Medical Center, Vanderbilt Kennedy Center	Author	Assisted with the conceptualization of the study, enrolled participants and collected study data, and critically reviewed and revised the manuscript.
Walter E. Kaufmann, MD	Greenwood Genetic Center, Center for Translational Research	Author	Assisted with the design of the study and interpretation of outcome measures, interpreted the data and critically reviewed and revised the manuscript.
Elizabeth Berry-Kravis, MD, PhD	Rush University Medical Center, Pediatrics, Neurological Sciences, and Biochemistry	Author	Enrolled participants and collected study data, interpreted the data, and critically reviewed and revised the manuscript.
Sean Condon, DPH	Vital Systems, Inc.	Author	Provided statistical advice, conducted the analysis of the safety and efficacy data, and reviewed the manuscript.
George Stoms, BS	Vital Systems, Inc.	Author	Provided statistical advice, conducted the analysis of the safety and efficacy data, and reviewed the manuscript.
Sean Oosterholt, MSc	Clinical Pharmacology & Therapeutics Group, University College London	Author	Conducted the pharmacokinetic (PK) and pharmacokinetic-pharmacodynamic (PKPD) analysis, interpreted the PK and PKPD data, and reviewed the manuscript.
Oscar Della Pasqua, MD, PhD	Clinical Pharmacology & Therapeutics Group, University College London	Author	Conducted the pharmacokinetic (PK) and pharmacokinetic-pharmacodynamic (PKPD) analysis, interpreted the PK and PKPD data, and reviewed the manuscript.
Larry Glass, BA	Neuren Pharmaceuticals, Ltd.	Author	Participated in the conceptualization and design of the study, participated in the analysis, interpreted the data, and critically reviewed and revised the manuscript.
Nancy E. Jones, PhD	Neuren Pharmaceuticals, Ltd.	Author	Participated in the conceptualization and design of the study, assisted with the coordination and implementation of the study, participated in the analysis, interpreted the data, and drafted the manuscript. N.J. takes responsibility for the article as a whole.
Alan Percy, MD	University of Alabama at Birmingham, Department of Pediatrics, Division of Neurology	Author	Alan Percy assisted with the conceptualization, design, and implementation of the study, enrolled participants and collected study data, interpreted the data, and critically reviewed and revised the manuscript.

Appendix 2 Coinvestigators

Name	Location	Role	Contribution
Arthur Beisang, MD	Gillette Children's Specialty Healthcare	Site investigator	Enrolled participants and collected study data. Provided advice on assessments.
Timothy Benke, MD, PhD	Children's Hospital Colorado	Site investigator	Enrolled participants and collected study data. Provided advice on assessments.

Appendix 2 *(continued)*

Name	Location	Role	Contribution
Timothy Feyma, MD	Gillette Children's Specialty Healthcare	Site investigator	Enrolled participants and collected study data.
Peter Heydemann, MD	Rush University Medical Center	Site investigator	Enrolled participants and collected study data.
Mary Jones, MD	UCSF Benioff Children's Hospital Oakland	Site investigator	Enrolled participants and collected study data.
Eric D. Marsh, MD, PhD	Children's Hospital of Philadelphia	Site investigator	Enrolled participants and collected study data.
Sarika Peters, PhD	Vanderbilt University Medical Center	Site investigator	Enrolled participants and collected study data.
Myron "Skip" Peterson, MD, PhD	Cato Research	Medical monitor	Monitored and reviewed safety data.
Mustafa Sahin, MD, PhD	Boston Children's Hospital	Site investigator	Enrolled participants and collected study data.
Steve Skinner, MD	Greenwood Genetic Center	Site investigator	Enrolled participants and collected study data.
Shannon Standridge, DO, MPH	Department of Pediatrics, University of Cincinnati College of Medicine, OH; Division of Neurology, Cincinnati Children's Hospital Medical Center, OH	Site investigator	Enrolled participants and collected study data.

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