CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217026Orig1s000

STATISTICAL REVIEW(S)

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION – ERRATA

CLINICAL STUDIES

NDA #:	NDA 217026
Supplement #:	N/A
Drug Name:	Trofinetide (ACP-2566)
Indication(s):	Treatment of Rett syndrome (in adults and pediatric patients 2 years of age and older)
Applicant:	Acadia Pharmaceuticals, Inc.
Date(s):	Submission date: 7/12/2022, PDUFA date: 3/12/2023
Review Priority:	Priority
Biometrics Division:	Division of Biometrics I
Statistical Reviewer:	Andrew N. Potter, PhD
Concurring Reviewers:	James Hung, PhD
Medical Division:	Division of Neurology I
Clinical Team:	Michael Dimyan, MD
Project Manager:	Brenda Reggettz, PharmD

Errata for the original Statistics Review of 02/04/2023.

This Review contains corrections to several typos in the original statistics review dated 02/04/2023.

In Section 3.2.2, the description of the stratification factors for Study 003 is missing age group (5-10 years old, 11-15 years old, and 16-20 years old).

Table 3 should have the final two rows deleted:
Table 1: Patient Disposition / Primary Analysis (ACP-2566-003)

Subject Disposition - Randomized Analysis Set

	Placebo N=94	**Trofinetide N=93**	**Total N=187**
Completion Status, n(%)			
Completed the Study	85 (90.4)	70 (75.3)	155 (82.9)
Early Termination	9 (9.6)	23 (24.7)	32 (17.1)
Reason for Early Termination, n(%)			
Adverse Event	2 (2.1)	16 (17.2)	18 (9.6)
Lack of Efficacy	0	1 (1.1)	1 (<1)
Non-Compliance with Study Drug	0	4 (4.3)	4 (2.1)
Protocol Deviation	1 (1.1)	0	1 (<1)
Subject Withdrew Consent	1 (1.1)	1 (1.1)	2 (1.1)
Other	5 (5.3)	1 (1.1)	6 (3.2)
~~Marked as Dropout but Had a Week 12 Visit, n(%)~~			
~~Completed the Study~~	~~93 (98.9)~~	~~86 (92.5)~~	~~179 (95.7)~~
~~Early Termination with Week 12 Visit~~	~~1 (1.1)~~	~~7 (7.5)~~	~~8 (4.3)~~

The Reviewer's Note above Table 4 should state eight patients instead of seven.

On page 10, the sentence beginning "In the trofinetide arm, there were only three subjects;" should read "In the **placebo** arm, there were only three subjects[.]"

On page 26, the "6.7 point greater improvement" should read "4.7 point greater improvement."

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/s/

ANDREW N POTTER
03/10/2023 11:21:16 AM

HSIEN MING J HUNG
03/10/2023 11:25:16 AM

U.S. Department of Health and Human Services
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Keywords: mixed models**,** sensitivity analyses, data imputation (except LOCF), multiple comparisons

Reference ID: 5119810

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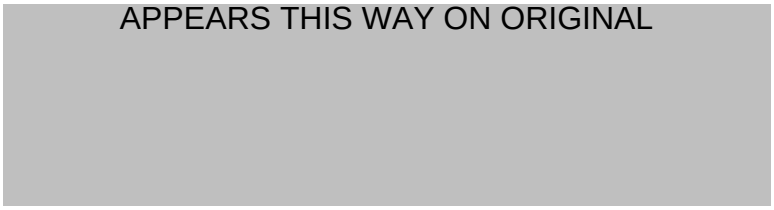

APPEARS THIS WAY ON ORIGINAL

1 EXECUTIVE SUMMARY

Acadia Pharmaceuticals, Inc., submitted two studies (Neu-2566-rett-002 and ACP-2566-003) in support of an original NDA 217026 for use of trofinetide for the treatment of Rett Syndrome. Rett Syndrome is a rare genetic disorder that affects brain development in girls. Study ACP-2566-003 studied the effect of a weight-based dosing of trofinetide on Rett Syndrome as measured by the Rett Syndrome Behavior Questionnaire and Clinical Global Impression - Improvement. In this study, there was statistical evidence that trofinetide improved both endpoints. Study 002 was an early phase, dose-finding study that explored multiple fixed doses of trofinetide and multiple endpoints. This study provided supportive evidence that trofinetide may have an effect on several endpoints at the highest dose. In summary, the statistical evidence supports approval of trofinetide.

2 INTRODUCTION

2.1 Overview

The Applicant submits Neu-2566-rett-002 and ACP-2566-003 studies to support the efficacy of treatment with oral trofinetide versus placebo in girls and women with Rett Syndrome (RTT). The original protocols were reviewed under IND114319.

Table 1: Summary of Trials to be Assessed in the Statistical Review

Trial ID	Design	Treatment/ Sample Size	Endpoint/Analysis	Preliminary Findings
Neu-2566-rett-002	Phase 2, randomized, double-blind, placebo-controlled, dose-ranging study of the safety and tolerability of oral trofinetide in pediatric and adolescent females with RTT. No control for multiplicity.	A fixed dose of trofinetide either 50mg/kg BID, 100mg/kg BID or 200mg/kg BID / N = 92 would enable randomization of approximately 16 per group to the 50mg/kg and 100mg/kg groups and up to 30 per group for the 200mg/kg and placebo group.	MBA Total score, CGI-I, RSBQ Total score, RTTDSC Total score, and Caregiver Top 3 Concerns Total score / Data derived ANCOVA.	Trofinetide at the 200mg/kg BID dose level demonstrated improvement compared to placebo (p-value <0.05) in 3 of the 5 pre-specified core variables, from 3 different efficacy domains: Mean decrease from treatment baseline to Day 54 in RSBQ total score (Caregiver completed syndrome specific measure) (p=0.042). Mean score (representing change from treatment baseline to Day 54) on CGI-I (Clinician completed syndrome specific global measure) (p=0.029). Median decrease from treatment baseline to Day 54 in RTT-DSC total score (Clinician completed syndrome specific measure) (p=0.025).
ACP-2566-003	Phase3, randomized, double-blind, placebo-controlled, parallel-	Weight-based doses on trofinetide / Sample size of 174 subjects was to provide at least 95% power at a 2-sided	Primary: 1) Rett Syndrome Behavior Questionnaire (RSBQ) total score – change from	Primary: The change from Baseline to Week 12 in the RSBQ total score and CGI-I was statistically significantly greater in the trofinetide

	group study of trofinetide for the treatment of girls and women with Rett syndrome.	significance level of 0.05 for each individual hypothesis test within the family.	baseline to week 12. 2) Clinical Global Impression – Improvement (CGI-I) score at week 12. / Direct likelihood mixed-effect model for repeated measures (MMRM) method. Secondary: Change from Baseline to Week 12 in Communication and Symbolic Behavior Scales Developmental Profile™ Infant Toddler (CSBS-DP-IT) Checklist – Social Composite Score. / MMRM method.	group compared with the placebo group. RSBQ - MMRM LSM diff: -3.1; p=0.0175. CGI-I – MMRM LSM diff: -0.3; p=0.0030. Secondary: The change from Baseline to Week 12 in the CSBS-DP-IT was statistically significantly greater in the trofinetide group compared with the placebo group. MMRM LSM diff: 1.0; p=0.0064.

The Applicant submitted two studies supporting this NDA in a rare disease. Study ACP-2566-003 (referred to as Study 003) had a positive finding supporting the efficacy of trofinetide for the treatment of Rett syndrome. Study Neu-2566-rett-002 (referred to as Study 002) was an exploratory study indicating a potential for efficacy in the 200 mg/kg BID dose of trofinetide for the treatment of Rett syndrome.

This review focuses on studies 002 and 003 to determine if the Applicant submitted sufficient evidence of efficacy.

2.2 Data Sources

The following data sources were considered in this review:
Study Neu-2566-rett-002: adsl, admba, addsc, adcgi, adcttc, adrb
Study ACP-2566-003: adsl, adqrsbq, adqcgi

The electronic location of the submission is: \\CDSESUB1\evsprod\NDA217026\0001.

3 STATISTICAL EVALUATION

3.1 Data and Analysis Quality

The Applicant submitted all necessary analysis datasets and SAS programs. This reviewer found the datasets acceptable.

3.2 Evaluation of Efficacy

3.2.1 Statistical Review Issues

- Does the higher dropout rate in the trofinetide arm observed in study 003 have an impact on the efficacy signal in study 003?
- Does the higher use of loperamide or the greater incidence of diarrhea in the trofinetide arm impact the efficacy signal in study 003?

3.2.2 Study ACP-2566-003

Study 003 was a phase 3, double-blind, two arm, parallel group, placebo-controlled study comparing weight-based dosing of trofinetide to placebo. The weight-based dosing ranged from 6 mg BID to 12 mg BID, see Table 2. Subject were randomized at a 1:1 ratio to trofinetide or placebo. Randomization was stratified by baseline RSBQ severity (<35 total score vs ≥ 35 total score).

Table 2: Weight-Based Banded Dosing

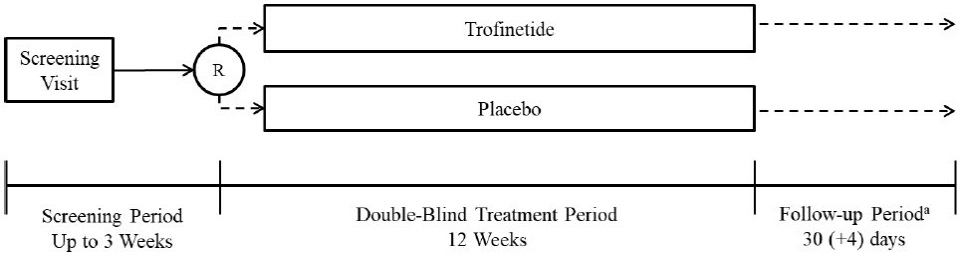
Body weight at Baseline	Dose	Total daily dose
12-20 kg	30 mL (6 g) BID	60 mL (12 g)
>20-35 kg	40 mL (8 g) BID	80 mL (16 g)
>35-50 kg	50 mL (10 g) BID	100 mL (20 g)
>50 kg	60 mL (12 g) BID	120 mL (24 g)

Abbreviations: BID=twice daily

Source: Applicant's Clinical Study Report for ACP-2566-003.

Study 003 followed subjects for 12 weeks of double-blind therapy, see Figure 1 for study schematic. The co-primary endpoints consisted of change from baseline to Week 12 (CFB12) in Rett Syndrome Behavior Questionnaire (RSBQ) and Clinical Global Impression – Improvement (CGI-I) at Week 12. RSBQ was observed at baseline, Week 2, Week 6, and Week 12. CGI-I was observed at weeks 2, 6, and 12 with baseline measurement of Clinical Global Impression – Severity (CGI-S). A key endpoint of CFB12 in CSBS-DP-IT Social Composite Score was collected at baseline, and week 2, 6, and 12.

Figure 1: Study Schematic for ACP-2566-003



Abbreviations: OLE=open-label extension; R=randomization

[a] If the subject continued into the OLE from the current study, she did not complete the Follow-up visit and rolled over into the OLE.

Source: Applicant's Statistical Analysis Plan for ACP-2566-003.

The Applicant planned to enroll 184 subject to provide at least 90% power to detect on both co-primary an assumed treatment effect (trofinetide minus placebo) for CFB12 in RSBQ of -4.4

points with a standard deviation (SD) of 8 and a treatment effect of -0.5 with an SD of 0.7 with an assumed dropout rate of 5% and a type I error rate of 0.05. For each endpoint, Study 003 had at least 95% power to detect the assumed treatment effects with no dropouts.

The primary endpoints were analyzed using a mixed effects model for repeated measured (MMRM) with fixed effects of treatment group, baseline RSBQ severity (<35 total score vs ≥ 35 total score), visit, baseline value (RSBQ for RSBQ endpoint and CGI-S for the CGI-I endpoint), age group (5-10 years old, 11-15 years old, and 16-20 years old), treatment by visit interaction terms, and baseline value by visit interaction terms with an unstructured covariance matrix. Treatment effects and standards errors are estimated with least square means (LS mean).

The Applicant controlled for multiple comparisons by requiring both co-primary endpoints to have a p-value < 0.05 to declare success in the study. Also, the key secondary endpoint was only tested if both co-primary endpoints were significant at alpha = 0.05.

The Applicant explored the impact of violations of the missing at random assumption for the missing data using a reference-based imputation model where missing data from the trofinetide arm was imputed using an imputation model built from the placebo completer data.

Reviewer's Note: The design and statistical analysis plan of Study 003 is adequate to assess the efficacy of trofinetide. The Applicant included both RSBQ score at baseline and RSBQ severity strata at baseline in the model analysis model because of the randomization plan. Having both terms in the model may be redundant

3.2.2.1 Protocol Amendments

In amendment 2 to the SAP, the Applicant removed the tipping point sensitivity analyses. In addition, a sensitivity analysis exploring the impact of COVID-19 was added.

3.2.2.2 Patient Disposition, Demographic and Baseline Characteristics

In Study 003, 187 randomized subjects in the intent-to-treat (ITT) population were randomized with 94 subjects in the placebo arm and 93 patients in the trofinetide arm. A differential dropout rate was observed with 24.7% of subjects dropping out of the trofinetide arm compared to 9.6% in the placebo arm. However, 7 subjects in the trofinetide arm who were marked as dropouts by the Applicant had a Week 12 RSBQ and CGI-I score recorded. These subjects were included in the primary analysis. Additional details of patient disposition are presented in Table 3.

Table 3: Patient Disposition / Primary Analysis (ACP-2566-003)

Subject Disposition - Randomized Analysis Set

	Placebo N=94	**Trofinetide N=93**	**Total N=187**
Completion Status, n(%)			
Completed the Study	85 (90.4)	70 (75.3)	155 (82.9)
Early Termination	9 (9.6)	23 (24.7)	32 (17.1)
Reason for Early Termination, n(%)			
Adverse Event	2 (2.1)	16 (17.2)	18 (9.6)
Lack of Efficacy	0	1 (1.1)	1 (<1)

Non-Compliance with Study Drug	0	4 (4.3)	4 (2.1)
Protocol Deviation	1 (1.1)	0	1 (<1)
Subject Withdrew Consent	1 (1.1)	1 (1.1)	2 (1.1)
Other	5 (5.3)	1 (1.1)	6 (3.2)
Marked as Dropout but Had a Week 12 Visit, n(%)			
Completed the Study	93 (98.9)	86 (92.5)	179 (95.7)
Early Termination with Week 12 Visit	1 (1.1)	7 (7.5)	8 (4.3)

Subjects Included in Various Analysis Sets

	Placebo N=94	**Trofinetide N=93**	**Total N=187**
Randomized Analysis Set [1], n(%)			
Yes	94 (100.0)	93 (100.0)	187 (100.0)
No	0	0	0
Safety Analysis Set [2], n(%)			
Yes	94 (100.0)	93 (100.0)	187 (100.0)
No	0	0	0
Full Analysis Set [3], n(%)			
Yes	93 (98.9)	91 (97.8)	184 (98.4)
No	1 (1.1)	2 (2.2)	3 (1.6)
Per-protocol Analysis Set [4], n(%)			
Yes	90 (95.7)	89 (95.7)	179 (95.7)
No	4 (4.3)	4 (4.3)	8 (4.3)

[1] The Randomized Analysis Set consisted of all subjects who were randomized.
[2] The Safety Analysis Set consisted of all randomized subjects who received at least one dose of study drug.
[3] Full Analysis Set consists of subjects who were randomized, received at least one dose of study drug, and had both a Baseline value and at least one postbaseline value for the RSBQ total score or had at least one CGI-I score after taking study medication.
[4] The Per-protocol Analysis Set consisted of all subjects in the Full Analysis Set who did not have a major protocol violation that would affect interpretation of the efficacy data and who had adequate treatment compliance (greater or equal to 75%).
Source: Statistical Analyst; adsl.xpt.

In study 003, the majority of subjects dropped after Week 6, see Table 4. Between weeks 2 and 6, 7 subjects dropped out of the trofinetide arm compared to 2 in the placebo arm. Between weeks 6 and 12, an additional 7 subjects dropped out in the trofinetide, and 7 subjects dropped out of the placebo arm.

Reviewer's Note: The number of subjects who were determined to be dropouts by the Applicant was caused by 7 subjects both dropping out and providing RSBQ and CGI-I data at the Week 12 visit. These seven subjects were included in the primary analysis.

Table 4: Number of Subjects per Study Visit (based on RSBQ)

	Placebo	**Trofinetide**
BASELINE	93	91
WEEK 2	90	90
WEEK 6	92	83
WEEK 12	85	76

Source: Statistical Analyst; adrsbq.xpt, adsl.xpt.

Study 003 was conducted in female subjects aged 5 to 20 years, see Table 5. These subjects

were predominately white (95.7%) and not Hispanic or Latino (10.6%).

Table 5: Demographics - Randomized Analysis Set

	Placebo N=94	Trofinetide N=93	Total N=187
Sex, n (%)			
Female	94 (100.0)	93 (100.0)	187 (100.0)
Age (years)			
Mean (SE)	10.9 (0.47)	11.0 (0.49)	10.9 (0.34)
SD	4.57	4.69	4.62
Median	10.0	10.0	10.0
Min, Max	5, 20	5, 20	5, 20
Age categories, n (%)			
5 to <12 Years	55 (58.5)	53 (57.0)	108 (57.8)
12 to <17 Years	24 (25.5)	23 (24.7)	47 (25.1)
≥ 17 Years	15 (16.0)	17 (18.3)	32 (17.1)
Primary race, n (%)			
White	90 (95.7)	82 (88.2)	172 (92.0)
Black or African American	1 (1.1)	1 (1.1)	2 (1.1)
Asian	1 (1.1)	5 (5.4)	6 (3.2)
Native Hawaiian or Other Pacific Islander	0	1 (1.1)	1 (<1)
Other	2 (2.1)	4 (4.3)	6 (3.2)
Race Group			
Non-White	4 (4.3)	11 (11.8)	15 (8.0)
White	90 (95.7)	82 (88.2)	172 (92.0)
Ethnicity, n (%)			
Hispanic or Latino	10 (10.6)	7 (7.5)	17 (9.1)
Not Hispanic or Latino	84 (89.4)	86 (92.5)	170 (90.9)
Height (cm)			
Mean (SE)	127.6 (1.67)	128.4 (1.70)	128.0 (1.19)
SD	15.97	16.28	16.09
Median	127.5	128.1	127.6
Min, Max	92, 171	94, 170	92, 171
Missing	2	1	3
Weight (kg)			
Mean (SE)	29.2 (1.07)	30.5 (1.31)	29.9 (0.84)
SD	10.37	12.61	11.53
Median	27.3	29.6	28.1
Min, Max	13, 57	13, 78	13, 78
BMI ($kg/m^{2)}$			
Mean (SE)	17.2 (0.36)	17.7 (0.46)	17.4 (0.29)
SD	3.46	4.43	3.97
Median	16.6	16.8	16.7
Min, Max	12, 28	10, 34	10, 34
Missing	3	1	4
Is the subject of childbearing potential?, n (%)			
Yes	35 (37.2)	34 (36.6)	69 (36.9)

No	59 (62.8)	59 (63.4)	118 (63.1)
Has the subject reached menarche?, n (%)			
Yes	35 (37.2)	33 (35.5)	68 (36.4)
No	59 (62.8)	60 (64.5)	119 (63.6)

Abbreviations: BMI=body mass index; max=maximum; min=minimum; SD=standard deviation; SE=standard error
Source: Statistical Analyst; adrsbq.xpt, adsl.xpt.

At baseline, both the trofinetide and placebo had similar disease severity (derived RSBQ scores equal to approximately 44 and CGI-S score equal to 4.9), see Table 6.

For one of the two co-primary efficacy endpoints (change from baseline to week 12 (CFB12) in RSBQ total score), the trofinetide arm showed evidence of efficacy with p = 0.0157, see Table 6. For other of the two co-primary efficacy endpoints (CGI-I at Week 12), the trofinetide arm showed evidence of efficacy with p = 0.0018, see Table 7. The CFB12 in RSBQ had a placebo subtracted treatment difference (ΔΔRSBQ) of -3.2 and CGI-I at Week 12 had a difference of -0.3 point. P-values were compared to threshold of 0.05. Throughout this Section, negative change indicates improvement.

Table 6: Primary Analysis of RSBQ Total Score and Change from Baseline by Visit (MMRM) - Full Analysis Set

	Placebo	Trofinetide
Baseline		
n	93	91
Mean (SE)	44.5 (1.26)	43.7 (1.21)
SD	12.20	11.52
Median	43.0	42.0
Min, max	14, 69	21, 74
Week 12		
n	85	76
Mean (SE)	42.8 (1.42)	39.9 (1.38)
SD	13.05	12.02
Median	41.0	40.5
Min, max	16, 69	9, 69
Change from Baseline to Week 12		
n	85	76
Mean (SE)	-1.7 (0.98)	-5.1 (0.99)
SD	9.05	8.67
Median	-2.0	-3.5
Min, max	-31, 40	-34, 10
MMRM analysis[1]		
LS mean (SE)	-1.7 (0.90)	-4.9 (0.94)
95% CI	(-3.5, 0.0)	(-6.7, -3.0)
Difference from placebo		
LS mean difference (SE)		-3.2 (1.30)
95% CI		(-5.7, -0.6)

p-value	0.0157

Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; MMRM=mixed effects model for repeated measures; RSBQ=Rett Syndrome Behavior Questionnaire; SD=standard deviation; SE=standard error
Note: Baseline was the latest non-missing value prior to the first dose of study drug.
[1] The MMRM included age group, baseline RSBQ severity, planned treatment, study visit, treatment-by-visit interaction, Baseline-by-visit interaction, and Baseline total score as fixed effects. An unstructured matrix was used to model within-subject errors. Sponsor used Kenward-Roger method for calculating the denominator degrees of freedom for tests of fixed effects. The statistics are slightly off by using R with approximate Satterwaite method for estimating the degrees of freedom.
Source: Statistical Analyst, adqrsbq.xpt.

Table 7: Primary Analysis of CGI-I Score by Visit (MMRM) - Full Analysis Set

	Placebo	Trofinetide
Week 2		
n	90	90
Mean (SE)	3.8 (0.06)	3.7 (0.06)
SD	0.58	0.53
Median	4.0	4.0
Min, max	1, 5	2, 5
MMRM analysis[1]		
LS mean (SE)	3.8 (0.06)	3.8 (0.06)
95% CI	(3.6, 3.9)	(3.6, 3.9)
Difference from placebo		
LS mean difference (SE)		-0.0 (0.08)
95% CI		(-0.2, 0.2)
p-value		0.9857
Week 6		
n	92	83
Mean (SE)	3.7 (0.06)	3.6 (0.08)
SD	0.59	0.74
Median	4.0	4.0
Min, max	2, 5	2, 5
MMRM analysis[1]		
LS mean (SE)	3.7 (0.07)	3.7 (0.07)
95% CI	(3.6, 3.9)	(3.5, 3.8)
Difference from placebo		
LS mean difference (SE)		-0.1 (0.10)
95% CI		(-0.3, 0.1)
p-value		0.4657
Week 12		
n	86	77
Mean (SE)	3.8 (0.06)	3.5 (0.08)
SD	0.55	0.74
Median	4.0	4.0
Min, max	2, 5	2, 5
MMRM analysis[1]		
LS mean (SE)	3.8 (0.07)	3.5 (0.07)
95% CI	(3.7, 4.0)	(3.4, 3.7)
Difference from placebo		
LS mean difference (SE)		-0.3 (0.10)
95% CI		(-0.5, -0.1)

p-value	0.0018

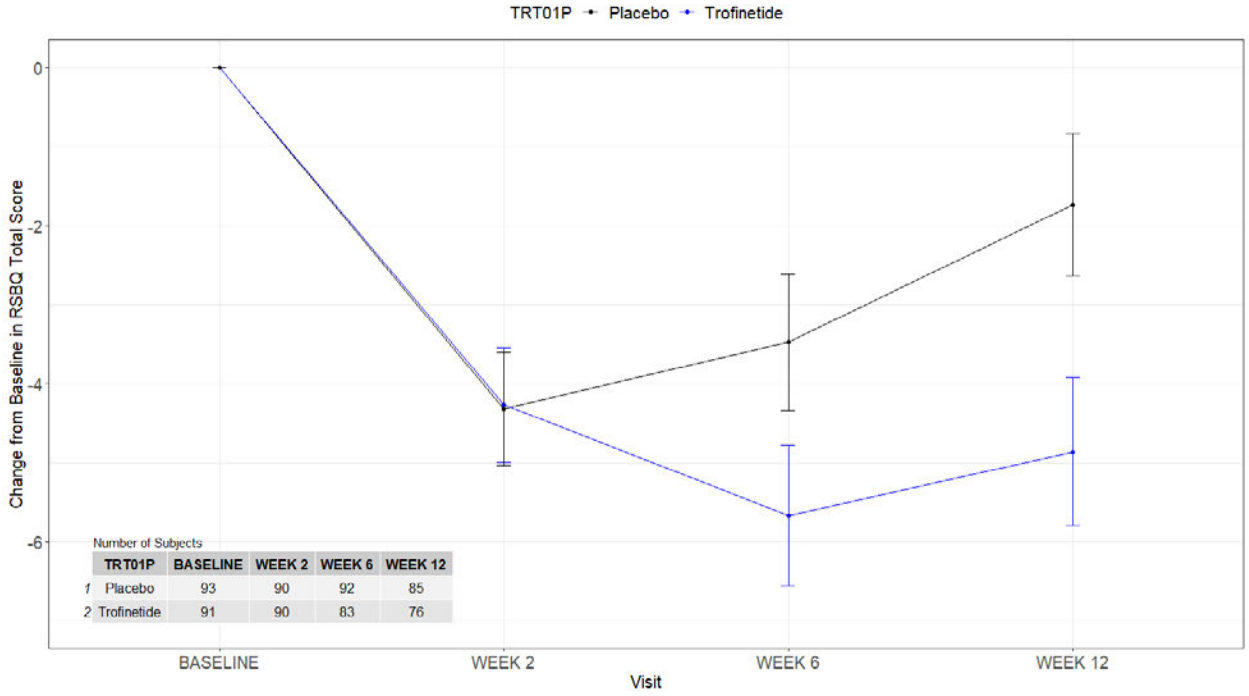
Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; LS=least squares; max=maximum; min=minimum; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behavior Questionnaire; SD=standard deviation; SE=standard error

[1] The MMRM included age group, baseline RSBQ severity, planned treatment, study visit, treatment-by-visit interaction, Baseline CGI-S-by-visit interaction, and Baseline CGI-S as fixed effects. An unstructured covariance matrix was used to model within-subject errors. Sponsor used Kenward-Roger method for calculating the denominator degrees of freedom for tests of fixed effects. The statistics are slightly off by using R with approximate Satterwaite method for estimating the degrees of freedom.

Source: Statistical Analyst, adqrsbq.xpt.

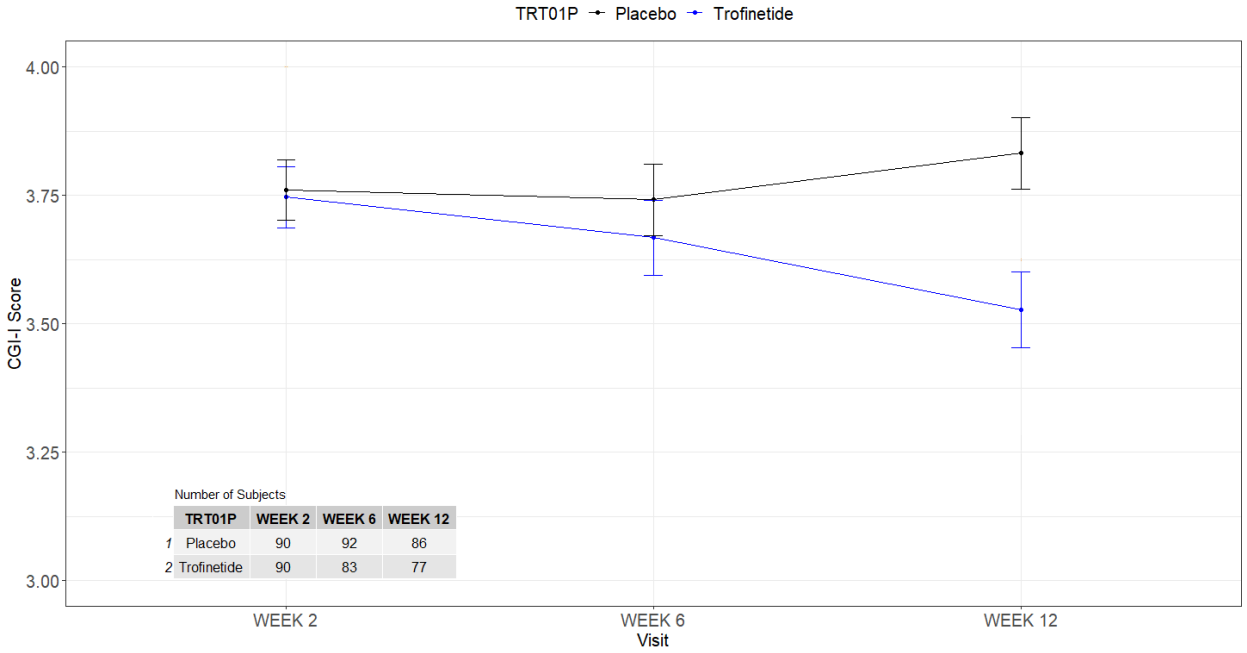
In both arms, RSBQ total score declined from baseline to week 2, see Figure 2. The trofinetide arm continued to decrease until Week 6. Between weeks 6 and 12, RSBQ scores increased by about 1 point. However, the placebo arm RSBQ score increased from Week 2 to Week 12. At Week 12, placebo arm's RSBQ total score was about 2 points lower than the baseline score.

Figure 2: Mean Rett Syndrome Behavior Questionnaire Total Score Change from Baseline by Visit (Full Analysis Set)



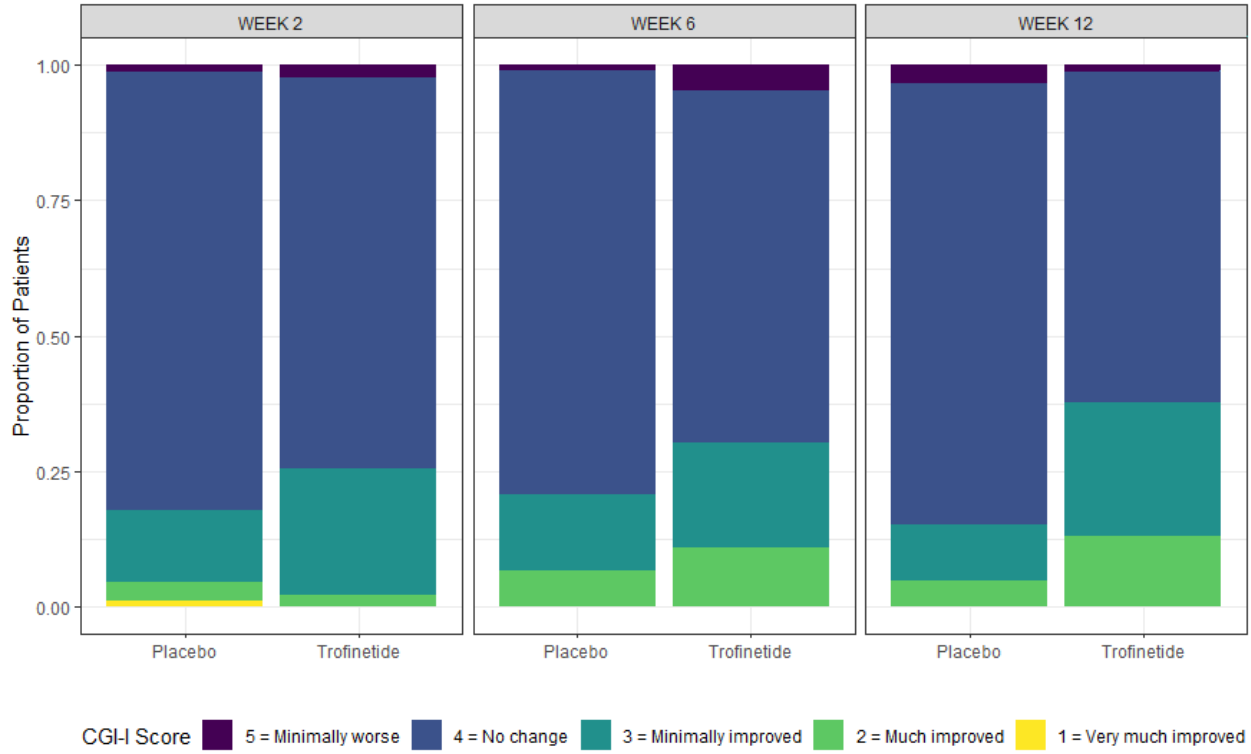
In the trofinetide arm, CGI-I score declined from baseline to week 12, see Figure 3. However, the placebo arm CGI-I score increased from baseline to Week 12. At Week 12, placebo arm's CGI-I score showed that the placebo arm worsened from baseline. In Figure 4, the percentage of subject in each CGI-I category are plotted by study visit with blue and purple indicating a CGI-I of worsening and greener and yellower colors indicating improvement. Note that the trofinetide arm has a greater percentage of subjects with a CGI-I score of 3 or less at all study visits. Therefore, more trofinetide subjects have no worsening or improvement compared to placebo. Throughout the study, the most common CGI-I score is 4 indicating some worsening in majority of subjects from clinician's global impression.

Figure 3: Mean Clinical Global Impression - Improvement at Each Visit (Full Analysis Set)



Source: Statistical Analyst; adcgi.xpt.

Figure 4: Percentage of Subjects in Each Clinical Global Impression - Improvement Category by Study Visit (FAS Population)



Source: Statistical Analyst; adcgi.xpt.

Reviewer's Note: *While the placebo arm worsens after week 6 for both endpoints, this placebo worsening is unlikely to completely explain the observed treatment effect because the trofinetide arm still showed improvement from baseline. However, if raters (either parents or clinicians) suspected which treatment that the subject was assigned to, this may impact the scoring with trofinetide scores being lower than placebo. This could be possible because of the common side effect of diarrhea in the trofinetide arm and the higher use of loperamide or other antipropulsives in the trofinetide arm.*

For the key secondary endpoint of CSBS-DP-IT Social Composite Score, the trofinetide showed a greater improvement in CSBS-DP-IT Social Composite Score compared to placebo at Week 12 (1.0 point change with p-value = 0.0064), see Table 8. During the Week 12 double-blind period, the trofinetide arm showed no improvement or worsening. In Figure 5, notice that both the trofinetide and placebo arms showed worsening through Week 6 with the placebo arm continuing to worsen from Week 6 to Week 12.

Reviewer's Note: *The CSBS-DP-IT endpoint is based on a screening checklist. This endpoint has not been validated for use as clinical study endpoint. Therefore, while the studied showed a statistically significant difference between trofinetide and placebo, it is not known how to interpret the observed difference.*

Table 8: Key Secondary Analysis of CSBS-DP-IT Social Composite Score and Change from Baseline by Visit (MMRM) – Full Analysis Set

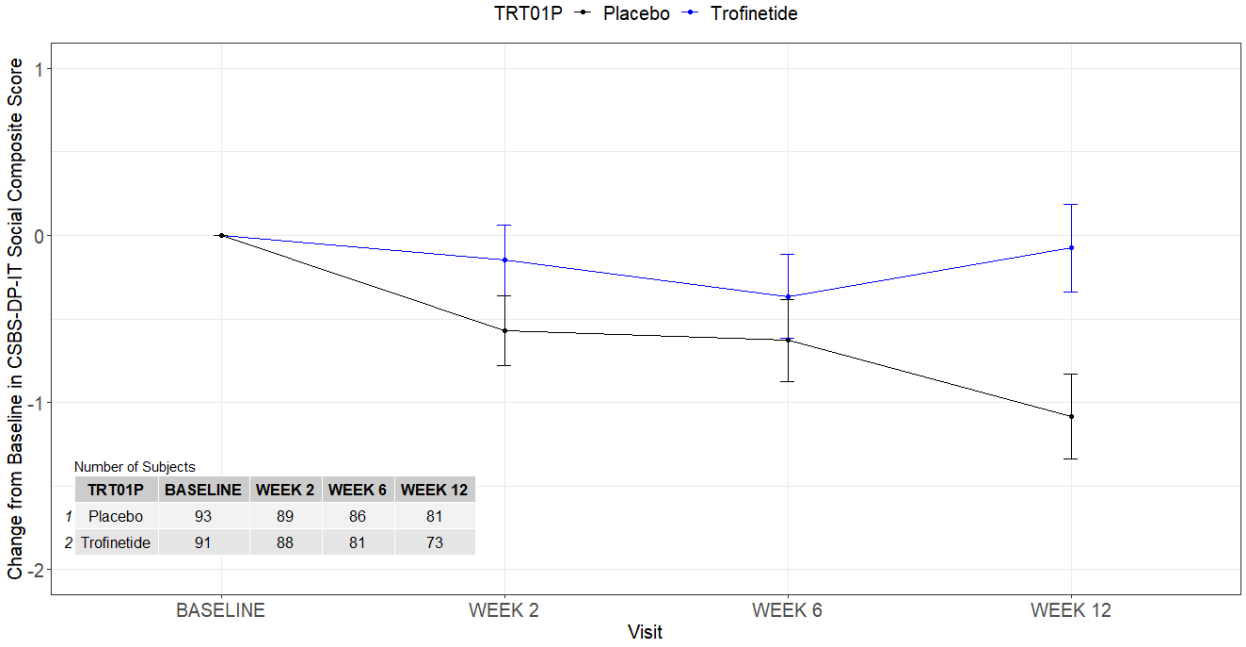
	Placebo	Trofinetide
Baseline		
n	93	91
Mean (SE)	8.8 (0.34)	8.7 (0.35)
SD	3.24	3.31
Median	9.0	9.0
Min, max	2, 16	2, 16
Week 12		
n	81	73
Mean (SE)	7.5 (0.33)	8.9 (0.44)
SD	2.99	3.74
Median	7.0	8.0
Min, max	2, 18	3, 19
Change from Baseline to Week 12		
n	81	73
Mean (SE)	-1.1 (0.28)	-0.1 (0.28)
SD	2.55	2.38
Median	-1.0	0.0
Min, max	-9, 4	-5, 7
MMRM analysis[1]		
LS mean (SE)	-1.1 (0.25)	-0.1 (0.26)
95% CI	(-1.6, -0.6)	(-0.6, 0.4)
Difference from placebo		
LS mean difference (SE)		1.0 (0.37)
95% CI		(0.3, 1.7)
p-value		0.0064

Abbreviations: CI=confidence interval; CSBS-DP-IT=Communication and Symbolic Behavior Scales Developmental Profile™ Infant-Toddler; LS=least squares; max=maximum; min=minimum; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behavior Questionnaire; SD=standard deviation; SE=standard error

Note: Baseline was the latest nonmissing value prior to the first dose of study drug.

[1] The MMRM included age group, baseline RSBQ severity, planned treatment, study visit, treatment-by-visit interaction, Baseline-by-visit interaction, and Baseline CSBS-DP-IT Social Composite Score as fixed effects. An unstructured matrix was used to model within-subject errors. Sponsor used Kenward-Roger method for calculating the denominator degrees of freedom for tests of fixed effects. The statistics are slightly off by using R.
Source: Statistical Analyst.

Figure 5: CSBS-DP-IT Social Composite Score and Change from Baseline by Visit



Source: Statistical Analyst.

Sensitivity Analyses

Missing Data

The Applicant conducted jump to reference (J2R) sensitivity analyses for both primary endpoints (CFB RSBQ and CGI-I). Their J2R analysis imputed missing observations in trofinetide subjects by creating an imputation model from the placebo subjects who completed the study. With this imputation model, the applicant imputed 50 datasets which were then analyzed using an ANCOVA model that adjusted for the same baseline factors as the primary analysis. The results of these 50 analyses were combined using Rubin's Rules, see Appendix for details.

Table 9 presented the results of multiple sensitivity analyses. The J2R sensitivity analyses are contained in the row labeled PMM-MNAR. For both the CFB12 in RSBQ and CGI-I at Week 12, both p-values are larger than the MMRM analysis (first row in Table 9). However, this increased p-value is still below 0.05. Therefore, the small numbers missing data (effective rate of about 10%) may not impact overall efficacy conclusions.

Table 9: Sensitivity and Supportive Analyses – Full Analysis Set

Endpoint Analyses	Mean (SD) at Baseline	LSM (SE) change from Baseline at Week 12	Trofinetide group comparison (PBO-TROF)

		PBO N=93	TROF N=91	PBO N=93	TROF N=91	Model	LS mean difference (SE)	2-sided p-value	Effect size (Cohen's *d*)
RSBQ coprimary analysis	RSBQ	44.5 (12 20)	43.7 (11.52)	-1.7 (0.90)-	-4.9 (0.94)	MMRM	-3.2 (1.30)	0.0157	0.37
RSBQ sensitivity and supportive analyses	PMM-MNAR	- -	- -	-1.9 (1.24)	-4.7 (1.27)	ANCOVA	-2.7 (1.29)	0.0338	NA
	PMM COVID-19-PHE-MAR variant	- -	- -	-2.0 (1.20)	-4.7 (1.24)	ANCOVA	-2.7 (1.30)	0.0406	NA
	Derived BL RSBQ randomization strata	44.5 (12 20)	43.7 (11.52)	-1.7 (0.90)	-4.9 (0.93)	MMRM	-3.3 (1.29)	0.0129	0.38
	Per-protocol	44.6 (12 21)	43.6 (11.42)	-1.7 (0.91)	-4.8 (0.95)	MMRM	-3.1 (1.32)	0.0193	0.36
CGI-I coprimary analysis	CGI-I	- -	- -	3.8 (0.07)	3.5 (0.07)	MMRM	-0.3 (0.10)	0.0018	0.47
CGI-I sensitivity and supportive analyses	PMM-MNAR	- -	- -	3.9 (0.08)	3.6 (0.09)	ANCOVA	-0.3 (0.10)	0.0112	NA
	PMM COVID-19-PHE-MAR variant	- -	- -	3.9 (0.09)	3.6 (0.09)	ANCOVA	-0.3 (0.10)	0.0100	NA
	Derived BL RSBQ randomization strata	- -	- -	3.8 (0.07)	3.5 (0.07)	MMRM	-0.3 (0.10)	0.0017	0.47
	Per-protocol	- -	- -	3.8 (0.07)	3.5 (0.07)	MMRM	-0.3 (0.10)	0.0018	0.47

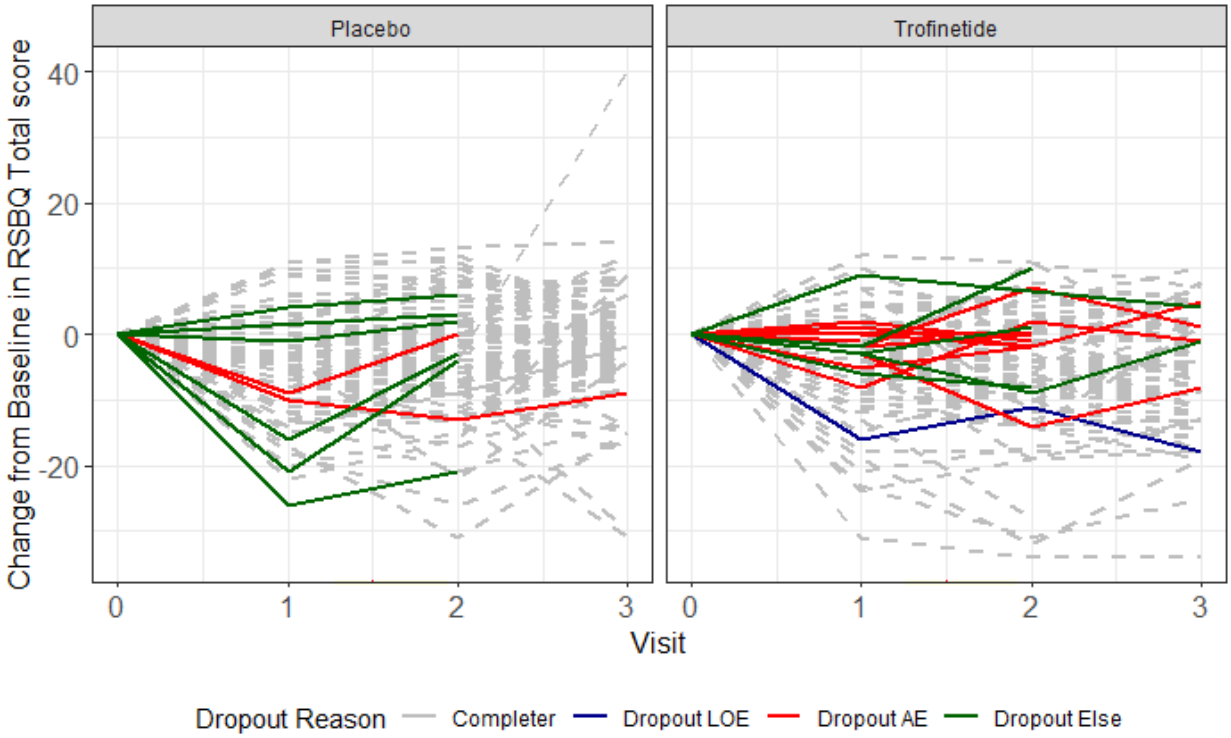
Sources: Applicant's CSR for Study 003. Results verified by Statistical Reviewer and Analyst.

Abbreviations: ANCOVA=analysis of covariance; BL=baseline; CGI-I=Clinical Global Impression Improvement; COVID-19=coronavirus disease 2019; LS=least squares; MAR=missing at random; MMRM=mixed-effect model for repeated measures; MNAR=missing not at random; NA=not applicable; PBO=placebo; PHE=public health emergency; PMM=pattern-mixture model; RSBQ=Rett Syndrome Behavior Questionnaire; SE=standard error; TROF=trofinetide

Note: Baseline was the latest nonmissing value prior to the first dose of study drug.

Note: Missing data were multiply imputed for 50 times based on the available nonmissing data of the placebo group.

Figure 6: Patient RSBQ Trajectories by Dropout Status (Study 003; mITT Population)



Source: Statistical Reviewer, adrsbq.xpt.

Reviewer's Note: *The Applicant's J2R sensitivity is useful to explore the impact of missing data under the assumption that the trofinetide dropouts look similar to the placebo completers. However, if the trofinetide dropouts do worse than the placebo completers, the J2R sensitivity may not fully explore the potential impact of the missing data. For Study 003, the small percentage of dropouts limits the impact of missing data.*

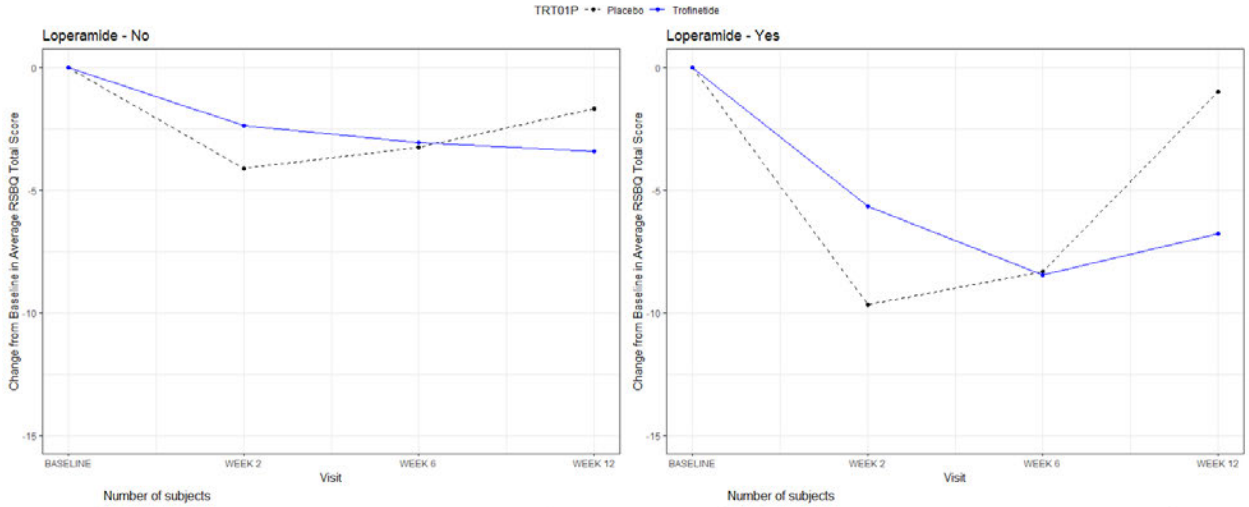
Analysis of Loperamide Usage

In consultation with the clinical team, they raised concerns about the impact of both diarrhea and loperamide usage as a concern about the efficacy of trofinetide. Specifically, there was concern that loperamide usage may cause functional unblinding in subjects who have diarrhea needing treatment with loperamide. In this case, functional unblinding would be caused by a change from constipation caused by Rett syndrome to diarrhea caused by starting the study. This change may have caused either parents or clinicians to suspect that the subject had received trofinetide. This suspicion could have an impact on either the parent rated (RSBQ) or clinician rated (CGI-I) endpoint. Therefore, the review team decided to conduct an exploration of the potential impacts of loperamide.

The Statistical Review team explored the impact of loperamide through both descriptive plots and a post-hoc mediation analysis. During these analyses, it was found that the placebo arm only had three subjects used loperamide. Therefore, there is limited information about the effects on loperamide in subjects not using trofinetide.

In Figure 7 and Figure 8, the mean RSBQ and CGI-I trajectories were plotted for each treatment arm by loperamide use status (ever used loperamide vs. never used loperamide). For RSBQ, trofinetide subjects on loperamide had a greater improvement in RSBQ compared to trofinetide subjects not taking loperamide. In the placebo arm, subjects who never took loperamide looked identical to the overall placebo arm mean in the mean RSBQ trajectory; this was expected because only three placebo patients were not in this group. In the trofinetide arm, there were only three subjects; therefore, the observed trajectory may be a poor reflection of the underlying RSBQ trajectory. For the CGI-I endpoint, the trofinetide arm in both loperamide groups had a similar improvement. In the never loperamide group, the CGI-I is similar to the overall CGI-I trajectory, see Figure 3.

Figure 7: RSBQ Trajectories by Use of Loperamide (Study 003; mITT Population)



Number of subjects

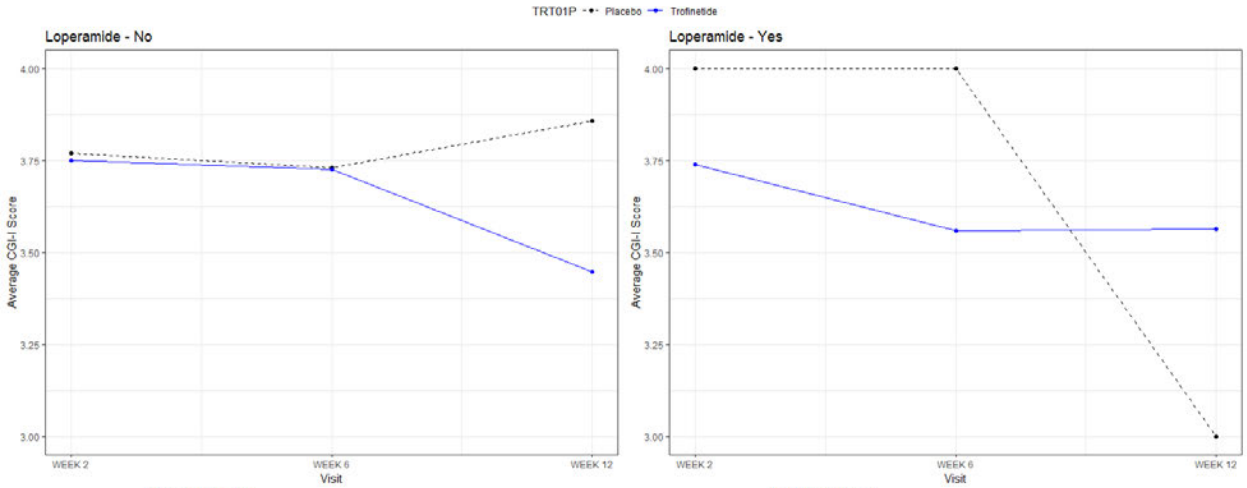
	TRT01P	n / N (%)	BASELINE	WEEK 2	WEEK 6	WEEK 12
1	Placebo	91 / 94 (96.8%)	90	87	89	83
2	Trofinetide	47 / 93 (50.5%)	45	44	40	38

Number of subjects

	TRT01P	n / N (%)	BASELINE	WEEK 2	WEEK 6	WEEK 12
1	Placebo	3 / 94 (3.2%)	3	3	3	2
2	Trofinetide	46 / 93 (49.5%)	46	46	43	38

Source: Statistical Analyst; adcm.xpt, adqrsbq.xpt.

Figure 8: CGI-I Trajectories by Use of Loperamide (Study 003; mITT Population)



Number of subjects

	TRT01P	n / N (%)	WEEK 2	WEEK 6	WEEK 12
1	Placebo	91 / 94 (96.8%)	87	89	84
2	Trofinetide	47 / 93 (50.5%)	44	40	38

Number of subjects

	TRT01P	n / N (%)	WEEK 2	WEEK 6	WEEK 12
1	Placebo	3 / 94 (3.2%)	3	3	2
2	Trofinetide	46 / 93 (49.5%)	46	43	39

Source: Statistical Analyst; adcm.xpt, adcgi.xpt.

The Statistical Reviewer conducted an exploratory mediation analysis to explore if or how the loperamide use may have mediated the relationship between trofinetide use and Rett Syndrome symptoms. This mediation analysis decomposed the total effect of trofinetide on Rett Syndrome into two paths – the direct path and the indirect path (includes loperamide use), see Figure 9. The path coefficients (α, β, and γ) were estimated using multiple regression models[1]:

$$logit(\Pr(lop = ever|trt = trofinetide)) = c_1 + \alpha \times trt$$
$$E(RSBQ|trt = trofinetide) = c_2 + \beta \times trt + \gamma \times lop + b \times Z.$$

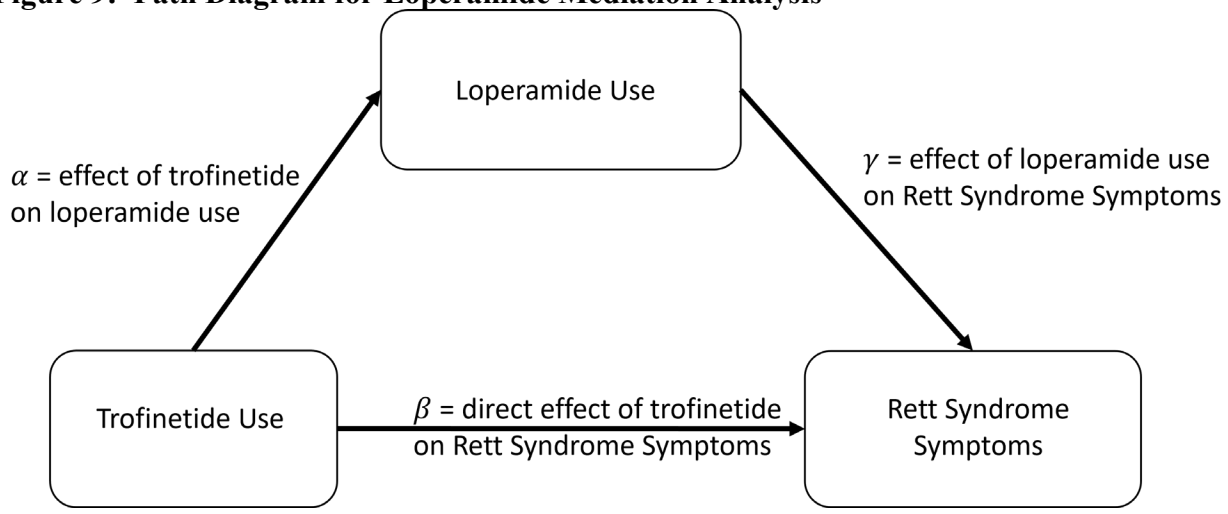
Here, *lop* is an indicator variable for ever or never used loperamide, *trt* indicates the randomized treatment, Z is a covariate matrix that includes the same baseline covariates as the primary analysis, c_i are the intercepts, and see Figure 9 for the definitions of α, β, and γ. The results of these regression are combined to form the estimates of the direct (NDE) and indirect (NIE) path effect estimates using:

$$NDE = \beta \times (trt_{trofinetide} - trt_{plb})$$
$$NIE = \gamma \times \left\{ \frac{e^{c_1+\alpha \times trt_{trofinetide}}}{1 + e^{c_1+\alpha \times trt_{trofinetide}}} - \frac{e^{c_1+\alpha \times trt_{plb}}}{1 + e^{c_1+\alpha \times trt_{plb}}} \right\}$$

No standard errors were reported the mediation analysis because it is a descriptive analysis to aid in the interpretation of the plots in Figure 7 and Figure 8.

[1] Noah A. Schuster, Jos W. R. Twisk, Martijn W. Heymans & Judith J. M. Rijnhart (2022): Causal Mediation Analysis with a Binary Mediator: The Influence of the Estimation Approach and Causal Contrast, Structural Equation Modeling: A Multidisciplinary Journal, DOI: 10.1080/10705511.2022.2104287

Figure 9: Path Diagram for Loperamide Mediation Analysis



Source: Statistical Reviewer.

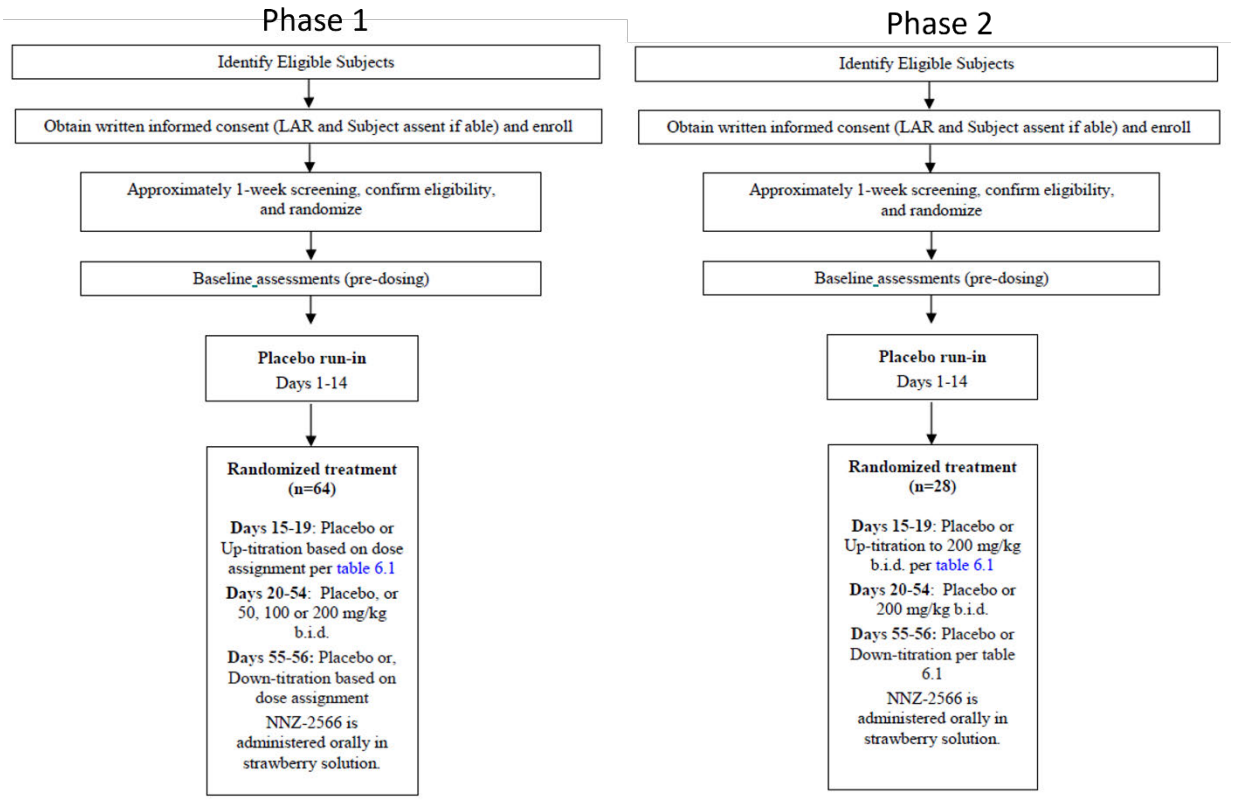
For both coprimary endpoints, the total effect of trofinetide on the endpoint was the same as the primary analysis (RSBQ: -3.2 and CGI-I: -0.3). This implied that the mediation models were useful for exploring the effects of loperamide. For RSBQ, the direct effect of trofinetide on Rett syndrome symptoms measured by RSBQ is -2.1 point and the indirect effect is -1.1 points. Therefore, while there may be a small effect of loperamide on RSBQ, it was not large enough to explain the observed treatment effect. For CGI-I, the direct effect of trofinetide on Rett syndrome symptoms measured by CGI-I is -0.3 point and the indirect effect is 0 points. Therefore, the CGI-I endpoint may not have any of its effect through the loperamide path.

The results of this mediation analysis did not suggest that loperamide completely explained the observed effects of trofinetide on Rett Syndrome symptoms.

3.2.3 Neu-2566-rett-002

Study 002 was a phase 2, dose-ranging study comparing three doses (50 mg/kg, 100mg/kg, and 200 mg/kg bid) of trofinetide to placebo. The study had a duration of 42 days of double-blind treatment with either trofinetide or placebo. The study consisted of two phases. The first phase randomized 64 subjects to each trofinetide arm and placebo at a 1:1:1:1 ratio. The second phase randomized an additional 28 subjects to either 200 mg/kg trofinetide or placebo at a 1:1 ratio, see Figure 10.

Figure 10: Study Neu-2566-RETT-002 Study Schema



Source: Applicant's Study Protocol.

In Study 002, all subjects were followed for 54 days where Days 1 – 14 were a placebo run-in period and Days 15 – 54 were a double-blind treatment period. Subjects were randomized before the placebo run-in period. Observations taken on Day 14 were used as the study baseline for all endpoints.

Reviewer's Note: *The Applicant's decision to have a placebo run-in period after randomization and use observations at the end of the placebo run-in for the primary efficacy baseline could lead to primary study comparisons that are potentially confounded by the Rett Syndrome symptom trajectories during the placebo run-in period.*

Study 002 measured eight clinician and caregiver reported efficacy outcomes. These outcomes measures included:

- The Clinical Global Impression of Severity (CGI-S) will be assessed at Screening, and at Days 14, 21, 28, 42, 54 and 66.
- The Clinical Global Impression of Improvement (CGI-I) will be assessed at Days 14, 21, 28, 42, 54 and follow up at Day 66
- Symptom severity according to the clinician rated Rett Syndrome Natural History Motor Behavior Assessment (MBA) (total score, subscale scores and modified change index scores). The MBA will be assessed at Baseline, and Days 14, 28, 42, 54 and at Follow-Up on Day 66.

- Clinician Rated Domain Specific Concerns VAS will be assessed at Baseline, and Days 14, 28, 42, 54 and at Follow-up at Day 66.
- Symptom severity according to the caregiver rated Rett Syndrome Behavior Questionnaire and the Caregiver Top 3 Concerns via a Visual Analogue Scale (VAS) which will be assessed at Baseline, and Days 14, 28, 42, 54 and at Follow-up at Day 66
- Caregiver stress according to the Rett Syndrome Caregiver Burden Inventory which will be assessed at Days 14, 28, 42, and 54 and at Follow-up at Day 66
- A semi-structured Caregiver Diary will be completed during the 1-week Screening period and collected and reviewed before the Baseline visit, and by caregivers during an approximately two-week period during treatment and collected on Days 14, 21, 28, 42, 54 and 66.

This Review focused on the CGI-I and RSBQ because these endpoints were the co-primary endpoints in Study 003. The analysis results for the other endpoints are included for completeness.

Statistical Analysis Plan

All statistical analyses were conducted on the modified Intent-to-Treat (mITT) population. The mITT population is the who received at least one dose of double-blind study medication. This population will be analyzed according to the treatment they actually received.

Reviewer's Note: *The Applicant's proposal to analyze the mITT population with the as received treatment instead of the as randomized may introduce bias into the statistical inference depending on if there were significant deviations from the randomized treatment assignments in the actual treatment assignments.*

The change from baseline (CFB) in RSBQ endpoint analyzed using a linear model that included a treatment baseline (Day 14 measurement), a treatment baseline by arm interaction, placebo response (change from Day 1 measurement to Day 14 measurement), and placebo response by arm only if these terms were statistically significant at $p \leq 0.10$. In addition, the Applicant tested if the outcome distribution was normal. If not, the Applicant used a non-parametric model to test if the endpoint.

Reviewer's Note: *The Applicant's plan to use the same data to both test for inclusion of covariates, test normality of outcome distribution, and to test the difference between trofinetide and placebo may inflate the type I error. This SAP was not reviewed during the IND because this was an exploratory study.*

There was no plan to control for multiple comparisons.

Reviewer's Note: *The lack of multiple comparison control was acceptable for an early phase learning study.*

3.2.3.1 Patient Disposition, Demographic and Baseline Characteristics

In Study 002, 82 randomized subjects in the intent-to-treat (ITT) population were randomized with 24 subjects in the placebo arm, 15 subjects in the 50 mg/kg arm, 16 subjects in the 100 mg/kg arm, and 27 patients in the 200 mg/kg arm, see Table 9. Only 1 subject dropped out of

the 200 mg/kg arm; no other dropouts were observed. Additional details of patient disposition are presented in Table 9.

Table 10: Patient Disposition / Core Efficacy (Neu-2566-RETT-002- mITT population)

	Placebo N=24	50 mg/kg N=15	100 mg/kg N=16	200 mg/kg N=27	Total N=82
Intent-to-Treat Population, n(%)					
Yes	24 (100)	15 (100)	16 (100)	27 (100)	82 (100)
No	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Modified Intent-to-Treat Population, n(%)					
Yes	24 (100)	15 (100)	16 (100)	27 (100)	82 (100)
No	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Per-Protocol Population, n(%)					
Yes	24 (100)	15 (100)	16 (100)	26 (96)	81 (99)
No	0 (0)	0 (0)	0 (0)	1 (4)	1 (1)
Completers Population, n(%)					
Completed the Study	24 (100)	15 (100)	16 (100)	26 (96)	81 (99)
Early Termination	0 (0)	0 (0)	0 (0)	1 (4)	1 (1)

Source: Statistical Analyst.

Study 002 was conducted in female subjects aged 5 to 16 years, see Table 5. These subjects were predominately white (93.9%).

Table 10: Demographics - Randomized Analysis Set

	Placebo N=24	50 mg/kg N=15	100 mg/kg N=16	200 mg/kg N=27	Total N=82
Age, Years					
Mean (SE)	9.38 (0.66)	10.06 (0.82)	10.81 (0.77)	9.23 (0.75)	9.73 (0.38)
SD	3.26	3.18	3.10	3.88	3.43
Median	9.64	9.54	9.66	7.49	9.41
Min, Max	5.1, 14.2	5.7, 15.4	6.1, 15.9	5.2, 15.7	5.1, 15.9
Ethnicity					
Hispanic or Latino	0	1 (6.7)	1 (6.2)	6 (22.2)	8 (9.8)
Not Hispanic or Latino	24 (100.0)	14 (93.3)	14 (87.5)	21 (77.8)	73 (89.0)
Not Reported	0	0	1 (6.2)	0	1 (1.2)
Race					
Asian	1 (4.2)	0	0	2 (7.4)	3 (3.7)
Black or African American	0	0	1 (6.2)	0	1 (1.2)
White	22 (91.7)	15 (100.0)	15 (93.8)	25 (92.6)	77 (93.9)
Other	1 (4.2)	0	0	0	1 (1.2)

Abbreviations: max=maximum; min=minimum; SD=standard deviation; SE=standard error
Source: Statistical Analyst.

The treatment baseline values for the two efficacy endpoints (RSBQ total score and CGI-I) were reported in Table 11. For RSBQ total score, note that the treatment baseline means varied from

39.5 to 44.7. The CGI-I endpoint had less variation with means ranging from 3.8 to 3.9.

Reviewer's Note: *The Applicant's choice to start double-blind therapy 14 days may bias the primary analysis.*

Table 11: Core Efficacy Variables at Treatment Baseline (Day 14) (mITT Population)

	Placebo N=24	50 mg/kg N=15	100 mg/kg N=16	200 mg/kg N=27
RSBQ Total Score				
Mean (SE)	39.5 (2.42)	44.7 (3.50)	40.3 (2.82)	42.2 (2.11)
SD	11.83	13.57	11.26	10.99
Median	40.5	47.0	40.5	42.0
Min, Max	16, 61	13, 67	20, 59	20, 69
CGI-I Score				
Mean (SE)	3.8 (0.10)	3.8 (0.11)	3.9 (0.11)	3.9 (0.12)
SD	0.48	0.41	0.44	0.62
Median	4.0	4.0	4.0	4.0
Min, Max	3, 5	3, 4	3, 5	2, 6
MBA Total Score				
Mean (SE)	48.8 (1.63)	46.6 (2.26)	48.6 (2.21)	46.6 (2.52)
SD	7.99	8.77	8.82	13.10
Median	47.0	49.0	45.5	44.0
Min, Max	34, 66	25, 58	37, 65	27, 72
RTT-DSC Total Score				
Mean (SE)	446.2 (20.36)	450.4 (20.76)	444.2 (19.98)	495.0 (18.71)
SD	99.75	80.39	79.91	97.21
Median	473.3	450.0	445.3	516.6
Min, Max	260, 637	243, 619	339, 588	270, 640
Caregiver Top 3 Concerns Total Score				
Mean (SE)	223.9 (11.13)	237.7 (16.52)	211.6 (10.65)	245.9 (9.45)
SD	54.51	63.97	42.60	49.12
Median	236.8	247.0	204.1	259.1
Min, Max	98, 300	64, 300	155, 292	91, 300

Abbreviations: RBSQ=Rett Syndrome Behavioral Questionnaire; CGI-I=Clinical Global Impression of Improvement; MBA=Motor Behavior Assessment; RTT-DSC=Rett Syndrome Domain Specific Concerns; mITT=modified intent-to-treat; n=number of subjects; SD=standard deviation.

Source: Statistical Analyst.

For change from baseline to Day 54 (CFB) in RSBQ total score, the 200 mg/kg trofinetide arm showed evidence of efficacy with 6.7 point greater improvement ($p = 0.035$) compared to placebo, see Table 6. The Applicant included placebo response but not the treatment baseline. CGI-I at Day 54 showed a -0.5-unit difference from placebo ($p = 0.029$) favoring 200 mg/kg of trofinetide. The 50 mg/kg and 100 mg/kg doses of trofinetide did not show any improvement compared to placebo.

Table 12: Summary of Primary Analyses from Treatment Baseline (Day 14) to Day 54 (mITT Population)

	Placebo N=24	50 mg/kg N=15	100 mg/kg N=16	200 mg/kg N=27
RSBQ Total				
D14 Treatment Baseline	39.5	44.7	40.3	42.2
Change D14-D54 (LSmean)	-2.0	-3.0	-1.5	-6.7
P-value vs Placebo		0.680	0.841	0.035
CGI-I				
D14 Treatment Baseline	3.8	3.8	3.9	3.9
D54 Treatment	3.5	3.3	3.4	3.0
P-value vs Placebo		0.391	0.703	0.029
MBA Total				
D14 Treatment Baseline	48.8	46.6	48.6	46.6
Change D14-D54 (LSmean)	-2.9	-2.6	-2.8	-2.6
P-value vs Placebo		0.853	0.953	0.870
RTTDSC Total				
D14 Treatment Baseline	473.3	450.0	445.3	516.6
Change D14-D54 (Exact Median Test)	-43.9	-53.0	-3.2	-71.3
P-value vs Placebo		0.842	0.524	0.019
Top 3 Caregiver Concerns				
D14 Treatment Baseline	223.9	237.7	211.6	245.9
Change D14-D54 (LSmean)	-12.5	-16.6	-2.1	-18.5
P-value vs Placebo		0.776	0.455	0.619

Abbreviations: RBSQ=Rett Syndrome Behavioral Questionnaire; CGI-I=Clinical Global Impression of Improvement; MBA=Motor Behavior Assessment; RTT-DSC=Rett Syndrome Domain Specific Concerns; mITT=modified intent-to-treat; n=number of subjects; SD=standard deviation.
In the analysis of the RSBQ, placebo response was included as a covariate. In the analysis of the MBA, placebo response and treatment baseline were included as covariates. The remaining three outcome measures were unadjusted.
The SAP specified that, if the distribution of data violated assumptions of normality for the GLM, non-parametric methods would be substituted. The distribution of data in the RTTDSC was non-normal. Consequently, group medians were used in the analysis of this endpoint and statistical significance was determined by the Exact Median Test.
Source: Statistical Analyst.

3.3 Evaluation of Safety

This review does not evaluate safety. Please refer to the clinical review for an evaluation of safety.

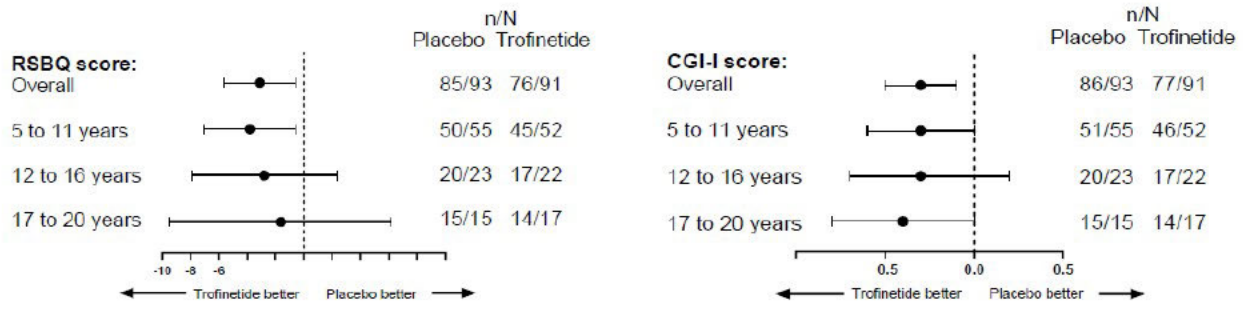
4 FINDINGS IN SPECIAL/SUBGROUP POPULATIONS

This section contains the results of the Applicant's subgroup analyses for Study 003. The Reviewer and Statistical Analyst verified these analyses. All subgroup analyses are post hoc, exploratory analyses and should be interpreted with care.

4.1 Gender, Race, Age, and Geographic Region

Age: The co-primary endpoints were analyzed by the age subgroups: 5 to 11, 12 to 16, and 17 to 20 years. For age, all subgroups had either a LS mean RSBQ or CGI-I indicating that the trofinetide arm is superior to placebo, see Figure 11.

Figure 11: Forest Plot of LSM Treatment Difference With 95% CI (MMRM) for Coprimary Endpoints by Age Group - Full Analysis Set



Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behavior Questionnaire
Source: Applicant's CSR, verified by Statistical Analyst.

Gender: All subjects were female.

Geographic Region: All study sites were in the United States.

4.2 Other Subgroups

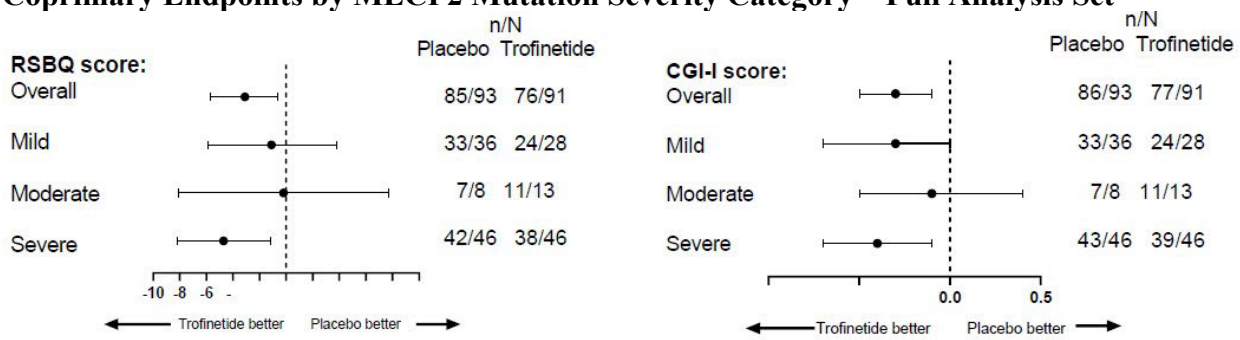
Baseline Rett Syndrome Severity: The co-primary endpoints were analyzed by the RSBQ severity subgroups: < 35 total score and ≥ 35 total score. and MECP2 mutation category (mild, moderate, and severe). For baseline RSBQ severity, both severity subgroups showed improvement; however, there were few (18) subjects per treatment arm in the RSBQ < 35, see Figure 12.

Figure 12: Forest Plot of LSM Treatment Difference With 95% CI (MMRM) for Coprimary Endpoints by Baseline RSBQ Score - Full Analysis Set

Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behavior Questionnaire
Source: Applicant's CSR, verified by Statistical Analyst.

MECP2 Mutation Severity: The co-primary endpoints were analyzed by MECP2 mutation category (mild, moderate, and severe). For baseline MECP2 mutation severity, all severity subgroups showed improvement; however, there were few subjects in the moderate subgroup (8 on placebo and 13 on trofinetide) see Figure 13.

Figure 13: Forest Plot of LSM Treatment Difference With 95% CI (MMRM) for Coprimary Endpoints by MECP2 Mutation Severity Category – Full Analysis Set



Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; LSM=least squares mean; MECP2=methyl-CpG-binding protein 2 gene (in humans); MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behavior Questionnaire
Source: Applicant's CSR, verified by Statistical Analyst.

5 SUMMARY AND CONCLUSIONS

5.1 Statistical Issues

There were two major statistical review issues in this original NDA. They were:

- Does the higher dropout rate in the trofinetide arm observed in study 003 have an impact on the efficacy signal in study 003?
    - In study 003, the higher observed higher dropout rate in the trofinetide arm had a minimal impact on the results of 003 because the higher dropout rate was low (approximately 10%).
- Does the higher use of loperamide or the greater incidence of diarrhea in the trofinetide arm impact the efficacy signal in study 003?
    - While analysis of the use of loperamide was limited by the observation that only 3 subjects in the placebo arm used loperamide, several exploratory analyses suggested that the effects of trofinetide persisted regardless of whether loperamide was used.

5.2 Collective Evidence

The Applicant supported their NDA for the rare disease of Rett Syndrome with two studies, Study 003 and Study 002. Study 003 is an approximately 200 subject randomized, double-blind, placebo-controlled, phase 3 study that had positive results on two primary endpoints, RSBQ and CGI-I. Therefore, Study 003 supported the efficacy of trofinetide for the treatment of Rett syndrome. The Applicant also submitted an early phase, dose-finding study (Study 002) as supportive evidence. While there were several design issues with Study 002, it provided supportive evidence for the efficacy of trofinetide the treatment of Rett Syndrome.

5.3 Conclusions and Recommendations

The Applicant presented sufficient evidence that trofinetide is effective in treating symptoms or Rett Syndrome. We recommend approval of trofinetide.

APPENDIX

1. Pattern-Mixture Models Assuming Missing Not At Random (PMM-MNAR)

The sensitivity analysis is implemented for Full Analysis Set using multiple imputations that are based on the distribution of placebo group responses over time to account for the intercurrent event of treatment discontinuations and missing assessments. The underlying assumption is that subjects with missing data due to early withdrawal evolve in the same way as placebo subjects that remain in the study.

The following steps are involved, and the imputed values will be constrained to be within the limits of 0 - 90 for RSBQ total score and 1 - 7 for CGI-I score:

- Non-monotone (intermediate) missing data at Baseline (not applicable for CGI-I score), Week 2, Week 6, and Week 12 will be multiply imputed using the Markov chain Monte Carlo (MCMC) method to create 50 monotone datasets. The imputation models for post baseline RSBQ total score and CGI-I score will include effects as follows:

  Post baseline RSBQ total score: age group (5-10 years old, 11-15 years old, and 16-20 years old), Baseline RSBQ severity (<35 total score and ≥35 total score), Baseline RSBQ total score, and treatment group CGI-I score: age group (5-10 years old, 11-15 years old, and 16-20 years old), Baseline RSBQ severity (<35 total score and ≥35 total score), Baseline CGI-S score, and treatment group.

- The monotone missing data will be imputed using a parametric, sequential linear regression method in which the missing data are imputed only on data from the placebo arm. A single imputation will be performed sequentially at each visit for each of the 50 imputed datasets. The predictors and their order in the PROC MI VAR statement for each visit are summarized in Table 5. The following SAS codes are to impute missing observations using the control-based pattern imputation method.

For RSBQ:

```
proc mi data=xxxx seed=2566003 nimpute=1 out=outname;
  by _Imputation_;
  class Trt Baseline_RSBQ_severity Age_group;
  monotone reg (/details);
  mnar model( W2_RSBQ W6_RSBQ W12_RSBQ / modelobs= (Trt='Placebo')); var
Age_group Baseline_RSBQ_severity Baseline_RSBQ W2_RSBQ W6_RSBQ
W12_RSBQ;
```

run;

For CGI-I:
by _Imputation_;
proc mi data=xxxx seed=2566003 nimpute=1 out=outname;
class Trt Baseline_RSBQ_severity Age_group;
monotone reg (/details);
mnar model(W2_CGI-I W6_CGI-I W12_CGI-I / modelobs= (Trt='Placebo')); var Age_group Baseline_RSBQ_severity Baseline_CGI-S W2_CGI-I W6_CGI-I W12_CGI-I;
run;

Table 5 Imputation Predictors

Visit	RSBQ Total Score - Predictors	CGI-I Score - Predictors
Week 2	Age group, Baseline RSBQ severity, Baseline RSBQ total score	Age group, Baseline RSBQ severity, Baseline CGI-S score
Week 6	Age group, Baseline RSBQ severity, Baseline RSBQ total score, Week 2 RSBQ total score	Age group, Baseline RSBQ severity, Baseline CGI-S score, Week 2 CGI-I score
Week 12	Age group, Baseline RSBQ severity, Baseline RSBQ total score, Week 2 RSBQ total score, Week 6 RSBQ total score	Age group, Baseline RSBQ severity, Baseline CGI-S score, Week 2 CGI-I score, Week 6 CGI-I score

Note: Age group (5-10 years old, 11-15 years old, and 16-20 years old); Baseline RSBQ severity (<35 total score and ≥35 total score).

- The change from Baseline to each post baseline visit values will then be calculated and analyzed for each of the 50 fully imputed datasets using the analysis of co-variance (ANCOVA) model. The ANCOVA model for RSBQ total score will have treatment group, Baseline RSBQ severity, and age group as fixed factors and Baseline RSBQ total score as a covariate; the ANCOVA model for CGI-I score will have treatment group, age group, and Baseline RSBQ severity as fixed factors and Baseline CGI-S score as a covariate.
- The results will be summarized by post baseline visit using the SAS MIANALYZE procedure to yield a combined estimate for treatment effect with its associated 95% confidence interval (CI) and p-value.

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/s/

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