CLINICAL REVIEW

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<tr><td>Application Type</td><td>NDA</td></tr>
<tr><td>Application Number(s)</td><td>217026</td></tr>
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<tr><td>Submit Date(s)</td><td>July 12, 2022</td></tr>
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<tr><td>Reviewer Name(s)</td><td>Michael Dimyan</td></tr>
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<tr><td>Established/Proper Name</td><td>Trofinetide</td></tr>
<tr><td>(Proposed) Trade Name</td><td>Daybue</td></tr>
<tr><td>Applicant</td><td>Acadia Pharmaceuticals Inc</td></tr>
<tr><td>Dosage Form(s)</td><td>Solution 200 mg/mL</td></tr>
<tr><td>Applicant Proposed Dosing Regimen(s)</td><td>Twice daily dosing according to patient weight:
Patient Weight

<table><tr><td>Patient Weight</td><td>DAYBUE Dose</td></tr><tr><td>9 kg to <12 kg</td><td>25 mL twice daily</td></tr><tr><td>≥12 kg to <20 kg</td><td>30 mL twice daily</td></tr><tr><td>≥20 kg to <35 kg</td><td>40 mL twice daily</td></tr><tr><td>≥35 kg to <50 kg</td><td>50 mL twice daily</td></tr><tr><td>≥50 kg</td><td>60 mL twice daily</td></tr></table></td></tr>
<tr><td>Applicant Proposed Indication(s)/Population(s)</td><td>For the treatment of Rett syndrome in adults and pediatric patients 2 years of age and older</td></tr>
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APPEARS THIS WAY ON ORIGINAL

Glossary

AC	advisory committee
AE	adverse event
ADR	adverse drug reaction
BID	twice daily
BLA	biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CGI-I	Clinician Global Impression of Improvement
ClinRO	clinician reported outcome
CMC	chemistry, manufacturing, and controls
CNS	central nervous system
COA	clinical outcome assessment
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSBS-DP-IT-SCS	Communication and Symbolic Behavior Scale Developmental Profile – Infant and Toddler – Social Composite Score
CSS	Controlled Substance Staff
DSMB	Data Safety and Monitoring Board
ECG	electrocardiogram
EDC	electronic data capture
eCTD	electronic common technical document
ETASU	elements to assure safe use
FAS	full analysis set
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GPE	glycine proline glutamate
GRMP	good review management practice
ICH	International Council for Harmonization

IGF-1	Insulin Like Growth Factor 1
IND	Investigational New Drug Application
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
LS	Least Square
MBA	Rett syndrome Natural History Motor Behavior Assessment
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
ObsRO	observer reported outcome
OCS	Office of Computational Science
OLE	Open Label Extension
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PerfO	performance outcome
PI	prescribing information or package insert
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PT	preferred term
PPI	patient package insert
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
REMS	risk evaluation and mitigation strategy
RTT	Rett syndrome
RTT-CBI	Rett Caregiver Burden Inventory
RTT-COMC	Rett syndrome Clinician Rating of Ability to Communicate Choices
RTT-CSS	Rett syndrome Clinical Severity Score
RTT-DSC	Rett syndrome Domain Specific Concerns
RSBQ	Rett syndrome Behavioral Questionnaire
SAE	serious adverse event
SAP	statistical analysis plan

SGE	special government employee
SOC	standard of care
SDTM	Study Data Tabulation Model
TEAE	treatment emergent adverse event
ULN	upper limit of normal
WHO	World Health Organization

1. Executive Summary

1.1. Product Introduction

Acadia Pharmaceuticals Inc, hereby referred to as the Applicant, submitted NDA 217026 for the marketing of DAYBUE (trofinetide) for the treatment of Rett syndrome in adults and pediatric patients 2 years of age and older. Trofinetide is a new molecular entity (NME) with no prior approvals. Trofinetide is the synthetic analogue of the naturally occurring N-terminal tripeptide of insulin-like-growth-factor-1 (IGF-1), glycine-proline-glutamate (GPE). Trofinetide is developed as a solution for oral or gastrostomy tube infusion in a 200 mg/mL concentration. The Applicant proposed dosing in bands according to body weight:

Table 1: Applicant's Recommended Weight Based Dosing Regimen

Patient Weight	Trofinetide Dose
9 kg to <12 kg	25 mL twice daily
≥12 kg to <20 kg	30 mL twice daily
≥20 kg to <35 kg	40 mL twice daily
≥35 kg to <50 kg	50 mL twice daily
≥50 kg	60 mL twice daily

Source: Applicant 1.14.1.2 Annotated Draft Labeling Text

1.2. Conclusions on the Substantial Evidence of Effectiveness

The placebo-controlled study ACP-2566-003 was an adequate and well-controlled study that supports approval of trofinetide for the treatment of children and adults with Rett syndrome. ACP-2566-003 was a multicenter study that provided reliable and statistically significant evidence that treatment with trofinetide for 3 months demonstrated benefit on the co-primary endpoints of the Rett Syndrome Behavior Questionnaire (RSBQ) and clinician rating of the Clinician Global Impression of Improvement (CGI-I). The study also demonstrated a statistically significant difference favoring trofinetide on the prespecified secondary endpoint of the Communication and Symbolic Behavior Scales Developmental Profile – Infant and Toddler – Social Composite Score (CSBS-DP-IT-SCS). This scale, developed as a screening tool to alert clinicians to potential communication deficits in infants and toddlers, is not fit for purpose for determining a treatment-related benefit in the complex concept of social reciprocity and communication (b) (4); however, the finding of a benefit on this endpoint in trofinetide

patients is still supportive of overall effectiveness.

Further confirmatory evidence of effectiveness was obtained in study NEU-2566-RETT-002, a multisite Phase 2 single-blind placebo run-in, randomized double-blind, placebo-controlled dose ranging clinical trial. The objective of NEU-2566-RETT-002 was to investigate the safety, tolerability, and pharmacokinetics of treatment with 3 different doses of oral trofinetide in girls ages 5 to 15 with Rett syndrome. A total of 82 subjects were enrolled and five outcome measures explored, but the primary support for effectiveness came from comparison of RSBQ and CGI-I in 24 placebo-treated subjects and 27 subjects receiving 200 mg/kg twice daily of trofinetide. Although formal statistical testing for efficacy was not prespecified, the least square means difference between trofinetide and placebo in RSBQ met nominal significance (p=0.042) as did CGI-I (P=0.029).

1.3. Benefit-Risk Assessment

Benefit-Risk Integrated Assessment

Trofinetide is a new molecular entity (NME) with no prior approvals. Trofinetide is the synthetic analogue of the naturally occurring N-terminal tripeptide of insulin-like-growth-factor-1 (IGF-1), glycine-proline-glutamate (GPE) and is developed as a solution for oral or gastrostomy tube infusion in a 200 mg/mL concentration. Trofinetide is indicated for the treatment of Rett syndrome in adults and pediatric patients 2 years of age and older.

Rett syndrome is a serious and life-threatening pediatric condition leading to severe disability and early death in adults. Rett syndrome is marked by initially normal development, followed at 18 to 30 months by severe loss of language, fine motor, and gross motor skills. Development of face, hand, and body stereotypies, epilepsy, non-epileptic spells, anxiety, and growth impairment occur in most subjects. The vast majority of patients with Rett syndrome are dependent on a caregiver for most activities of daily living and they suffer from the multiple complications that occur with impaired mobility and dependency, such as scoliosis, contractures, and pneumonia amongst others. Patients with Rett syndrome have a reduced life expectancy into the forties or fifties.

Rett syndrome has substantial unmet medical need. There are no therapies indicated specifically for the treatment of Rett syndrome. All the treatments currently used are solely for management of the numerous complications of the disorder. These treatments include medications for epilepsy, constipation, and other systemic features, physical therapies to compensate for neurological impairment, and surgical therapies for dysphagia, contracture, and scoliosis.

The Applicant has met the evidentiary standard of substantial evidence of effectiveness for trofinetide in the treatment of Rett syndrome in adults and children aged two and above. The primary evidence is based on one adequate and well-controlled trial of the use of trofinetide for 12 weeks in 187 females with Rett syndrome ages 5 to 20. In that pivotal study, a clinical benefit was demonstrated on the co-primary endpoints, Rett syndrome Behavioral Questionnaire (RSBQ) and Clinical Global Impression of Improvement (CGI-I). In ACP-2566-003, the trofinetide group had a least square (LS) mean decrease in RSBQ at 12 weeks of 4.9 points compared to 1.7-points for the placebo group (p=0.0175). The week 12 CGI-I LS mean scores were 3.5 for the trofinetide group and 3.8 for placebo (p=0.0030), favoring trofinetide. The study also demonstrated a statistically significant difference favoring trofinetide on the prespecified secondary endpoint, the Communication and Symbolic Behavior Scales Developmental Profile – Infant and Toddler – Social Composite Score (CSBS-DP-IT-SCS). While placebo subjects demonstrated an LS mean worsening of -1.1-point on the CSBS-DP-IT-SCS, trofinetide subjects' LS mean worsening was -0.1 (p=0.0064). No effects were seen

at the 50 mg/kg or 100 mg/kg twice daily doses. Confirmatory evidence of effectiveness was provided in NEU-2566-RETT-002, a phase 2 placebo-controlled dose-range-finding study in 82 patients treated for 56 days. In this study, 27 subjects received 200 mg/kg twice daily trofinetide and 24 patients received placebo. Although formal statistical testing for efficacy was not prespecified, the least square mean difference in RSBQ change from baseline between trofinetide and placebo subjects (-4.7-points) met nominal significance (p=0.035) as did CGI-I (-0.5-units) (p=0.029). The evidence for effectiveness in subjects with Rett syndrome ages 2 through 4 years was provided by the bridging PK study, ACP-2566-009 in which 10 subjects completed 12 weeks of treatment with trofinetide at banded-weight-based-dosing. Effectiveness can be extrapolated from the older/larger subjects to younger/smaller subjects since the pathophysiology of Rett syndrome doesn't differ in these similar age groups, and exposure-response relationships should also be similar. In ACP-2566-009, adjusted banded-weight-based dosing achieved comparable exposure in the 2 through 4 age range as had been achieved and was effective in the 5 through 20 age range in ACP-2566-003.

The evidence of effectiveness has some limitations, including concerns with the quality of the primary outcome measure, the RSBQ, and disproportionate adverse events, drug discontinuations, and concomitant medication use in the trofinetide group. However, application of regulatory flexibility allows for the determination of substantial evidence of effectiveness based on one adequate and well-controlled study with confirmatory evidence given the unmet need in this rare, severe, and life-threatening disorder.

The safety profile of trofinetide is acceptable to support approval. The two most common adverse reactions, diarrhea and vomiting were very frequent and led to withdrawal of 40% of subjects in long term studies. Though generally rated as mild to moderate in severity, labeling will ensure that prescribers, caregivers, and patients are aware of these adverse reactions. For immobile patients, chronic diarrhea has the potential to lead to multiple serious complications if not anticipated, prepared for, and treated. The potential for weight loss, or inadequate weight gain during development, along with a potential for worsening baseline seizures may also be safety concerns for trofinetide but are monitorable conditions. The high rate of concomitant use of antidiarrheals should also be monitored given that they may carry their own risks. However, these safety concerns are monitorable and addressable.

In conclusion, the Applicant has met the evidentiary standard of substantial evidence of effectiveness. Safety risks are monitorable and treatable. In the Voice of the Patient report of the externally-led patient focused drug development meeting of March 11, 2022, patients and caregivers expressed a dire unmet therapeutic need for treatments that directly address Rett

syndrome, to improve communication and hand use, and their willingness to try anything to lessen the suffering of patients. Given that Rett syndrome is a rare, severe, and life-threatening condition, it is appropriate to apply regulatory flexibility in accepting one well controlled and adequate study along with confirmatory evidence to establish the effectiveness of trofinetide for the treatment of Rett syndrome. Therefore, this reviewer recommends approval of trofinetide. Trofinetide will be the first FDA approved treatment for adults and pediatric patients 2 years of age and older with Rett syndrome.

Benefit-Risk Dimensions

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Rett syndrome is a rare genetic disorder affecting approximately 1 in 15,000 predominantly female live births. • The vast majority of Rett syndrome cases are due to a mutation on the X chromosome affecting the MeCP2 gene, which codes for a critically important methyl DNA binding transcription factor that mediates neurogenesis, migration, and patterning. • Rett syndrome accounts for 10% of profound intellectual disability of genetic origin in females • Patients with Rett syndrome experience normal growth until ages 18-30 months when major regression begins to occur. • Rett syndrome is marked by severe loss of language skills, fine and gross motor skills, and presence of face, hand, and body stereotypies, epilepsy, non-epileptic spells, anxiety, and growth impairment. • Most patients with Rett syndrome are dependent for most activities of daily living, suffer from the multiple complications that occur with such dependency and impaired mobility, and have a reduced life expectancy into the forties or fifties.	Rett syndrome is a serious and life-threatening pediatric condition leading to severe disability and early death in adults. Rett syndrome has substantial unmet medical need.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• A Voice of the Patient Report based on an externally-led patient focused drug development meeting identified the inability to communicate and inability to use their hands functionally as two of a vast array of severe symptoms that drastically reduced quality of life in patients with Rett syndrome.	
Current Treatment Options	• There are no FDA approved therapies specifically indicated for the treatment of Rett syndrome. • Patients with Rett syndrome are prescribed numerous therapies to assist with management of their epilepsy, chronic constipation, and spastic muscles, amongst others. • Physical, occupational, speech, and recreational therapy can be provided to attempt to reduce the speed of regression and prevent complications of mobility dependency. However, these therapies primarily work to compensate for inevitable neurological impairment. • Surgical therapies for dysphagia, contracture, and scoliosis are common.	There are no therapies indicated specifically for the treatment of Rett syndrome. All the treatments currently used are solely for management of the numerous complications of the disorder. These treatments include medications for epilepsy, constipation, and other systemic features, therapies to compensate for neurological impairment, and surgical therapies for dysphagia, contracture, and scoliosis.
Benefit	• ACP-2566-003 was a double-blind, placebo-controlled trial of trofinetide in 187 female subjects with Rett syndrome aged 5 to 20. Trofinetide was dosed in a banded-weight-based manner from 5 g twice daily to 12 g twice daily for 12 weeks. Co-primary endpoints were the RSBQ, a caregiver reported outcome measure of frequency and severity of behavioral symptoms in Rett syndrome, and the CGI-I, a clinician rating of improvement. • In ACP-2566-003, the trofinetide group had a least square (LS)	The Applicant has met the evidentiary standard of substantial evidence of effectiveness for the treatment of Rett syndrome in adults and children aged two and above. The primary evidence is based on one adequate and well-controlled trial of the use of trofinetide for 12 weeks in females with Rett syndrome ages 5 to 20. In ACP-2566-003, the trofinetide group had a least square (LS) mean

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	mean decrease in RSBQ at 12 weeks of 4.9 points compared to 1.7 points for the placebo group (p=0.0175). The week 12 CGI-I LS mean scores were 3.5 for the trofinetide group and 3.8 for placebo (p=0.0030), favoring trofinetide. • Supportive evidence of effectiveness was provided by the secondary endpoint, CSBS-DP-IT-SCS, an infant communication screening scale used to assess for changes in non-verbal communication during the trial. While placebo subjects demonstrated an LS mean -1.1-point worsening on the scale, trofinetide subjects' LS mean was -0.1 (p=0.0064). • Confirmatory evidence of trofinetide's benefit is provided by a dose-range-finding phase 2 study, NEU-2566-RETT-002. This study enrolled 82 subjects; however, the evidence came from comparison of 24 placebo subjects and 27 subjects who received 200 mg/kg twice daily which was the highest dose given in that study. In a non-prespecified analysis, placebo subjects had a 2-point decrease in RSBQ compared to 6.7-point decrease in the trofinetide group (p=0.042). The CGI-I scores were 3.5 in placebo and 3.0 in trofinetide (p=0.029). No effects were seen at the 50 mg/kg or 100 mg/kg twice daily doses. • The evidence for effectiveness in subjects with Rett syndrome ages 2 through 4 years was provided by the bridging PK study, ACP-2566-009 in which 10 subjects completed 12 weeks of treatment with trofinetide at banded-weight-based-dosing and achieved comparable exposure as was effective in ACP-	decrease in RSBQ at 12 weeks of 4.9 points compared to 1.7 points for the placebo group (p=0.0175). The week 12 CGI-I LS mean scores were 3.5 for the trofinetide group and 3.8 for placebo (p=0.0030), favoring trofinetide. Evidence of effectiveness in patients between 2 and 4 years of age was established by the bridging PK study, ACP-2566-009. Supportive evidence of effectiveness was provided by examining the Communication and Symbolic Behavior Scales Developmental Profile – Infant and Toddler – Social Composite Score (CSBS-DP-IT-SCS), which demonstrated a statistically significant difference favoring trofinetide over placebo. Confirmatory evidence was provided in a phase 2 placebo-controlled study of lower doses of trofinetide treatment for 56 days in a smaller sample of subjects. Though not part of a prespecified analysis, nominally significant benefit was found in RSBQ and CGI-I in this study as well. The evidence of effectiveness has some limitations including concerns with the quality of the primary outcome measure, RSBQ, and disproportionate adverse events, drug discontinuations, and concomitant medication use in the trofinetide group.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	2566-003. • The limitations of the evidence are the 1) reliance on one single adequate and well controlled study with confirmatory evidence, 2) the limitations of the RSBQ as a tool to measure functional improvement, 3) the disproportionate study withdrawal rate (16 trofinetide subjects versus 8 placebo subjects), 4) the disproportionate and rapid onset of diarrhea in the trofinetide arm along with the disproportionate use of loperamide in the trofinetide arm, with a risk for functional unblinding, and 5) confirmatory evidence coming from a post-hoc statistical analysis. • Altogether, despite limitations, the finding of improved RSBQ and CGI-I scores in trofinetide-treated subjects in ACP-2566-003, with confirmatory evidence from study NEU-2566-RETT-002 are compelling and clinically meaningful. Application of regulatory flexibility allows for the determination of substantial evidence of effectiveness based on a single adequate and well-controlled study plus confirmatory evidence given the unmet need in this rare, severe, and life-threatening disorder.	
Risk and Risk Management	• The safety database is based on the 187 subjects enrolled in the placebo-controlled trial ACP-2566-003 and supplemented with the long-term safety data from the 154 subjects who continued into ACP-2566-004 open-label long-term extension, and, as of NDA submission, the 47 subjects who continued into ACP-2566-005. Safety in ages 2 to 4	The safety profile of trofinetide is acceptable to support approval. The two most common adverse effects, diarrhea and vomiting were very frequent and led to withdrawal of 40% of subjects in long-term studies. Though generally rated as mild to moderate in severity, labeling

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	years is based on the 13 subjects enrolled in the open label ACP-2566-009. • The most commonly observed adverse events associated with the use of trofinetide in the 12-week placebo-controlled study (≥5% incidence and ≥2% greater than placebo) were diarrhea (87%), vomiting (29%), fever (9%), seizure (9%), anxiety (8%), decreased appetite (8%), fatigue (8%), and nasopharyngitis (5%). • In long term studies, 84% of trofinetide-treated subjects experienced diarrhea. • Treatment emergent adverse effects led to withdrawal of study drug in 40.4% of subjects in the placebo-controlled and open-label long term extension studies, the majority of which were due to chronic diarrhea on trofinetide. • Due to the frequency of diarrhea, concomitant therapy with loperamide was initiated in over 50% of subjects in the placebo-controlled and open-label long-term extension studies. • In ACP-2566-003, 12% of trofinetide-treated subjects experienced weight loss of >7% compared to 4% of placebo patients. • Serious adverse events included two events of seizure that are possibly be related to trofinetide; one case of urosepsis from urinary tract infection that occurred in the setting of diarrhea was deemed possibly related by the investigator; and a number of infections and respiratory conditions that	will ensure that prescribers and patients are aware of these adverse reactions. For immobile patients, chronic diarrhea has the potential to lead to multiple serious complications if not anticipated, prepared for, and treated. The potential for weight loss, or inadequate weight gain during development, along with a potential for worsening baseline seizures may also be safety concerns for trofinetide but are monitorable conditions. The high rate of concomitant use of antidiarrheals needs to be accounted for given that they may carry their own risks. Together, these safety concerns are monitorable and addressable and therefore do not outweigh the potential for benefit in this serious and life-threatening disorder with unmet medical need.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	occur frequently in the background of Rett syndrome and so cannot be clearly attributed to trofinetide. • There were two deaths in the clinical development program for Rett syndrome that did not appear to be related to treatment with trofinetide based on this reviewer's assessment. One death was due to severe vomiting, aspiration, and respiratory arrest the night after placement of a percutaneous gastrostomy tube. The second was in a subject who suffered multiple gastrointestinal hemorrhages and perforation after chronic treatment with ibuprofen for post-spinal surgery pain.	

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

☒	The patient experience data that was submitted as part of the application include:			Section where discussed, if applicable
	☒	Clinical outcome assessment (COA) data, such as		Sec 6.1 Study endpoints
✓		☐	Patient reported outcome (PRO)	
		☒	Observer reported outcome (ObsRO)	Sec 6.1 Study endpoints
		☒	Clinician reported outcome (ClinRO)	Sec 6.1 Study endpoints
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	☐	Other: (Please specify)		
☒	Patient experience data that were not submitted in the application, but were considered in this review:			
		☐	Input informed from participation in meetings with patient stakeholders	
		☒	Patient-focused drug development or other stakeholder meeting summary reports	Sec 2 Therapeutic Context
		☐	Observational survey studies designed to capture patient experience data	
		☐	Other: (Please specify)	
☐	Patient experience data was not submitted as part of this application.			

2. Therapeutic Context

2.1. Analysis of Condition

Rett syndrome is a rare genetic disorder with an incidence of approximately 1 in 15,000 live

births, predominantly female. Classical or Typical Rett syndrome (>95% of cases) is caused by mutations in the methyl-CpG binding protein 2 (MeCP2) gene on the X chromosome. MeCP2 protein is a methyl-DNA binding protein, likely leading to multiple transcription factor roles for the protein, including suppression of initiation of gene regions with high cytosine-guanine methylation levels. Therefore, loss of MeCP2 leads to altered transcription of BDNF, CREB1, FOXO3 and likely other transcriptional and post-transcriptional alterations. Through these mechanisms, MeCP2 activity mediates neurogenesis, migration, and patterning. MeCP2 gene mutations are thought to disrupt normal excitatory-inhibitory balance in the cortex with findings of elevated gamma-amino-butyric-acid-A (GABA-A) and N-methyl-D-aspartic acid receptors (NMDA) receptors in knockout mice (Zhang, Shi et al. 2022). A related disorder, MeCP2 Duplication syndrome, which also manifests with neurodevelopmental disorders, demonstrates that MeCP2 overexpression is also harmful. The clinical manifestations of Rett syndrome can be heterogeneous due to the various genetic mutations that can cause RTT and also because of X-inactivation. Atypical Rett syndrome cases generally have a limited phenotype, and only about 75% are found to have MeCP2 mutations.

Rett syndrome accounts for 10% of cases of profound intellectual disability of genetic origin in females. Rett syndrome is characterized by apparently healthy development up until approximately 6 to 18 months, at which point regression of early milestones occurs. Development of language, motor control, and eye contact halt and begin to regress. Over time, hand stereotypies (clapping, wringing) and other cortical symptoms and signs such as anxious appearance, non-epileptic “Rett spells” (characterized by breath holding, unusual eye movements or staring), and epileptic seizures occur. Between 18 and 30 months, further loss of fine and gross motor control occur. There is usually either lack of gait or decline of any previously acquired gait skills with emergence of ataxia. After 40 months, many Rett syndrome patients enter a ‘plateau’ phase characterized by prominent hand stereotypies and intense eye gaze for communication (Stallworth, Dy et al. 2019). In young adulthood, Parkinsonian features and progressively worsening motor tone occur leading to scoliosis and contractures. Prominent respiratory dysrhythmias, and less common cardiac tachycardia, decreased beat to beat variability, QTc prolongation, and vasomotor and sudomotor abnormalities are indicative of a more general dysautonomia. While primarily a neurological disorder, cholesterol metabolism pathology and general growth impairment are present, and the nervous system effects are observed in gastrointestinal and renal symptoms. Also, due to severe neurological impairment and subsequent dependencies, systemic disorders such as osteoporosis, aspiration pneumonia, pressure ulcers, and infectious disorders occur (Neul and Chang 2020, Romero-Galisteo, Gonzalez-Sanchez et al. 2022, Panayotis, Ehinger et al.). Life expectancy is reduced to the 40s or 50s.

An externally-led Patient Focused Drug Development meeting was co-hosted by the International Rett syndrome Foundation and the Rett syndrome Research Trust on March 11, 2022. A Voice of the Patient report was published August 9, 2022, based on the content of this

meeting and online comments submitted afterward (Coenraads, Hehn et al. 2022). This meeting emphasized the diverse array of dynamic symptoms that make living with Rett syndrome difficult for both patients and caregivers. The inability to communicate was identified as the top area of concern, as Rett patients appeared to their caregivers to be cognitively aware and distressed by their communication impairments. Patients and caregivers expressed a dire unmet therapeutic need for treatments that directly address Rett syndrome and to improve communication and hand use. They also expressed a willingness to try anything to lessen the suffering of patients with Rett syndrome. In conclusion, Rett syndrome is a serious condition with unmet medical need.

2.2. Analysis of Current Treatment Options

There are currently no FDA approved therapies directly targeting the treatment of Rett syndrome. Specifically, there are no approved therapies directed at correcting the MeCP2 genetic mutations most common in typical Rett syndrome or to modifying the downstream effects of those mutations. While research is ongoing targeting gene replacement therapies, gene/RNA editing strategies, and reactivation of the inactivated X chromosome, therapy for Rett syndrome patients is currently supportive for the various symptoms and complications.

Because current therapy for Rett syndrome is symptomatic and does not directly treat the pathophysiology of the disease, clinical management is complex. Patients require care from multidisciplinary teams of neurologist, cardiologists, gastroenterologists, orthopedists, geneticists, speech, physical, and occupational therapists (Romero-Galisteo, Gonzalez-Sanchez et al. 2022). Standard antiepileptic therapy is generally successful in reducing the frequency of seizures in Rett syndrome, though up to 19% of patients require polytherapy for intractable seizures (Krajnc, Zupancic et al. 2011). Chronic constipation is common and can be severe and is treated with various laxatives. Progressive dysphagia often leads to placement of gastrostomy tubes for maintenance of nutrition. In higher income countries, patients with Rett syndrome are generally involved in regular programs of physical, occupational, and communication therapy which may employ bracing, splinting, and assistive technologies for mobility and communication. These therapies improve quality of life and preserve autonomy (Fonzo, Sirico et al. 2020). Surgical treatment of scoliosis and contractures is common.

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Trofinetide is a new molecular entity not currently marketed in the U.S. The Applicant has an active IND for (b) (4)

(b) (4)

Reviewer Comment: No substantial regulatory action has occurred on these INDs that are relevant to the current application.

3.2. Summary of Presubmission/Submission Regulatory Activity

Neuren Pharmaceuticals, Ltd., the original Sponsor of the trofinetide IND, had a pre-investigational new drug (IND) application meeting with the FDA on May 1, 2012, at which time the Sponsor was advised regarding its nonclinical and clinical development programs. The Sponsor was advised to "explore potential clinically meaningful endpoints, specific populations, and expected time course for evaluation of specific endpoints". The Sponsor subsequently submitted IND 114319 on November 20, 2012.

Trofinetide for the treatment of Rett syndrome was granted Fast Track designation on June 4, 2013, Orphan Drug Designation on February 11, 2015, and Rare Pediatric Disease Designation on March 02, 2020, all predicated on the fact that Rett syndrome is a serious, rare condition with an unmet medical need. Trofinetide was denied Breakthrough Therapy Designation on February 24, 2015, (b) (4) and again on December 18, 2018, (b) (4)

At the end-of-phase 2 (EOP2) meeting on October 12, 2017, the Applicant proposed a subsequent Phase 3 study that would be a double-blind, placebo-controlled, parallel-group study design. The Agency advised that there were multiple concerns with the RSBQ as a primary endpoint. At the encouragement of the Agency, the Applicant agreed to study the Clinical Global Impression-Improvement (CGI-I) scale as an anchoring functional measure as a co-primary endpoint with the Rett syndrome Behavior Questionnaire (RSBQ). The Sponsor believed that the RSBQ was the most relevant, validated, and useful endpoint in Rett syndrome. At this meeting also, the Sponsor proposed that total exposure for the safety database would include at least 35 subjects exposed for 12 months. The Agency advised that any secondary endpoints studied with the intention to be included (b) (4) should assess domains that are distinct from those evaluated by the primary endpoints.

When open-label extension Study ACP-2566-004 was submitted, the Agency advised that the blinding be maintained for all subjects who progressed from the placebo-controlled, double-blind ACP-2566-003 to ACP-2566-004 and the Sponsor agreed.

In written responses to Sponsor questions dated December 8, 2020, the Sponsor proposed Study ACP-2566-009 as an open-label study in subjects ages 2 through 4. The Agency advised

that the distribution of subjects should ensure adequate sample sizes in the youngest age groups (2 and 3 years old). The Agency advised that the Sponsor should provide a justification that any findings of effectiveness in older pediatric and young adult subjects from the pivotal trial (ACP-2566-003) can be extrapolated to subjects 2 through 4 years of age.

Regarding the statistical analysis plan (SAP) for the pivotal trial ACP-2566-003, on January 29, 2021, the Agency advised that sensitivity analyses accounting for the COVID19 Public Health Emergency should treat remote assessments and missing assessments differently.

Regarding chemistry, manufacturing, and controls (CMC), the Agency advised in a Type C written responses only letter dated August 10, 2021, that 12 months of long-term data and 6 months of accelerated stability data for three registration batches be submitted as part of the NDA.

In meeting minutes dated September 13, 2021, the Sponsor proposed that nonclinical studies were not required to investigate abuse potential. The Sponsor was asked to clarify the inhibitory action of trofinetide at the NMDA receptor and explain why no correlative abuse-related adverse events were observed in clinical studies in healthy adults.

The Sponsor reported to the Agency a cybersecurity incident that caused an outage of the contract research organization (CRO) management of ECG Central analysis between September 20, 2020, and October 7, 2020. The CRO created a work-around by which collected ECGs could be transmitted via fax and this resulted in minor protocol deviations for 6 visits in 5 subjects in the Study -003 and 3 visits for 3 subjects in Study -004. One major protocol violation occurred for ACP-2566-004 (b) (6) who was enrolled in Study -004 prior to ECG eligibility being evaluated and whose post-dose change in QTcF was not centrally evaluated due to the outage.

At the pre-NDA meeting dated March 24, 2022, the Agency noted the large number of dropouts in the trofinetide group of Study 003 and recommended that all prespecified analyses in the SAP should be presented in the NDA submission. The Agency also advised that any secondary endpoints intended for inclusion (b) (4) should assess domains that are distinct from those evaluated by the primary endpoint and should be statistically controlled for Type I error. The Agency noted that the controlled effectiveness data from Study 003 would be the primary source of the data to determine effectiveness. In reference to the Type C Written Response dated November 1, 2021, the Sponsor's proposed pooling for the integrated summary of safety (ISS) while keeping Study 003 as the primary source of safety data. The Agency also emphasized the need for at least 35 subjects being exposed to drug for 12 months as the minimum number of subjects expected for the application to be reviewed. The Sponsor explained that (b) (4) for subjects aged 2 to 5 years, Study 009 data would be submitted with the NDA containing data from 10 subjects treated for 12 weeks and 5 subjects treated for less than 12 weeks, with at least 4 subjects under 4 years of age.

3.3. Foreign Regulatory Actions and Marketing History

Not Applicable

4. Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

OSI clinical site visits were conducted primarily based on risk ranking in the clinical investigator site selection tool, numbers of enrolled subjects, impact on primary effectiveness analyses, and prior inspection history. The conclusions of clinical inspection summary were that the study was conducted adequately, and the data generated by the inspected sites and submitted by the Sponsor appeared acceptable in support of the respective indication.

4.2. Product Quality

The chemistry, manufacturing, and controls (CMC) review was not available at the time of this review; however, in multiple discussions with the CMC team, no substantial issues were identified. Please refer to the CMC review for further details and analysis.

4.3. Clinical Microbiology

The Integrated Quality review has found that the application is adequate from the standpoint of product quality microbiology.

4.4. Nonclinical Pharmacology/Toxicology

The nonclinical review was not available at the time of this review; however, in multiple discussions with the nonclinical team, no substantial issues were identified. Please refer to the nonclinical review for further details and analysis.

4.5. Clinical Pharmacology

See the clinical pharmacology review for details. In brief, trofinetide is the synthetic analogue of the naturally occurring N-terminal tripeptide of insulin-like-growth-factor-1 (IGF-1), glycine-proline-glutamate (GPE). Trofinetide is developed as a solution for oral or gastrostomy tube infusion in a 200 mg/mL concentration. Pharmacokinetic (PK) studies of trofinetide reveal that it exhibits linear kinetics with no time or dose dependent effects. Bioavailability by oral administration was estimated at 80%. While rate of absorption was mildly reduced by administration with a high fat meal, the concentration-time-curve (AUC) was unaffected. Trofinetide elimination is primarily renal. (b) (4)

4.6. Devices and Companion Diagnostic Issues

Not applicable.

4.7. Consumer Study Reviews

Not applicable.

5. Sources of Clinical Data and Review Strategy

5.1. Table of Clinical Studies

Study ID	Study phase	Number of study centers (locations)	Study dates, status	Study objectives	Study and control drugs dose, regimen	Number of subjects enrolled by arm/ (FAS/mITT/Safety Analysis Set)[a]	Treatment duration	Inclusion criteria - main eligibility criteria	Efficacy endpoints and analyses
Primary Efficacy Study									
Study 003 NCT0 41817 23	3	US: 28 planned/ 21 enrolled	Nov 2019 to Oct 2021 Completed	Efficacy, safety	Trofinetide or Placebo Oral or G-tube Single trofinetide dose group by weight: 12-20 kg: 30 mL (6 g) BID >20-35 kg: 40 mL (8 g) BID >35-50 kg: 50 mL (10 g) BID >50 kg: 60 mL (12 g) BID; Placebo	Trof: 93/91 Pbo: 94/93	12 weeks	5-20 years old Meets clinical criteria for Rett syndrome Documented disease causing mutation in the *MECP2* gene. Post-regression at Screening RTT-CSS score 10-36 CGI-S ≥4	Change from Baseline to Week 12: RSBQ (coprimary) At Week 12: CGI-I (coprimary) Change from Baseline to Week 12: CSBS-DP-IT Social Composite Score (key secondary)
Supportive Efficacy Study									

Study RETT -002 NCT0 27151 15	2	US: 12	Mar 2016 to Jan 2017 Completed	Safety	Trof: 200 mg/kg BID Trof: 100 mg/kg BID Trof: 50 mg/kg BID; Placebo Oral or G-tube	Trof 200 mg/kg BID: 27/27 Trof 100 mg/kg BID: 16/16 Trof 50 mg/kg BID: 15/15 Pbo: 24/24	42 days	5-15 years old Meets clinical criteria for Rett syndrome Proven mutation of the *MECP2* gene Post-regression Hagberg Stage 3 or 4 RTT-CSS score 10- 36 CGI-S ≥4	Change from Baseline to Week 6 (Day 54) RSBQ, MBA RTT-DSC Caregiver Top 3 Concerns Actual values at Week 6 (Day 54): CGI-I
Phase 1 Study Supporting Safety									
Study RETT -001 NCT0 17035 33	2	US: 3	Apr 2013 to Sep 2014 Completed	Safety	Trof: 70 mg/kg BID Trof: 35 mg/kg BID Placebo Oral or G-tube	Cohort 0 Trof 35 mg/kg BID: 5/5 Pbo: 4/4 Cohort 1 Trof 35 mg/kg BID: 13/13 Pbo: 5/5 Cohort 2 Trof 70 mg/kg BID: 18/17 Pbo: 11/11	14 days (Cohort 0) or 28 days (Cohorts 1 and 2)[b]	16-45 years old Meets clinical criteria for Rett syndrome Proven mutation of the *MECP2* gene Not undergoing regression RTT-CSS score 10-36 CGI-S≥4	Change from Baseline to end of treatment: MBA,CSS, Caregiver Top 3 Concerns, ABC modified apnea index, EEG Actual values at end of treatment: CGI-I
Open Label Studies for Safety									

Study 004 NCT042793 14	3	US: 21	Jan 2020 to 15 Feb 2022 (Interim) Ongoing	Safety	Trofinetide Oral or G-tube Open-label 12-20 kg: 30 mL (6 g) BID >20-35 kg: 40 mL (8 g) BID >35-50 kg: 50 mL (10 g) BID >50 kg: 60 mL (12 g) BID;	Trof: 154	40 weeks	Completed 003 or Within the past 12 months, Completed 003, but did not directly rollover to 004 due to COVID-19 PHE or Within the past 12 months, discontinued from Study 003 due to COVID-19 PHE	Primarily a safety study, but also explored Change from Baseline of Study 003 and Study 004 at Weeks 2, 12, 26, 40 in RSBQ and CGI by Visit
Study 005 NCT04776746	3	US: 21	November 2020 to Ongoing (Interim Data Cutoff March 04, 2022)	Safety	Trofinetide Oral or G-tube Open-label 12-20 kg: 30 mL (6 g) BID >20-35 kg: 40 mL (8 g) BID >35-50 kg: 50 mL (10 g) BID >50 kg: 60 mL (12 g) BID	Trof: 47 (of planned 153)	139 weeks	Completed 40 weeks in ACP-2566-004	Primarily a safety study but also exploring RSBQ change from baseline and CGI-I
Open Label Study for Safety and Support Extrapolation of Efficacy									

Study 009 NCT0 49888 67	2/3	US: 7	Sep 2021 to 14 Mar 2022 (Interim) Ongoing	Safety	Trofinetide Oral or G-tube Open-label Week 1: 10 mL (2 g) BID Week 2: 20 mL (4 g) BID Week 4: ≥9 to <12 kg: 25 mL (5 g) BID 12 to <20 kg: 30 mL (6 g) BID	Trof: 14	Period A: 12 Weeks Period B: 21 months	2-4 years old, ≥9 kg and <20 kg at Screening 5 years old, ≥9 kg and <12 kg at Screening Meets clinical criteria for Rett syndrome Proven mutation of the *MECP2* gene CGI-S≥4	Exploratory: Period A: CGI-I at Weeks 2, 4, 8, 12 Change from Baseline in CGI- S at Weeks 2, 4, 8, 12 CaGI-I at Week 12 Change from Baseline in Overall QoL of ICND at Week 12

5.2. Review Strategy

The primary effectiveness determination was made by evaluating the results of ACP-2566-003, the placebo-controlled, 12-week study of trofinetide in subjects with Rett syndrome ages 5 to 20 years. Confirmatory evidence for effectiveness was evaluated from the 200 mg/kg dose cohort in NEU-2566-RETT-002, a placebo-controlled Phase 2 dose-range-finding study of 40 days of trofinetide treatment in subjects with Rett syndrome ages 5 to 15 years. Although the Applicant has submitted NEU-2566-RETT-001 for further supportive evidence, the very low exposures in this study (35 and 75 mg/kg) were considered inadequate to provide sufficient evidence of effectiveness. Extrapolation of effectiveness to ages 2 through 4 years was based on bridging PK data from study ACP-2566-009, an ongoing open label study of trofinetide in that age group.

The safety analyses and determination were made based on evaluating data from both placebo-controlled and long-term open-label exposure data from studies NEU-2566-RETT-001, NEU-2566-RETT-002, ACP-2566-003, ACP-2566-004, ACP-2566-005, and ACP-2566-009.
This review presents the effectiveness results and reports of the Applicant, confirmed by the biometrics reviewer with commentary by this reviewer. The safety analyses are composed of the Applicant's reports in addition to tabulations and analyses conducted by the Division clinical data scientist (CDS) and this reviewer.

6. Review of Relevant Individual Trials Used to Support Efficacy

6.1. ACP-2566-003: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study of Trofinetide for the Treatment of Girls and Women with Rett syndrome

6.1.1. Study Design

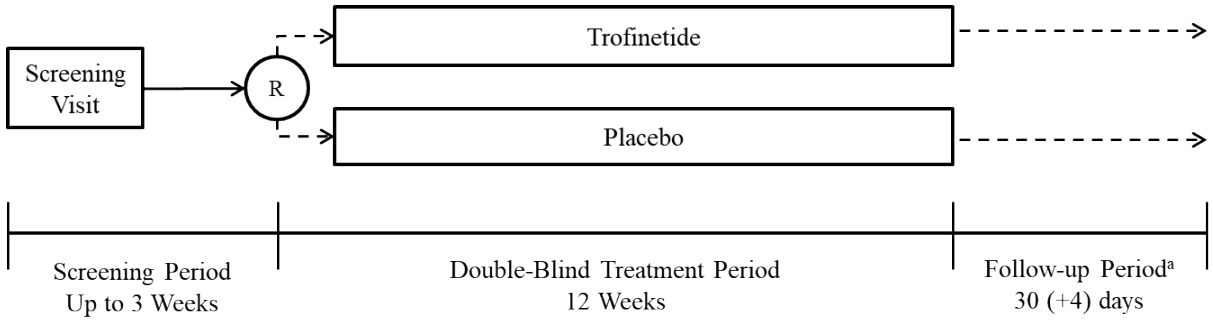
Overview and Objective

ACP-2566-003 was a Phase 3, multi-site, double-blind, placebo-controlled clinical trial to determine the effectiveness of oral trofinetide in girls and women with Rett syndrome. The purpose was to evaluate the effectiveness, safety, and tolerability of weight-based daily dosing of trofinetide. The objectives were to measure effectiveness via two co-primary endpoints, the Rett syndrome Behavior Questionnaire (RSBQ) and the Clinical Global Impression of Improvement score (CGI-I).

Trial Design

Basic Study Design

Figure 1: ACP-2566-003 Study Design Schematic



Source: Study 003 Clinical Study Report (CSR) Figure 9-1
Abbreviations: OLE=open-label extension; R=randomization
[a]If the subject continued into the OLE from the current study, the subject did not complete the follow-up visit, and instead, the last treatment period visit in Study 003 was also the first study visit in OLE Study 004.

ACP-2566-003 was a multicenter, parallel group, randomized, double-blind, placebo-controlled, cohort designed study. The study population were females aged 5 to 20 years old with a genetically confirmed diagnosis of Rett syndrome. They were screened for inclusion, underwent a baseline visit at which time they were randomized 1:1 to trofinetide weight-based daily dosing or placebo. They underwent treatment for 12 weeks with outcome visits at week 2, 6, and 12. If the subject completed the study but did not enroll in the open-label extension (OLE), Study ACP-2566-004, then they had a 30-day post-treatment-end follow-up visit.

REVIEWER COMMENT: This was an appropriate design for a phase 3 effectiveness trial.

Trial Location

The trial took place in the United States between 21 different clinical sites.

Choice of Control Group

There are no FDA approved therapies for the general indication of Rett syndrome. Treatments used in Rett are usually targeting one or a few symptoms, such as anti-epileptics for seizures, sedatives, antidepressants or other central nervous system (CNS) active medications for various neurobehavioral manifestations. Various forms of physical, occupational, speech, recreational, or other therapies are used to attempt to maintain or improve function. Therefore, trofinetide was compared to placebo.

REVIEWER COMMENT: For a clinical trial of an NME with a broad indication of treating multiple symptoms of Rett syndrome, placebo was the appropriate control for this trial.

Diagnostic Criteria

Females aged 5 to 20 years with a diagnosis of typical or classical Rett syndrome by 2010 diagnostic criteria were included. Documentation of a known disease-causing mutation of the MeCP2 gene was required from a College of American Pathologists or Clinical Laboratory Improvement Act/Amendment certified lab. Participants were stratified into 3 age groups (5 to 10, 11 to 15, 16 to 20) and by baseline RSBQ (<35 or ≥ 35).

Key Inclusion/Exclusion Criteria

Key Inclusion Criteria at Baseline:

- Weight ≥12 kg
- Rett syndrome Clinical Severity Score (RTT-CSS) score 10 to 36
- Clinical Global Impression-Severity (CGI-S) score ≥4
- No loss or degradation within 6 months of the following:
    - ambulation (gait, coordination, independence of walking/standing)
    - hand function
    - speech
    - nonverbal communication
    - social skills (using eye gaze, body, social attentiveness to indicate communicative intent)
    - stable seizure pattern or no seizures within 8 weeks
    - concomitant anticonvulsants and psychoactive medications stable for 4 weeks or discontinued at least 2 weeks or 5 half-lives (whichever is greater) prior
    - concomitant non-pharmacological therapy stable for 4 weeks or discontinued at least 2 weeks prior

Key Exclusion Criteria at Baseline:

- Free of treatment with the following within 12 weeks of Baseline:
    - growth hormone
    - IGF-1
    - insulin
- Current clinically significant diseases within these systems:
    - cardiovascular
    - endocrine
    - renal
    - hepatic
    - respiratory

  - gastrointestinal
  - cerebrovascular
  - brain trauma
  - uncorrected visual or hearing
  - malignancy, current or history
- Plan for surgery during trial
- Abnormal basic laboratory tests

REVIEWER COMMENT: The inclusion and exclusion criteria were appropriate for the study of the most common manifestation of classic Rett syndrome in the U.S. In one of the largest natural history studies of Rett syndrome (Tarquinio, Hou et al. 2015), the median RTT-CSS for classic Rett patients was 25 amongst those who survived during the 9-year natural history study, and 35 in the 0.3% of the population that died during that same time. In ACP-2566-003, the mean (standard error) Rett-CSS was 24.1 (0.48) at baseline. Aside from the lack of racial and ethnic diversity in ACP-2566-003, the two samples were comparable to each other with regards to Rett syndrome features.

Dose Selection

Based on the results of NEU-2566-RETT-002, the Applicant's dose-range-finding study, they had determined that children of lower weight had reduced overall exposures as compared to children of higher weight when using a single mg/kg weight-based dosing approach. The Applicant also used exposure-response modeling and simulation that confirmed higher exposure was associated with greater improvement in RSBQ and CGI-I. After determining that the target exposure for effectiveness was an AUC(0-12) of 800-1000 microg*hr/mL, the Sponsor determined that banded weight-based dosing would more effectively achieve that exposure.

Therefore, dosing of trofinetide in ACP-2566-003 was a banded weight-based dosing:
Table 2: Trofinetide Weight Based Dosing

Patient Weight	DAYBUE Dose
9 kg to <12 kg	25 mL twice daily
≥12 kg to <20 kg	30 mL twice daily
≥20 kg to <35 kg	40 mL twice daily
≥35 kg to <50 kg	50 mL twice daily
≥50 kg	60 mL twice daily
Source: Applicant ACP-2566-003 CSR Table 9-3	

Accommodations were planned for dose reductions due to diarrhea as that was a known risk at the time of the study. These included reducing the dose by up to half the assigned dose. Up to

four doses total (consecutive or not) could be withheld within the first six weeks. The goal was to reach the highest dose tolerable close to the assigned weight-based dose.

With regards to exposure-response, the Applicant built exposure response models from 264 subjects from studies NEU-2566-RETT-002 and ACP-2566-003. It should be noted that NEU-2566-RETT-002 used much lower doses. The Applicant summarized that the effects of trofinetide AUC_{0-12}, C_{max}, and C_{avg} as linear functions on slope were statistically significant and produced similar magnitudes of change on RSBQ total score (Applicant Trofinetide Clinical Pharmacology Summary Aid).

Study Treatment

The doses used were weight based as previously shown in Table 2. The drug product is a ready-to-use liquid formulation with concentration of 200 mg/mL and was given to subjects twice daily via mouth or gastrostomy tube as appropriate for the subject. Placebo was administered in the same manner. Concomitant therapies were recorded, and every effort was made to maintain stable regimens. Prohibited treatments were IGF-1, growth hormone, and insulin. Advice and caution were given to avoid QT interval lengthening medications and to keep stable any baseline psychoactive medications.

Assignment to Treatment

Randomization 1:1 was stratified by age group (5 to 10, 11 to 15, 16 to 20) and baseline RSBQ (<35 or ≥35). A minimum of 12 subjects were required to be randomized for each age group. A pre-generated permuted-block randomization schedule was implemented via an interactive response technology system.

Blinding

The Applicant claimed treatment assignments were blinded to subjects, caregivers, investigators, raters, site personnel, and Applicant personnel. Trofinetide and placebo were both strawberry flavored liquid formulations. The study database was locked on November 11, 2021, and unblinding occurred on November 12, 2021. The Applicant did not have a method of assessing maintenance of the blind; however, in their results the Applicant performed an analysis of the coprimary effectiveness endpoints by diarrhea status as that was the most common AE and disproportionately occurred in the trofinetide group. Diarrhea was known as a likely adverse drug reaction (ADR) based on the results of NEU-2566-RETT-002, and it appeared very early in this trial. The data safety and monitoring board (DSMB) noted in their first data review meeting on April 20, 2020, that the rates of diarrhea were likely compromising the blind.

The two co-primary endpoints of change in RSBQ and final rating of CGI-I at the 12 week timepoint are both susceptible given their subjective nature.

> *REVIEWER COMMENT: Given the high frequency of AEs in the trofinetide group, there is a concern for maintenance of the blind and a risk that the blind was not maintained. However, an assessment of maintenance of the blind was not conducted. Given that both coprimary endpoints were dependent on caregiver and clinician rating, such functional unblinding may have had effect on the results of the study. The Applicant was aware that the drug product had changed osmolality between the NEU-2566-RETT-002 and ACP-2566-003, as documented in a graph presented by the Applicant to the DSMB in a meeting on April 24, 2020 (Table 3). Although the AE of diarrhea is likely an ADR, maintaining iso-osmolality in the drug product may have reduced the frequency or severity of diarrhea.*

Table 3: Study Treatment Osmolality

	Study 002 Placebo	Study 002 50 mg/kg BID Dose	Study 002 100 mg/kg BID Dose	Study 002 200 mg/kg BID Dose	Study 003 Active Dose Arm (Average ~300 mg/kg BID)	Study 003 Placebo
Trofinetide Concentration (mg/ml)	0	25	50	100	200	0
Neuren Estimated Osmolality (mOsm/kg)	50	90	180	360	740	NA
ACADIA Estimated Osmolality (mOsm/kg)	NA	NA	NA	NA	956	279

Source: Applicant DSMB Presentation April 20, 2020

Dose Modification/Dose Discontinuation

Does modification/reduction to as low as half the prescribed dose was allowed to manage adverse events during the first 6 weeks of treatment. Up to four doses (consecutive or not) could be withheld in the setting of an AE in the first 6 weeks. If the assigned dose could not be tolerated, the investigator could assign the highest tolerated dose. Diarrhea was anticipated based on study NEU-2566-RETT-002 and found to be very frequent by the time of enrollment of 25% of subjects in ACP-2566-003 in April of 2020 (first randomization had occurred in October 2019). For this reason, the protocol was amended March 12, 2021, with the following recommendations being circulated to study investigators:

1. Stop all bowel medications the day the subject is randomized. If no diarrhea occurs during double-blind treatment, hold all bowel medications the first day of open-label dosing.
2. Ask caregivers to contact the coordinator at the first episode of diarrhea, even if it is just softening of stool. Do not wait for the stool to become watery.
3. Immediately start loperamide (IMODIUM®) and psyllium (Metamucil®). Tell caregivers to use the loading dose of IMODIUM® (which is usually 15 mL), then have them use the secondary dose daily (which is usually 7.5 mL). The Metamucil® is daily and dosed per the packaging instructions.
4. If there is no bowel movement for 2 to 3 days, hold the IMODIUM® until a bowel movement occurs, then use the IMODIUM® as needed until the subject displays a good balance.
5. Metamucil® continues even if the diarrhea resolves.
6. A diet of cereal or bananas, rice, applesauce, and toast may help with the diarrhea as well.

Note: If the subject stops all their regular bowel medications and does NOT have diarrhea or a bowel movement for 2 to 3 days, resume all their normal bowel medications so the subject does not become constipated.

REVIEWER COMMENT: As previously reviewed, the risk of unblinding of caregivers and clinicians (the raters of the co-primary endpoints) was concerning given the rates of diarrhea and nausea/vomiting on trofinetide. Given that a formalized process was implemented to manage diarrhea, which occurred early after exposure to trofinetide, it would not be unreasonable to conclude that functional unblinding may have occurred within the first 4 weeks after randomization.

Administrative Structure

A number of vendors were contracted with Acadia Pharmaceuticals for various aspects of the conduct of ACP-2566-003. Most critically, (b) (4) created the subject randomization schedule and supported the DSMB and (b) (4) was the clinical research organization (CRO).

An independent data and safety monitoring board (DSMB) was chartered October 18, 2019, consisting of 5 members who were completely independent of the Applicant, CRO, IRBs and investigators. The DSMB committee was convened to periodically review unblinded safety data

during the conduct of both the pivotal study ACP-2566-003, and the open-label extensions ACP-2566-004 and ACP-2566-005. The provision of unblinded data was facilitated by an independent statistical group, comprising study-independent statisticians and programmers, who served as a firewalled communication gateway for data queries and additional data/analyses (if applicable) between the Applicant and the DSMB, ensuring that all Acadia study personnel remained blinded to treatment assignments. The DSMB met for 4 scheduled meetings and 2 ad hoc meetings over the course of the study. The DSMB was supposed to schedule a closed meeting after the 4th data unblinding meeting with the Applicant; however, no records were provided for that.

Source documentation consisted of source notes captured by site personnel, caregiver diaries, lab, ECG, and other electronic source data. These were entered and validated into an electronic data capture (EDC) database by trained site personnel. The clinical study report (CSR) indicated that the Applicant's monitor inspected the eCRFs at regular intervals throughout the study to verify adherence to the protocol amongst other quality measures. Three of the 21 sites participating in the study were audited by the Applicant to assess trial conduct and compliance with the protocol, International Conference on Harmonization guidance on Good Clinical Practice, and other regulatory requirements. The study database was locked on November 11, 2021, and unblinding occurred on November 12, 2021.

REVIEWER COMMENT: The administrative structure of ACP-2566-003 was appropriate for a Phase 3 pivotal clinical trial.

Procedures and Schedule

Table 4: Study Schedule for ACP-2566-003

Period	Screening	Baseline	Double-blind Treatment Period			Safety Follow-up[b]
Visit week		0	2[a]	6	12/EOT/ET	EOT/ET+ 30 days
Visit number	1	2	3	4	5	
Visit window (days)	N/A	N/A	±3	±4	+3	+4
Type of visit[k]	Clinic or Off-site					Telephone or Telemedicine
Informed consent	X				X[c]	
Inclusion/exclusion criteria	X	X				
Medical history and demographics	X					
Confirm documented Rett diagnosis and *MECP2* mutation	X					
Rett syndrome history	X					
Rett syndrome Clinical Severity Scale	X					
Physical examination[k]	X	X	X	X	X	
Vital signs[d]	X	X	X	X	X	
Height	X				X	
Weight	X	X	X[k]	X[k]	X[k]	
12-lead ECG[e]	X	X[e]	X	X	X	
Clinical laboratory tests (hematology, chemistry)	X	X	X	X	X	
Urinalysis	X	X	X		X	
TSH, Free T3, Free T4	X	X			X	
HbA_{1c}	X					
Serum pregnancy test[f]	X		X	X	X	
Blood samples for pharmacokinetics		X[g]	X[h]	X[h]	X[h]	

Blood sample for optional analysis for biomarkers[i]		X			X	
Rett syndrome Behaviour Questionnaire (RSBQ)	X	X	X	X	X	
Period	Screening	Baseline	Double-blind Treatment Period			Safety Follow-up[b]
Visit week		0	2[a]	6	12/EOT/ET	EOT/ET+ 30 days
Visit number	1	2	3	4	5	
Visit window (days)	N/A	N/A	±3	±4	+3	+4
Type of visit[k]	Clinic or Off-site					Telephone or Telemedicine
Clinical Global Impression–Improvement (CGI-I)			X	X	X	
Clinical Global Impression–Severity (CGI-S)	X	X	X	X	X	
Communication and Symbolic Behavior Scales Developmental Profile™ Infant-Toddler (CSBS-DP- IT) Checklist		X	X	X	X	

Source: Adapted from Applicant ACP-2566-003 CSR Table 9-1

Abbreviations: ECG=electrocardiogram; EOT=end of treatment; ET=early termination; HbA_{1c}=glycosylated hemoglobin; *MECP2*=methyl-CpG-binding protein 2 gene (in humans); OLE=open-label extension; PK=pharmacokinetics; RTT-COMC=Rett syndrome Clinician Rating of Ability to Communicate Choices; TSH=thyroid stimulating hormone

[a] Timing of postbaseline visits was calculated from the first day of dosing (Day 1) (i.e., the Week 2 visit occurred 2 weeks [±3 days] after the first day of dosing).

[b] Subjects who rolled over into the OLE study did not have the safety follow-up telephone call.

[c] For subjects who decided to continue into the OLE study, informed consent for the OLE was obtained prior to performing the Week 12/EOT procedures.

[d] Vital signs included body temperature, resting respiration rate, sitting systolic and diastolic blood pressure, and pulse rate. The sitting blood pressure was measured after the subject had been sitting for ≥3 minutes.

[e] ECGs were completed in triplicate at Visit 1 (Screening), at Visit 2 (Baseline) both before dosing and 2 to 3 hours after dosing, and at Visit 5 (Week 12/EOT/ET). A single ECG was completed at Visit 3 (Week 2) and Visit 4 (Week 6).

[f] For subjects who reached menarche and had not had surgical sterilization.

[g] A predose PK blood sample was collected before administration of study drug. A postdose PK blood sample was collected at the end of ECG assessment 2 to 3 hours after study drug administration.

[h] PK samples at Visits 3, 4, and 5 were collected at one of the following time intervals: 1) 2 to 3 hours after dosing OR 2) 4 to 6 hours after dosing OR 3) 7 to 11 hours after dosing. Every effort should have been made to collect the PK samples at discrete time intervals during Visits 3, 4, and 5. However, if the interval was the same across these visits, then the collection time should have varied within that interval.

[i] Participation in the effort to identify biomarkers was an optional component of the study requiring a separate informed consent, which may have been obtained at any time during the study. If consent was obtained after Baseline, only the sample at Visit 5 (or upon early termination) was taken.

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

[k] Study visits may have been done off-site rather than in the clinic with the prior approval of the Applicant or Medical Monitor. Screening, Baseline, and EOT visits should have been done in the clinic whenever possible. When a study visit took place off-site, the physical examination was not be required. Weight was measured whenever possible at off-site visits. The RTT-COMC was to be completed, if possible, but it was not required.

Dietary Restrictions/Instructions

The study started with medication dosing to occur during fasting. However, an amendment was made on August 7, 2020, after results from a completed healthy volunteer (HV) study demonstrated minimal difference on trofinetide pharmacokinetics by food. As noted in 6.1.1 Study Design – Dose Modification/Dose Discontinuations, a suggested protocol was implemented after April 2020 due to the frequency of diarrhea that included anti-diarrheal measures, including fiber enriched diet. While ketogenic diet was not excluded, subjects had to be stable and remain on the diet if they were to participate in the study.

> *REVIEWER COMMENT: Given the known ADR of diarrhea from the highest dose cohort of NEU-2566-RETT-002, the dietary plan regarding diarrhea management should have been developed prior to start of ACP-2566-003. Once implemented, these dietary guidelines were appropriate. However, it is also concerning that such a dietary plan would not have been implemented for subjects who did not experience diarrhea, and hence a clear unblinding variable was introduced.*

Concurrent Medications

There were no run-in or wash-out periods with the study drugs. Concomitant therapies (which are all symptomatic in Rett syndrome) were allowed except for treatment with the following within 12 weeks of the baseline visit:

- growth hormone
- IGF-1
- insulin

All other concomitant therapies were to be stable within 4 weeks, 2 weeks, or 5 half-lives before the baseline visit. After a protocol amendment in August 2020, specific suggestions were made to mitigate the risk of diarrhea, including instructions on dosing of loperamide.

Treatment Compliance

Initial study drug was dispensed at the site at the baseline visit. Subsequent study drug shipments were made directly to the subject's home from a central pharmacy. Cold chain/refrigerated conditions had to be maintained. Patient families were instructed to return all bottles of medication at subsequent visits and this return allowed for treatment compliance accounting which was recorded in the eCRF. The caregiver diary was also used by family members to record missed or modified doses.

The study drug was provided in liquid form supplied in a 500 mL bottle. The total volume of drug taken was calculated as total drug dispensed less total drug returned. The volume of drug returned was estimated based on height of the liquid in centimeters of drug remaining in the

bottle and recorded in the eCRF. The estimated conversion was based on an Applicant created estimate seen in Table 5.

Table 5: Conversion of Height of Remaining Liquid (cm) into Volume (mL)

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

Source: Applicant ACP-2566-003 CSR

The study drug compliance was calculated as a percentage of the total volume of drug taken divided by the total volume of drug expected to be taken. The total volume of drug expected to be taken was based on the duration of exposure and dosing schedule based on weight-based dosing. However, if there was any dose modification prescribed by the investigator due to intolerance, the total drug expected to be taken was adjusted accordingly to account for the modified prescribed dose schedule as recorded in the EDC. Treatment compliance was also summarized as a categorical variable. The number and percentage of subjects within each of the following compliance levels was tabulated: <80%, 80 to 120% and >120%.

> *REVIEWER COMMENT: This method of calculating compliance, using the volume of liquid in the bottle, without also cross-referencing with the caregiver diary regarding daily dose was problematic, as evidenced by the report of compliance rates over 100%. The concern is that rather than indicating simply amount of drug used, this method also accounts for liquid spilled, potentially vomited or otherwise lost, and may overestimate compliance, especially if related to a gastrointestinal AE.*

Rescue Medications

As there are no FDA-approved or off-label standard of care therapies for Rett syndrome, there was no rescue medication option.

Subject Completion/Discontinuation/Withdrawal

The following analysis set definitions were used by the Applicant, as articulated in their statistical analysis plan:

> Randomized Analysis Set
> The randomized analysis set consisted of all subjects who were randomized. Subjects were classified according to the randomized treatment assignment.
> Safety Analysis Set
> The safety analysis set consisted of all randomized subjects who received at least one dose of study drug. The safety analysis set was analyzed according to the actual treatment received.
> Full Analysis Set
> The full analysis set (FAS) consisted of all subjects who were randomized, received at least one dose of study drug, and had both a baseline value and at least one post-baseline value for the RSBQ total score or had at least one CGI-I score after taking study medication. The FAS was analyzed according to the randomized treatment assignment regardless of the actual treatment received.
> Per-protocol Analysis Set
> The per-protocol analysis set consisted of the subjects in the full analysis set who did not have a major protocol violation that would affect interpretation of the effectiveness data. The per-protocol analysis set was defined prior to study unblinding. The per-protocol analysis set was analyzed according to randomized treatment assignment.
> Pharmacokinetics (PK) Analysis Set
> The PK analysis set consisted of the subjects in the safety analysis set with at least one measurable trofinetide whole blood concentration. Subjects were classified according to the actual treatment received.

Patients were allowed to withdraw consent for the study at any time. Patients could be removed by the Investigator for the use of prohibited medications (IFG-1, growth hormone, insulin) or for major changes in CNS medications or other somatic treatment regimens. Patients could be discontinued from the study for AEs, death, increase in post-baseline QTcF, lack of effectiveness, non-compliance with study drug, physician decision, pregnancy, protocol deviation, study termination by Applicant, or other. Planned sample size calculations were anticipating a discontinuation rate of up to 5%.

Study Endpoints

The co-primary endpoints for ACP-2566-003 were the RSBQ and CGI-I. As documented in section 3.2 of this review, these endpoints were proposed by the Applicant at the end-of-phase 2 (EOP2) meeting of October 12, 2017, with the CGI-I encouraged by the Agency to assist with evaluation of clinical meaningfulness of the RSBQ. The RSBQ is a 45-item observer rated outcome (ObsRO) that asks caregivers to rate a set of symptom occurrences over the previous two weeks as "not true as far as you know," "somewhat or sometimes true," or "very true or often true." All but 1 of the 45 items are worded as a predominant pathological symptom of Rett syndrome, and hence higher scores represent more or worse symptoms of the disease. Because item 31 of the RSBQ rates the subject on ability to use "eye gaze to convey feelings, needs, and wishes", the numerical ratings of 1, 2,3 are opposite in quality to the rest of the RSBQ. Therefore, after administration, the scoring of this item was reversed to calculate the derived RSBQ which was used as the co-primary endpoint. Of note, this reversal was not used as part of the stratification of subject randomization by baseline RSBQ. In scoring for ACP-2566-003, the 45 items were divided into 8 domains (Table 6) per Mount, et al (Mount, Charman et al. 2002). It should be noted that in the prior study, NEU-2566-RETT-002 which is reviewed for confirmatory evidence, items 11 and 26 were included in the body rocking and expressionless face subscale and item 31 was not included in any subscale (Kaufmann, Tierney et al. 2012). The RSBQ was administered by trained personnel at baseline and visits 3, 4, 5.

Table 6 RSBQ Subscales and Assigned Items in ACP--2566-003

RSBQ subscale	Number	RSBQ subscale items (Description)
General mood	2	spells of screaming for no apparent reason during the day
	14	abrupt changes in mood
	15	certain periods when performs much worse than usual
	16	times when appears miserable for no apparent reason
	22	screams hysterically for long periods of time and cannot be consoled
	29	times when irritable for no apparent reason
	30	spells of inconsolable crying for no apparent reason during the day
	36	vocalizes for no apparent reason
Breathing problems	1	times when breathing is deep and fast
	5	times when breath is held
	6	air or saliva expelled from mouth with force
	19	swallows air
	25	abdomen fills with air and sometimes feels hard
Hand behaviors	18	does not use hands for purposeful grasping
	20	hand movements uniform and monotonous
	21	has frequent naps during the day
	24	restricted repertoire of hand movement
	35	has difficulty in breaking/stopping hand stereotypies
	43	amount of time spent looking at an object is longer than time spent manipulating or holding

Repetitive face movements	4	makes repetitive movements involving fingers around tongue
	28	makes mouth grimaces
	32	makes repetitive tongue movements
	34	makes grimacing expressions with face
Body rocking/ expressionless face	12	expressionless face
	17	seems to look through people into the distance
	31	uses eye gaze to convey feelings, needs and wishes (reversed)
	33	rocks self when hands are prevented from moving
	40	tendency to bring hands together in front of chin or chest
	41	rocks body repeatedly
Night-time behaviors	13	spells of screaming for no apparent reason during the night
	37	spells of laughter for no apparent reason during the night
	42	spells of inconsolable crying for no apparent reason during the night
Fear/anxiety	7	spells of apparent anxiety/fear in unfamiliar situations
	9	seems frightened when sudden changes in body position
	10	times when parts of body held rigid
	38	spells of apparent panic
Walking/Standing	39	Walks with stiff legs
	23	Although can stand independently tends to lean on objects or people
Items not included in subscales	3	Makes repetitive hand movements with hands apart
	8	Grinds teeth
	11	Shifts gaze with a slow horizontal turn of head
	26	Spells of laughter for no apparent reason during the day
	27	Has wounds on hands a result of repetitive hand movements
	44	Appears isolated
	45	Vacant 'staring' spells
Source: Applicant Created Table as part of response to information requestion from division regarding the use of subscale scores in the two studies ACP-2566-003 and NEU-2566-RETT-002		

The CGI-I is a clinician rated outcome (CRO) measuring global clinical impression, specifically of improvement. The CGI-I is administered as a 7-point scale where the clinician rates the subject's condition in the previous week from 1 (very much improved) to 7 (very much worse). It was administered at Visits 3, 4, 5 along with the CGI-S which was a secondary measure. Training of CGI-I raters included a standard presentation, quiz, and discussion of 6 vignettes with gold-standard ratings and a quiz with 2 vignettes on which concordance with gold-standard raters was required (1 point on 1 vignette, gold standard on the other). The CGI-I was anchored to the raters' experience with the Rett syndrome population and required the rater to have a certain set of qualifications or to undergo specialized training.

REVIEWER COMMENT: The Agency expressed concern regarding the use of the RSBQ at the EOP2 meeting dated October 24, 2017. AIthough extensively used in Rett syndrome research, the RSBQ is a problematic observer reported outcome given that multiple items may reference similar constructs, multiple items require interpretation by the observer, multiple items assess disease signs rather than directly reflecting how a subject feels, functions, or survives. The CGI-I was directly recommended by the FDA as an anchoring measure of function to be used as a co-primary endpoint.

Secondary Endpoints

The main secondary endpoint for this study was the Communication and Symbolic Behavior Scales Developmental Profile Infant-Toddler Social Composite Score (CSBS-DP-IT-SCS). The CSBS-DP-IT is a caregiver rated outcome checklist of 24 items broken into 7 subscales of behavior. The first 13 items in 3 subscales, "emotion and eye gaze", "communication", and "gestures" make up the social composite score. Those 3 subscales ask the primary caregiver to rate the frequency of particular behaviors on a 3- point Likert-like scale of "not yet/sometimes/often", with higher scores indicating more normative nonverbal communicative behavior. Many of the questions begin with "Does your child" and then asks about a specific behavior. However, the first question asks, "Do you know when your child is happy and when your child is upset?". The CSBS-DP-IT was originally developed as a screening assessment of communication in otherwise healthy preverbal infants ages 6-24 months. The original intention of this screener was to detect potential communication deficits, not to categorize communication abilities or to monitor changes during development.

In ACP-2566-003, the entire 24 CSBS-DP-IT was administered to parents at baseline (Visit 2) and 3 time-points after treatment initiation (Visits 3, 4, 5). Study staff administered the CSBS-DP-IT to caregivers after receiving training, live or video, and passing a quiz and had to otherwise have experience with neurodevelopmental disorders or PROs/ObsROs. With regards to secondary endpoints, the Agency had advised the Applicant that use of the CSBS-DP-IT-SCS as a key secondary endpoint (b) (4) would require justification of its use in the older age group, justification regarding the importance of social communication in this population, individual level responses and rater information, and would be a matter of review. The Applicant cited the March 11, 2022, Patient Focused Drug Development meeting and scientific articles indicating the importance of communication for caregivers of patients with Rett syndrome and the ability of patients with Rett to use eye-gaze and gestures to communicate (Lavas, Slotte et al. 2006, Didden, Korzilius et al. 2010, Glaze, Percy et al. 2010, Urbanowicz, Downs et al. 2016). The Applicant also noted that after age 3, communication abilities are not related to age and that communication impairment in Rett syndrome was not dependent on age (Cass, Reilly et al. 2003, Bartolotta, Zipp et al. 2011, Urbanowicz, Downs et al. 2016, Townend, Bartolotta et al. 2020). The Applicant cited 3 instances of the CSBS-DP-IT-SCS being used specifically to assess communication in developmental disorders including Rett syndrome

(Anagnostou, Jones et al. 2015, O'Leary, Kaufmann et al. 2018) in order to justify its use in ACP-2566-003. The Applicant also posited that given that the RSBQ only has 1 of 45 items related to communication and eye gaze, that the CSBS-DP-IT-SCS assessed the domain of communication distinctly from what could be ascertained from the RSBQ.

REVIEWER COMMENT: As reviewed in Section 3.2, the Applicant was advised that the CSBS-DP-IT-SCS would need to measure a functional outcome distinct from those measured by the primary outcomes, for the results of this secondary outcome to be used (b) (4) *The Applicant was also advised to provide a rationale as to the use of this measure in the subject population. The Applicant argued that only item 31 of the RSBQ which asks about eye movement as a form of communication is directly related to the CSBS-DP-IT-SCS. The Applicant also explained that the CSBS-DP-IT has been used in older children with developmental disability including subjects with Rett syndrome.*

(b) (4) *The Applicant has demonstrated that the CSBS-DP-IT-SCS is an additional measure of non-verbal communication that contributes to the demonstration of effectiveness for trofinetide. However, this tool was originally created as a screening tool for pre-verbal infants. Its validation and reliability have been demonstrated in populations of otherwise healthy infants to identify those who may not be meeting communication milestones. Scoring and interpretation of a screening tool are generally significantly different than those of a measurement tool. While we acknowledge that the Applicant has referred to three studies in which the CSBS-DP-IT-SCS was used with older subjects with developmental disorders, these studies do not establish that it is a tool fit for the purpose of measuring communication and a treatment effect in a clinical trial. Furthermore, the 13-point SCS do not represent the full concept of social reciprocity in communication. Therefore, the results of this analysis may provide support for the findings on the co-primary endpoints,* (b) (4) *.*

Exploratory, Safety, and Other Endpoints

Other ObsROs used included the Rett syndrome Caregiver Burden Inventory (RTT-CBI) and Impact of Childhood Neurologic Disability Scale (ICND). Other CROs included the Rett syndrome Clinician Rating of Hand Function (RTT-HF), Rett syndrome Clinician Rating of Ambulation and Gross Motor Skills (RTT-AMB), Rett syndrome Clinician Rating of Ability to Communicate Choices (RTT-COMC), and Rett syndrome Clinician Rating of Verbal Communication (RTT-VCOM). All of these Clinician Rating scales are actually sub-scales of the Rett Syndrome Domain Specific Visual Analog Scale (RTT-DSC), a scale used in NEU-2566-RETT-002.

Patients also underwent physical examinations, ECG, basic laboratory evaluations, drug PK labs, and subject families were provided with a diary to record seizures and spells, dietary intake,

and medication use.

Statistical Analysis Plan

Version 1.0 of the statistical analysis plan submitted January 30, 2021, was reviewed by the Agency. The prespecified plan included defining the full analysis set (FAS) as all subjects who were randomized, received at least 1 dose of study drug, and had both baseline and at least one post-baseline value for RSBQ total score and 1 post-baseline CGI-I assessment. The FAS was to be analyzed according to the treatment assigned regardless of treatment received. The primary analysis method was direct likelihood mixed model for repeated measures (MMRM) assuming missing data are missing at random (MAR). A mixture of covariates was to be used and an unstructured covariance matrix was assumed with a plan to deal with convergence failure. Sensitivity analyses were also planned for data missing not at random (MNAR) and for data missing due to COVID-19 public health emergency. The two co-primary hypotheses were tested as gatekeepers with two-sided alpha = 5% without multiplicity adjustment. The secondary hypothesis regarding CSBS-DP-IT-SCS was tested also at two-sided alpha = 5%.

The Agency advised the Applicant to describe all possible intercurrent events including AEs and withdrawals and plans for maintenance of the blind. The Agency also advised to differentiate between missing data due to COVID-19 versus remote collected data due to COVID-19.

The final SAP was dated October 20, 2021.

Protocol Amendments

The protocol was amended April 27, 2020, subsequent to the pause of screening and enrollment on March 18, 2020, due to the COVID-19 public health emergency. This amendment allowed for off-site methods of performing safety and effectiveness assessments. This protocol also detailed how dose adjustments should be done for subjects who did not tolerate drug and added 2 study sites and multiple other amendments.

Amendment 2 was made to the protocol dated August 7, 2020, which increased the number of sites from 20 to 28 and made multiple editorial changes. More critically, as discussed in the section on dietary restrictions/instructions, this amendment also added a comprehensive plan for managing diarrhea.

> *REVIEWER COMMENT: As previously mentioned, the dissemination of the diarrhea management plan as part of Amendment 2 may have compromised the blinding of the study.*

6.1.2. Study Results

Compliance with Good Clinical Practices

The Applicant provided attestation of performance of the study in accordance with Good Clinical Practice and International Conference on Harmonization regulations and guidance.

Financial Disclosure

Financial disclosures provided indicate that none of the investigators had a conflict of interest that would influence the conduct or outcomes of the study.

Protocol Violations/Deviations

In Table 14.1.4.1 of the ACP-2566-003 CSR, page 199/1039, the Applicant presented the major protocol deviations which affected 24 of the 187 subjects randomized. The majority of these were in study procedures (18 of the 24).

> *REVIEWER COMMENT: While the sample size is small, there was no obvious discrepancy between trofinetide and placebo groups with regard to frequency of major protocol violations. This reviewer's analysis of the DV domain SDTM file provided by the Applicant indicates that there were 701 protocol deviations, the majority of which (677) were minor. This reviewer scanned the line listing of deviations and, overall, the majority appeared to be related to procedural deviations including missing of various tests or outcome measures and improper return of medication bottles. While only 42 protocol deviations occurred with the investigational product, a greater number (n=29, 69%) occurred in the trofinetide group, which is notable. It is also notable that this number of deviations is greater than the average of 100-200 deviations found across large pharmaceutical trials by metanalysis (Getz, Smith et al. 2022).*

Patient Disposition

The first subject was randomized on October 29, 2019. The study database was locked on November 11, 2021, and unblinding occurred on November 12, 2021.

The Applicant presented the data displayed in Table 7 of subject disposition in the CSR, which this reviewer confirmed with comments below.

Table 7 ACP-2566-003 Subject Disposition

Subject Disposition	Placebo (N=94) n (%)	Trofinetide (N=93) n (%)	Total (N=187) 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			
Adverse Event	2 (2.1)	16 (17.2)	18 (9.6)
Death	- -	- -	- -
Lack of Efficacy	- -	1 (1.1)	1 (0.5)
Lost to Follow-Up	- -	- -	- -
Non-Compliance with Study Drug	- -	4 (4.3)	4 (2.1)
Use of Prohibited Medication	- -	- -	- -
Physician Decision	- -	- -	- -
Pregnancy	- -	- -	- -
Protocol Deviation	1 (1.1)	- -	1 (0.5)
Study Terminated by Applicant	- -	- -	- -
Subject Withdrew Consent	1 (1.1)	1 (1.1)	2 (1.1)
Other	5 (5.3)	1 (1.1)	6 (3.2)
Other: COVID-19 Withdrawal of Consent	- -	- -	- -
Other: COVID-19 Quarantine Measures	5 (5.3)	1 (1.1)	6 (3.2)
Other: Not Related to COVID-19	- -	- -	- -

Source: Applicant ACP-2566-003 CSR Table 14.1.3.1

Patient distribution in the various analysis sets is displayed in Table 8.

Table 8 ACP-2566-003 Analysis Sets

Analysis set	Placebo n (%)	Trofinetide n (%)	Total n (%)
Randomized analysis set[a]	94	93	187
Safety analysis set[b]	94 (100.0)	93 (100.0)	187 (100.0)
Full analysis set[c]	93 (98.9)	91 (97.8)	184 (98.4)
Per-protocol analysis set[d]	90 (95.7)	89 (95.7)	179 (95.7)

Source: Applicant ACP-2566-003 CSR Table 14.1.2.1

Abbreviations: CGI-I=Clinical Global Impression-Improvement; RSBQ=Rett syndrome Behaviour Questionnaire

[a] The randomized analysis set was used as the denominator for calculating percentages within each treatment group. The randomized analysis set consisted of all subjects who were randomized.

[b] The safety analysis set consisted of all randomized subjects who received at least one dose of study drug.

[c] 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.

[d] 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 effectiveness data and who had adequate treatment compliance (≥75%).

REVIEWER COMMENT: On review of the narratives, this reviewer determined that the trofinetide subjects listed as withdrawing due to "Non-Compliance with Study Drug" and "Subject Withdrew Consent" had all experienced AEs prior to study drug non-compliance and withdrawal. Specifically, subjects ACP-2566-003- (b) (6) *and ACP-2566-003-*

(b) (6) *both experienced diarrhea prior to non-compliance with study drug and should be considered as having discontinued study drug due to the AE of diarrhea.*

Also, in collaboration with our Office of Biostatistics reviewer, we confirmed that 7 subjects on trofinetide and 1 subject on placebo discontinued study medication; however, subjects returned for end-of-study visits between day 64 and 115 which, based on the SAP, were used as primary outcome 12-week data. Therefore, completion of the study as defined by completing a 12-week visit occurred for n = 86 placebo subjects and n = 77 trofinetide subjects. These counts do not affect the Full Analysis Set, which counts all subjects randomized and with any post-baseline measurement of both RSBQ and CGI-I but is necessary to understand the difference between study withdrawals and medication discontinuations.

Patient Demographics

Table 9: ACP-2566-003 Demographics

	Placebo		Trofinetide		All	
Recruitment Stratified Age Groups	N	% of Total	N	% of Total	N	% of Total
5 to 10 Years	52	28%	49	26%	101	54%
11 to 15 Years	24	13%	25	13%	49	26%
16 to 20 Years	18	10%	19	10%	37	20%
All	94	50%	93	50%	187	100%
FDA Age Groups						
5 to <12 Years	55	29%	53	28%	108	58%
12 to <17 Years	24	13%	23	12%	47	25%
>= 17 Years	15	8%	17	9%	32	17%
Race						
WHITE	90	48%	82	44%	172	92%
ASIAN	1	1%	5	3%	6	3%
OTHER	2	1%	4	2%	6	3%
BLACK OR AFRICAN AMERICAN	1	1%	1	1%	2	1%
NATIVE HAWAIIAN OR OTHER PACIFIC ISLANDER	0	0%	1	1%	1	1%
All	94	50%	93	50%	187	100%
Ethnicity						
NOT HISPANIC OR LATINO	84	45%	86	46%	170	91%
HISPANIC OR LATINO	10	5%	7	4%	17	9%
All	94	50%	93	50%	187	100%

Source: Reviewer JMP Analysis Using ADSL

Reviewer Comment: The demographics of ACP-2566-003 were not well representative of racial and ethnic minorities of the United States, specifically of Black or African American, Asian, and Hispanic or Latino populations. However, there is no reason to anticipate that

efficacy or safety would be different in these populations based on the hypothesized mechanisms of the drug.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

The mean age at Rett syndrome diagnosis was 2.4 years, with mean age at first symptoms noticed of 0.8 years. The distribution of mutation types was similar between the placebo and trofinetide groups. The mutation distribution in ACP-2566-003 were similar to those reported in various Rett natural history studies and databases in the United States (Ehrhart, Jacobsen et al. 2021).

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The Applicant reports that of the 187 subjects in the safety analysis set, 182 subjects (97.3%) were ≥ 80% compliant with study drug, with 92 (97.9%) in the placebo group and 90 (96.8%) in the trofinetide group. The Applicant also reported that all subjects received at least 1 concomitant medication, with antiepileptics (68.4%) and drugs for constipation (65.2%) being the most frequently used by anatomical therapeutic chemical class in both treatment groups. Antipropulsives (50.5%) were the most frequently used concomitant medications by in the trofinetide group. (Source Applicant ACP-2566-003 CSR Table 11-5)

Reviewer Comment: Overall, disease characteristics were well balanced between the two study groups. As noted in Section 6.1.1 Treatment Compliance, the method of measuring individual compliance was problematic as it likely overestimated compliance as evidenced by the 55 (29.4%) subjects in the safety analysis set who had recorded compliance >100% (i.e., the total volume of drug taken was greater than the total volume of drug expected to be taken). The concern is that greater than expected loss of drug volume, when not reported as overdose, likely represented inadvertent loss. With the loss being greater in the trofinetide group (see Table 10), this may have been due to increased refusal/vomiting/spitting-up or other AE related loss not otherwise captured. This also implies that the per protocol set (which required >75% compliance) overestimated the number of subjects to include.

Table 10: Percent Treatment Compliance by Volume for 55 Patients with Compliance >100%

Placebo		Trofinetide	
N	Mean	N	Mean
25	103.7	30	110.4

Source: Reviewer via JMP, ADSL

With regards to treatment emergent concomitant therapies, this reviewer's analysis clearly highlights a difference between the treatment groups in the use of loperamide, plantago ovato, and oxcarbazepine. This could confound both effectiveness analysis via unblinding and adverse reaction analyses. Most concerning to this reviewer is the potential CNS consequences of the chronic use of loperamide, which can be sedating and potentially addictive (Lasoff, Koh et al. 2017). Also concerning is the increased use of the antiepileptic oxcarbazepine, which may indicate an increase in seizure rate in the trofinetide group not captured by AE reporting of seizures.

Table 11: Treatment Emergent Concomitant Medication 2x Relative Risk with Trofinetide and Use in Minimum of 4 Patients

		Trofinetide (N = 93)		Placebo (N = 94)		
Medication Class	Standardized Medication Name	Count	%	Count	%	Total
ANTIPROPULSIVES	LOPERAMIDE HYDROCHLORIDE	38	40.9%	1	1.1%	39
	LOPERAMIDE	11	11.8%	2	2.1%	13
HERBAL INTESTINAL ADSORBENTS	PLANTAGO OVATA	22	23.7%	.	.	22
CARBOXAMIDE DERIVATIVES	OXCARBAZEPINE	15	16.1%	5	5.3%	20
PIPERAZINE DERIVATIVES	CETIRIZINE	7	7.5%	2	2.1%	9
	CETIRIZINE HYDROCHLORIDE	6	6.5%	3	3.2%	9
CALCIUM	CALCIUM	4	4.3%	2	2.1%	6
OTHER COMBINATIONS OF NUTRIENTS	FISH OIL	5	5.4%	1	1.1%	6
OTHER ANTIEPILEPTICS	LACOSAMIDE	4	4.3%	1	1.1%	5
OTHER ANXIOLYTICS	ESCITALOPRAM OXALATE	3	3.2%	1	1.1%	4
BULK-FORMING LAXATIVES	PLANTAGO OVATA	.	.	1	1.1%	1

Source: Reviewer ACP-2566-003 JMP Clinical Concomitant Medication Report

Efficacy Results – Primary Endpoint

The Applicant presented, and the Agency biostatistical reviewer confirmed, a statistically significant difference favoring trofinetide over placebo as measured by a mixed model repeated measures (MMRM) analysis of change from baseline (CFB) in the RSBQ and in the CGI-I Score at

the 12-week timepoint. The details of these findings are available in Table 12 and Table 13.

Table 12 ACP-2566-003 Primary Analysis of RSBQ and its CFB in the Full Analysis Set

Visit	Placebo (N=93)	Trofinetide (N=91)
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[a]		
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)		-3.1 (1.30)
95% CI		(-5.7, -0.6)
Two-sided p-value		0.0175
Effect size (Cohen's *d*)		0.37

Source: Applicant ACP-2566-003 CSR Table 14.2.1.1

Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; MMRM=mixed-models for repeated measures; RSBQ=Rett syndrome Behaviour Questionnaire; SD=standard deviation; SE=standard error

Note: Baseline was the latest non-missing value prior to the first dose of study drug.[a] The mixed model for repeated measures (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. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

Table 13 ACP-2566-003 Analysis of Co-Primary Endpoint CGI-I Full Analysis Set

Visit	Placebo (N=93)	Trofinetide (N=91)
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		
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)
Two-sided p-value		0.0030
Effect size (Cohen's d)		0.47

Source: Applicant ACP-2566-003 CSR Table 14.2.2.1

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.[a] 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. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

The Applicant performed sensitivity analyses that were all prespecified. The pattern-mixture-model with missing-not-at-random assumptions calculated the trofinetide-placebo LS mean difference at -2.7 (compared to the MMRM model -3.1) which was still statistically significant at p=0.033. For the CGI-I the sensitivity analysis gave the same LS mean difference as the MMRM analysis of -0.3 with p=0.112. The Applicant also conducted sensitivity analyses to account for alterations due to the COVID-19 public health emergency which maintained statistical significance. A subgroup analysis of subjects who had remote assessments (trofinetide n=28, placebo n=29) demonstrated a smaller MMRM LS mean difference of -2.1 that did not meet nominal significance.

Prespecified subgroup analyses by age group (5-10, 11-15, 16-20) all favored trofinetide, though only nominally significant in the youngest age group (See Table 14).

Table 14 ACP-2566-003 Prespecified Stratified Age Group Analysis of 12 week CFB in RSBQ

AGE 5-10 Change from Baseline to Week 12	Placebo	Trofinetide

n	47	41
Mean (SE)	-0.4 (1.32)	-4.1 (1.29)
SD	9.02	8.28
Median	-2.0	-2.0
Min, Max	-16, 40	-25, 10
MMRM Analysis[1]		
LS Mean (SE)	-0.4 (1.17)	-4.4 (1.24)
95% CI	(-2.7, 1.9)	(-6.9, -1.9)
Difference from Placebo		
LS Mean Difference (SE)		-4.0 (1.71)
95% CI		(-7.4, -0.6)
Two-sided p-value		0.0215
Effect Size (Cohen's d)		0.50

AGE 11-15

Change from Baseline to Week 12	Placebo	Trofinetide
n	20	19
Mean (SE)	-1.4 (1.38)	-5.8 (1.55)
SD	6.15	6.74
Median	-1.5	-5.0
Min, Max	-18, 12	-19, 5
MMRM Analysis[1]		
LS Mean (SE)	-1.3 (1.43)	-4.6 (1.47)
95% CI	(-4.2, 1.6)	(-7.6, -1.7)
Difference from Placebo		
LS Mean Difference (SE)		-3.4 (2.06)
95% CI		(-7.5, 0.8)
Two-sided p-value		0.1098
Effect Size (Cohen's d)		0.51

AGE 16-20

Change from Baseline to Week 12	Placebo	Trofinetide
n	18	16
Mean (SE)	-5.4 (2.61)	-6.9 (2.87)
SD	11.07	11.49
Median	-1.5	-4.5
Min, Max	-31, 8	-34, 8
MMRM Analysis[1]		
LS Mean (SE)	-5.4 (2.53)	-6.2 (2.60)

95% CI	(-10.5, -0.2)	(-11.5, -0.9)
Difference from Placebo		
LS Mean Difference (SE)		-0.8 (3.63)
95% CI		(-8.2, 6.6)
Two-sided p-value		0.8233
Effect Size (Cohen's d)		0.08

Source: Applicant ACP-2566-003 CSR Excerpts from Table 14.2.1.1.1

Prespecified but unadjusted subgroup analysis by the stratified baseline RSBQ severity (<35 or >/=35) also favored trofinetide but was not nominally significant (See Table 15). Prespecified but unadjusted subgroup analysis by groupings of MECP2 mutations into mild, moderate, and severe (which the sample had not been stratified for), favored trofinetide, but only reached nominal significance in the severe group, which also had the largest baseline sample (46 subjects in each of the placebo and trofinetide groups). The same prespecified but unadjusted subgroup analyses were performed for the CGI-I, which again favored trofinetide, though few were nominally significant.

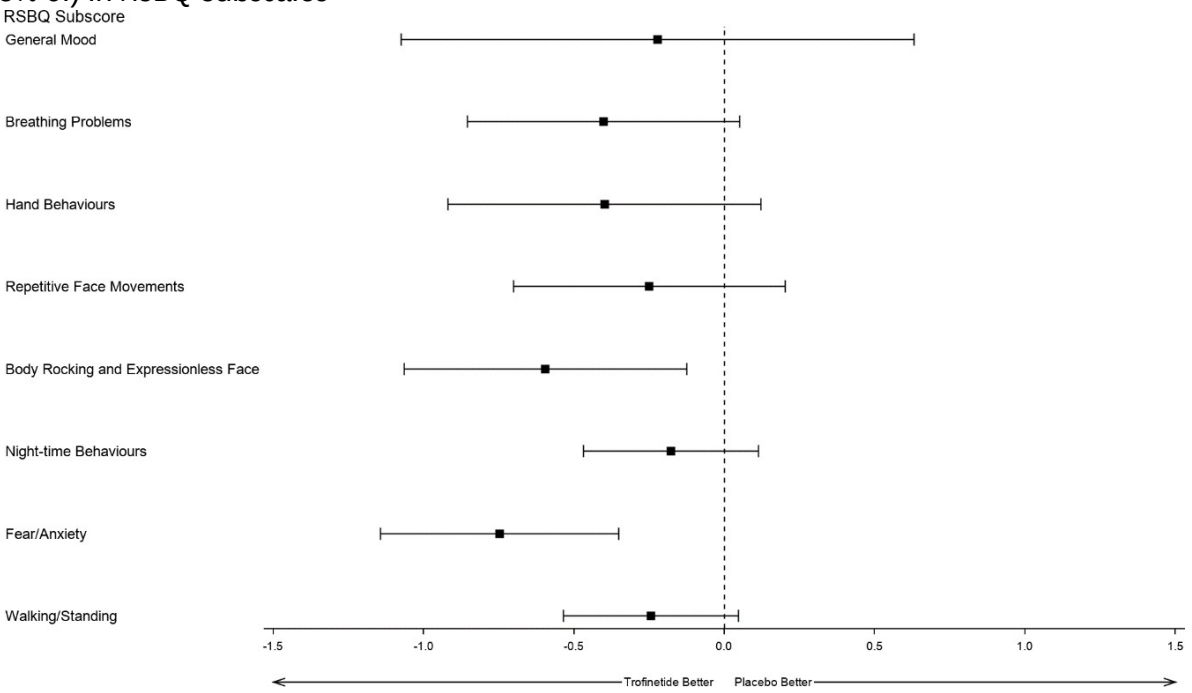
Table 15 ACP-2566-003 Prespecified Stratified Baseline RSBQ Severity Analysis of 12 week CFB in RSBQ

BASELINE RSBQ<35		
Change from Baseline to Week 12	Placebo	Trofinetide
n	16	12
Mean (SE)	3.4 (2.85)	-1.1 (1.32)
SD	11.42	4.58
Median	-0.5	-1.0
Min, Max	-7, 40	-12, 5
MMRM Analysis[1]		
LS Mean (SE)	3.5 (2.16)	-1.2 (2.48)
95% CI	(-1.0, 7.9)	(-6.3, 3.9)
Difference from Placebo		
LS Mean Difference (SE)		-4.6 (3.30)
95% CI		(-11.4, 2.1)
Two-sided p-value		0.1719
Effect Size (Cohen's d)		0.54
BASELINE RSBQ>=35		
Change from Baseline to Week 12	Placebo	Trofinetide
n	69	64
Mean (SE)	-2.8 (0.97)	-5.8 (1.13)
SD	8.06	9.07
Median	-2.0	-4.5
Min, Max	-31, 12	-34, 10
MMRM Analysis[1]		
LS Mean (SE)	-3.0 (0.99)	-5.7 (1.02)
95% CI	(-4.9, -1.0)	(-7.7, -3.7)
Difference from Placebo		
LS Mean Difference (SE)		-2.7 (1.42)
95% CI		(-5.5, 0.1)
Two-sided p-value		0.0571
Effect Size (Cohen's d)		0.33

Source: Adapted by reviewer from Applicant ACP-2566-003 CSR Table 14.2.1.2

Reviewer Comment: While the Applicant did demonstrate a significant effect of trofinetide compared to placebo on the RSBQ CFB as a whole, that change may have been primarily driven by two sub-domains of the RSBQ, "Body Rocking and Expressionless Face" and "Fear/Anxiety". The other 5 subdomains showed numerical trends in favor of trofinetide, but none of them reached nominal significance in post hoc testing (See Figure 2*).*

Figure 2 ACP-2566-003 Forest Plot of Trofinetide-Placebo Treatment Difference in LSM CFB (95% CI) in RSBQ Subscales



Source: Applicant ACP-2566-003 CSR Figure AH1.3.4 Abbreviations: CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett syndrome Behavior Questionnaire

As discussed in 8.0 Review of Safety, there was a disproportionate occurrence of diarrhea, and nausea vomiting, likely ADRs of trofinetide, that occurred early and persisted during study ACP-2566-003. And as noted in 6.1.1 Study Design, that exposure could lead to functional unblinding, most concerning for the caregivers and clinicians who were the raters for the two coprimary endpoints. The Applicant, investigating this concern, presented an unplanned subgroup analysis of the changes in RSBQ and CGI-I broken down by occurrence of diarrhea (Table 16). The Applicant argues that since the LS means favored trofinetide in the group of subjects that did not have diarrhea, that functional unblinding did not occur. However, this reviewer does not find that argument compelling given a) the number of subject dropouts in the group who experienced diarrhea, b) the fact that the small number of placebo subjects that experienced diarrhea had numerically better RSBQ CFB and CGI-I scores than the placebo group that did experience diarrhea. This was also substantially true for the trofinetide group for RSBQ, though it did not hold for the CGI-I, potentially indicating that potential functional unblinding could have different effects on caregivers than on clinicians. Treatment with loperamide did not seem to be a major factor in driving the difference between groups,

as demonstrated by a mediation analysis conducted by the Agency biostatistical reviewer (see the biostatistical review and section 7.3 for details).

Table 16 ACP-2566-003 Co-Primary Endpoint Analysis by Diarrhea Occurrence

Endpoint	Placebo		Trofinetide	
	Reported Diarrhea N=18	Did Not Report Diarrhea N=75	Reported Diarrhea N=73	Did Not Report Diarrhea N=18
RSBQ				
Baseline				
n	18	75	73	18
Mean (SE)	43.7 (2.93)	44.7 (1.41)	44.5 (1.39)	40.5 (2.31)
Change From Baseline to Week 12 (MMRM[a])				
n	16	69	60	16
LS Mean (SE)	-2.3 (2.01)	-1.7 (1.04)	-5.1 (1.01)	-4.0 (2.17)
CGI-I				
Week 12 MMRM[b]				
n	17	69	61	16
LS Mean (SE)	3.7 (0.17)	3.8 (0.07)	3.6 (0.09)	3.4 (0.14)

Sources: Applicant ACP-2566-003 CSR

Abbreviations: CGI-I=Clinical Global Impression-Improvement; RSBQ=Rett syndrome Behaviour Questionnaire; MMRM=mixed-effects model for repeated measures; LS=least squares; SE=standard error.

Note: Baseline was the latest non-missing value prior to the first dose of study drug.

[a] The MMRM for RSBQ 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 covariance matrix was used to model within-subject errors. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

[b] The MMRM for CGI-I 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. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

Data Quality and Integrity

The Applicant performed audits on 3 of 21 sites as part of its monitoring and auditing risk reduction plan. No data quality issues were identified.

Efficacy Results – Secondary and other relevant endpoints

The prespecified key secondary outcome measure was the CSBS-DP-IT Social Composite Score,

which demonstrated a statistically significant difference in favor of trofinetide, with placebo subjects worsening on the scale as compared to trofinetide subjects who maintained their score on average (Table 17). Further subgroup analyses, like those of the co-primary endpoints, tended to favor trofinetide, but not exclusively, with few reaching nominally significant differences from placebo.

The Applicant also studied 8 other ClinRO or ObsRO questionnaires as exploratory endpoints for which there was no prespecified plan to conduct a robust statistical analysis. The Applicant presented results of post hoc MMRM analyses that demonstrated that 1 of the 8 scales, the Rett syndrome Clinician Rating of Ability to Communicate Choices (RTT-COMC) was the only one that met nominal significance in favor of trofinetide (LS mean difference placebo-trofinetide = -0.3, p=0.02. Six of the remaining scales had LS mean differences favoring trofinetide, while the remaining Rett syndrome Clinician Rating of Verbal Communication (RTT-VCOM) and Caregiver Global Impression of Severity (CGI-S) had LS mean difference of 0.

Table 17 ACP-2566-003 Analysis of Key Secondary Endpoint CSBS-IT-SCS CFB at Week 12

Change from Baseline to Week 12	Placebo	Trofinetide
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[a]		
LS mean (SE)	-1.1 (0.25)	-0.1 (0.26)
95% CI	(-1.6, -0.6)	(-0.6, 0.5)
Difference from placebo		
LS mean difference (SE)		1.0 (0.37)
95% CI		(0.3, 1.7)
Two-sided p-value		0.0064
Effect size (Cohen's *d*)		0.43

Source: Applicant ACP-2566-003 CSR Table 14.2.3.1
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 Behaviour Questionnaire; SD=standard deviation; SE=standard error
Notes: Baseline was the latest non-missing value prior to the first dose of study drug.
[a] 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 covariance matrix was used to model within-subject errors. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

REVIEWER COMMENT: The CSBS-DP-IT is a 24-item ObsRO reported by caregivers originally designed to assess infants between 6 and 24 months in the pre-verbal stage.

The scale asks parent impressions regarding infant development in 7 domains of questions: emotion and eye gaze, communication, gestures, sounds, words, understanding, and object use. The CSBS-DP-IT was intended as a screening tool to help identify possible deficits in communication milestones during infant development. The questionnaire is a 3-level Likert-like scale where the parent answers "Not Yet", "Sometimes", or "Often" to questions such as "Do you know when your child is happy and when your child is upset", "When you are not paying attention to your child, does he/she try to get your attention", "Does your child nod his/her head to indicate yes?".

In attempting to adapt the CSBS-DP-IT to use for this study the Applicant cited previous work as demonstrating that the scale is useful in patients with developmental delay, including a study that used the first 14 items to assess patients with Rett syndrome. That subset of questions on emotion and eye gaze, communication, and gestures are the Social Composite Score (CSBS-DP-IT-SCS). It is the CSBS-DP-IT-SCS that the Applicant used as an endpoint in this study.

From a clinical perspective, there is concern that this instrument is not well validated for the population studied. There is concern that parents may not always be able to objectively assess a neurologically impaired child's non-verbal cues (Miller, Perkins et al. 2017). The concept of communication has numerous attributes that would need to be measured to be addressed adequately. The intent of the CSBS-DP-IT-SCS is to serve as a screening tool to identify potential communication issues. In our review of the instrument, we find that the CSBS-DP-IT-SCS does not evaluate various important aspects of communication in depth. Insufficient evidence was provided to justify the administration, scoring, and interpretation of the CSBS-DP-IT-SCS for the population of subjects with Rett syndrome studied. Finally, the 13-items that make the social composite score do not represent the full concept of social reciprocity in communication and this score should not suggest that it does. It should also be noted that the 1 communication item in the RSBQ, "Uses eye gaze to convey feelings, needs, and wishes" did not demonstrate a significant difference between trofinetide and placebo (Table 18).

Table 18 ACP-2566-003 "RSBQ Eye Gaze to Convey Feelings" Item Summary Statistic Tables

		Analysis Visit			
		BASELINE	WEEK 2	WEEK 6	WEEK 12
Trofinetide	N	93	90	83	76
	Mean	1.54	1.48	1.47	1.46
	Std Dev	0.50	0.64	0.61	0.62
	Min	0.00	0.00	0.00	0.00
	Median	1.50	2.00	2.00	2.00
	Max	2.00	2.00	2.00	2.00

		Analysis Visit			
		BASELINE	WEEK 2	WEEK 6	WEEK 12
Placebo	N	94	90	92	85
	Mean	1.59	1.51	1.52	1.51
	Std Dev	0.54	0.62	0.62	0.61
	Min	0.00	0.00	0.00	0.00
	Median	2.00	2.00	2.00	2.00
	Max	2.00	2.00	2.00	2.00

Source: Reviewer JMP Clinical Tabulation of RSBQ01 Observed Results

Dose/Dose Response

Dose-response or exposure-response were not addressed in ACP-2566-003 but rather are dependent on an integrated assessment across studies and hence are discussed in 7.1.

6.2. Neu-2566-RETT-002 A Randomized, Double-Blind, Placebo-Controlled, Dose-ranging Study of the Safety and Pharmacokinetics of Oral NNZ-2566 in Pediatric Rett Syndrome

6.2.1. Study Design

Overview and Objective

Neu-2566-RETT-002 was a single-blind placebo run-in, randomized double-blind placebo-controlled dose ranging clinical trial of trofinetide treatment in girls with Rett syndrome ages 5 to 15 years. The objective of this Phase 2 study was to investigate the safety, tolerability and pharmacokinetics of treatment with 3 different doses of oral trofinetide.

Trial Design

Basic Study Design

The core aspects of this study design were altered after 62 subjects had been recruited as this was initially designed as a Phase 2 dose finding study. Initially, 62 subjects were recruited into a 56-day study that included the following:

- two weeks of placebo run-in for placebo-response covariate identification (though without any further change in population)
- 1:1:1:1 randomization to placebo or trofinetide at 3 different doses

- 2-5 days of dose titration to goal trofinetide dose, depending on total goal dose (see Table 19 for details):
    - 2 days for 50 mg/kg BID, followed by 42 days of steady dosing
    - 3 days for 100 mg/kg BID, followed by 41 days of steady dosing
    - 5 days for 200 mg/kg BID, followed by 40 days of steady dosing
- 1-2 days of dose tapering to off for 100 mg/kg BID and 200 mg/kg BID cohorts
- Placebo-treated subjects received 42 days of placebo. There was no Applicant report of sham-titration/tapering for subjects randomized to placebo.
- After 62 subjects were enrolled, another 20 subjects (for a total of 82 subjects) were randomized 1:1 to placebo or 200 mg/kg BID after safety data was reviewed at the lower doses.

Trial Location
The study took place at 12 sites around the United States.

Choice of Control Group

This study was placebo-controlled as there are no indicated therapies for Rett syndrome.

Diagnostic Criteria

Neu-2566-RETT-002 enrolled female subjects ages 5-15 years with classic Rett syndrome with proven MeCP2 gene mutation.

Key Inclusion/Exclusion Criteria

The subjects had to be at Hagberg Stages 3 or 4 (post-regression stages of Rett syndrome), weigh between 15 to 100 kg, and have a CGI-S score of 4 or greater (moderate severity). Patients were excluded if they had an abnormal QT, unstable dosing of other medications, anticonvulsants with liver enzyme inducing effects, or concurrent treatment with insulin, IGF-1, or growth hormone.

Patients were stratified by age into two groups: 5 to 10 years of age and 11 to 15 years of age.

> *Reviewer Comment: While there are atypical types of Rett syndrome, usually with non-MeCP2 genetic mutations, and male sex can be affected, the diagnostic and inclusion/exclusion criteria were appropriate to address the greatest medical need and to provide a stable population to study.*

Dose Selection

Doses from 50 mg/kg BID to 200 mg/kg BID were selected based on extrapolation of doses found to be well-tolerated in nonclinical safety/toxicology, Phase 1 studies in adult healthy volunteers, and a small Phase 2 study in Rett subjects ages 16-45. Of note, prior studies in healthy volunteers had studied up to 100 mg/kg BID, and in subjects up to 70 mg/kg BID, therefore this study was at a higher dose, to achieve higher exposure and increase the chance of effect.

Reviewer Comment: As this was a Phase 2 dose finding study, it is appropriate that multiple doses were tested; however, it would have been ideal to have had the maximum dose given to subjects tested first in healthy volunteers for tolerability.

Study Treatment

Study drug was given in a liquid formulation either orally or by percutaneous gastrostomy tube as per the subject's individual routine care plan. The dose was calculated according to the body weight at the screening visit and the drug was given at least 2 hours after or 30 minutes before food intake.

The Applicant provided a table to illustrate the dose titration and tapering schedules:

Table 19: Neu-2566-RETT-002 Dose Titration Schedule

Dose Level	Day of Study	Dose (mg/kg BID)	Total Dose/Day (mg/kg)
Placebo	15-56	Placebo	0
50 mg/kg BID	15	8.5	17
	16	35	70
	17-56	50	100
100 mg/kg BID	15	17.5	35
	16	35	70
	17	50	100
	18 – 55	100	200
	56	50	100
200 mg/kg BID	15	17.5	35
	16	35	70
	17	50	100
	18	100	200
	19	150	300
	20-54	200	400

	55	100	200
	56	50	100

Source: Neu-2566-RETT-002 CSR Table 9-1

Assignment to Treatment

A web-based randomization system was used to assign treatment allocation. The randomization list was submitted as part of this application.

Blinding

An unblinded pharmacist administered the randomization system and prepared the study drug for single-blind administration by caregivers during the 14-day placebo run-in and then for double-blind administration during the remainder of the study. Both the study drug and placebo were liquid formulations with strawberry flavor, with trofinetide at a concentration of 100 mg/mL.

> *Reviewer Comment: As discussed in the section on basic study design, the lack of consistency with titration/tapering schedules leading to variance in volumes of study drug being administered by the caregiver on different days was a high risk for unblinding across treatment groups. Given that the outcome measures for this study are all based on caregiver surveys, they are highly susceptible to bias from unblinding.*

Dose Modification/Dose Discontinuation

No prespecified plans were made for subject or investigator dose modification. Patients and their legally authorized representatives (LARs) were free to withdraw consent and discontinue study medication at any time. The protocol specified that investigators or subjects could stop study drug administration for AEs, inter-current illness, opinion of the investigator for safety, requiring excluded medication,

Administrative Structure

All data collection and assessments were conducted by study staff, investigators, and patient caregivers. An effort was made to maintain the same investigator/caregiver raters/assessors throughout participation for each patient. Various CROs and other vendors were used for bioanalytical and other study components. A data safety and monitoring committee composed of 5 experts was chartered for this study. No interim analyses were conducted. After

amendment 3 was made to the protocol on August 12, 2013, the DSMB allowed the Applicant to increase the enrollment population to accommodate the enrollment of another 20 patients randomized 1:1 placebo:trofinetide-200 mg/kg BID.

Procedures and Schedule

Table 20 NEU-2566-RETT-002 Schedule of Procedures

Evaluation	Screening Approx. 1 week[1]	Baseline[2] Pre-placebo run-in	Treatment Baseline[2] Day 14 (+2) End of Placebo-run	Day 21 (+/- 1)	Day 28 (+/- 2)	Day 42 (+/- 2)	Day 54 (-2) EOA Before Down Titration	Day 66 (+/- 3) Follow-Up
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8
Informed Consent	X							
Inclusion / Exclusion criteria	X							
Demographics	X							
Medical / Psychiatric assessment	X							
Rett syndrome diagnosis incl. genotyping	X							
Physical examination	X	X	X	X	X	X	X	X
Concomitant medication query	X	X	X	X	X	X	X	X
Vital signs[3]	X	X	X	X	X	X	X	X
B-HCG Pregnancy test[4]	X		X				X	
Hematology including HbA1c	X		X	X	X	X	X	X
Urinalysis	X (Drug test)		X (Drug test)	X	X (Drug test)	X	X	X
Biochemistry including Thyroid panel (free T3, free T4, TSH)	X		X	X	X	X	X	X
Fundoscopy & Tonsil size		X	X	X	X	X	X	X
Pharmacokinetic assay					X		X	
ECG (12-lead)	X	X	X	X	X	X	X	X

Heart Rate and Respiratory Variability[5]		X	X			X	X	
Dispense Study Medication		X	X		X	X		
Evaluation	Screening Approx. 1 week1	Baseline2 Pre-placebo run-in	Treatment Baseline2 Day 14 (+2) End of Placebo-run	Day 21 (+/- 1)	Day 28 (+/- 2)	Day 42 (+/- 2)	Day 54 (-2) EOA Before Down Titration	Day 66 (+/- 3) Follow-Up
(For Outpatient Administration)6								
Take Single-blind placebo run in Medication6			X					
Take Double-Blind Study Medication6				X	X	X	X	
CGI-S	X		X	X	X	X	X	X
CGI-I			X	X	X	X	X	X
Clinician Rated Domain Specific Concerns VAS		X	X		X	X	X	X
CSS	X							
MBA		X	X		X	X	X	X
RSBQ		X	X		X	X	X	X
Caregiver Top 3 Concerns VAS		X	X		X	X	X	X
RTT CBI			X		X	X	X	X
Semi-structured Caregiver Diary	X	X	X	X	X	X	X	X
AE/SAE	X (SAE)	X(SAE)	X	X	X	X	X	X

Source: Applicant Neu-2566-RETT-002 CSR Table 9-2

1. Screening assessments were completed after consent during a period of approximately 1 week before randomization. The screening period was to be at least 5 days and no more than 10 days.
2. The Baseline visit was completed after eligibility was confirmed, and the patient was randomized, but before the first dose of study medication. Baseline visits could occur up to 48 hours before the first dosing day of the single-blind placebo run-in. Typically, the Baseline visit occurred the day before the first day of dosing, but this was not a requirement. Day 1 began after the first dose is administered for the placebo run-in. The double-blind dosing period began the day after Visit 3 (Day 14 visit-end of placebo run-in). Visit 3 was to occur before any double-blind medication was taken. Visits windows were determined from Day 1 of study medication.

3. Vital signs, including height, weight, blood pressure (systolic and diastolic n mmHg), heart rate (bpm), respiratory rate (rpm), and body temperature (ºC) were assessed at Screening. Weight, blood pressure, heart rate (bpm), respiratory rate (rpm), and body temperature (ºC) were also assessed at baseline (pre-dose), and after the morning dose of study medication on Days 14, 21, 28, 42 and 54, and anytime on Day 66 (Follow-Up visit). Height was captured at Screening and Day 54 only.
4. Females who had reached menarche had a serum pregnancy test (β-HCG) at Screening, Day 14, and Day 54, except those who had surgical sterilization procedures.
5. The heart rate and respiration rate assessment were done in-clinic during an up to 3-hour awake period using wireless data capture.
6. While visit windows of between +/- 1-2 days were allowed during treatment, the total days on double-blind randomized treatment was not to be more than 42 days.

Dietary Restrictions/Instructions

A food effect study had not been conducted prior to this study, so the patients were instructed to take the study drug 30 minutes before or 2 hours after eating. Patients using a dietary method to treat other symptoms (such as ketogenic diet for seizures) were required to be stable and remain stable on that diet.

Concurrent Medications

It was expected that multiple concomitant medications would be used by patients with Rett syndrome due to the numerous comorbid symptoms they have. No concomitant therapies were required with study drug. Excluded concomitant therapies were insulin, IGF-1, growth hormone, and enzyme inducing antiepileptic medications.

Treatment Compliance

Volume of drug delivered and returned from patients was recorded along with caregiver diary entries of study drug delivery to patients was all recorded. No analysis was provided by the Applicant regarding the measure of compliance.

> *Reviewer Comment: No analysis was conducted by the Applicant with regards to treatment compliance. We cannot make conclusions regarding compliance as no data from caregiver diaries or volume of drug returned was provided.*

Rescue Medications

As there are no therapies either indicated or standard of care for the overall treatment of Rett syndrome, there were no accommodations made for rescue medications.

Subject Completion/Discontinuation/Withdrawal

Patients and their legally authorized representatives were allowed to withdraw treatment at any time. There was no documented effort to maintain patients in follow-up for effectiveness or safety results after treatment withdrawal. Missing data at a scheduled visit was imputed with the median value for the subject's assigned dose group at that visit. The imputation was performed for individual instrument items; any subscale subtotals and overall totals for a given instrument were calculated based on the imputed individual items. No imputations were performed directly for subscale subtotals or overall totals.

Reviewer Comment:

Of the 82 patients who were randomized, only one patient withdrew in the 200 mg/kg group. While it did not likely have a major impact given that there was only one withdrawal in the study, using the median value of the cohort's score for any particular instrument to impute the missing data for a single subject is problematic as it does not take into account that particular patient's baseline or trend in the study on that particular instrument.

Study Endpoints

See Clinical Review Section 3: Summary of Presubmission/Submission Regulatory Activity, for discussion of endpoints with regards to Rett syndrome.

The primary objectives of NEU-2566-RETT-002 were safety and tolerability measures collected by investigators and their staff.

The secondary objective of effectiveness was explored widely with a number of study endpoints, primarily measured as a change from baseline (defined as measures obtained on visit 3, day 14 of the placebo-run in portion) to visit 7, day 54 of treatment in the 200 mg/kg cohort. Specifically, the following measures were assessed:

1. Clinician Reported Outcomes, specific: Rett syndrome Natural History Motor Behavior Assessment (MBA) (total score, subscale scores, and change index scores), Clinician-Rated Domain-Specific Concerns Visual Analogue Scale (RTT-DSC).
2. Clinician Reported Outcomes, global: Clinical Global Impression Scales – Severity and – Improvement (CGI-S and CGI-I).
3. Caregiver-Reported Outcomes, specific: Caregiver Top 3 Concerns, Rett syndrome Behavior Questionnaire (RSBQ) (total score, subscale scores), Rett Caregiver Burden Inventory (RTT-CBI) (burden total score and optimism index score)
4. Physiological Measures: Respiratory variability and 3-lead ECG heart rate and heart rate variability. Standard protocols for these assessments were followed at all sites.

Reviewer Comment: As previously reviewed, the Agency advised regarding the limitations of all the various caregiver and clinician reported endpoints and recommended use of a global measure of function, which in this study was the CGI-I. However, the use of coprimary endpoint does not itself reduce the problems with the various scales, which will be further commented on in this reviewer's review of the effectiveness results, Section 6.2.2.

Statistical Analysis Plan

The Applicant states in the statistical analysis plan that this is an exploratory study and hence no single prespecified endpoint analysis was designated to define effectiveness.

For the Applicant's exploratory analysis, the first 62 patients who had been randomized 1:1:1:1 were analyzed as cohort 1 and the remaining 20 patients randomized 1:1 to placebo:trofinetide 200 mg/kg BID were referred to as cohort 2.

The following populations were defined:

- Intent to treat (ITT): all patients randomized into the study, as treatment assigned
- Modified intent to treat (mITT): Patients in the ITT who received at least one dose of the study medication during the double-blind period.
- Per protocol: Patients in the mITT who did not have a major protocol violation. Defined prior to unblind. Used for sensitivity analyses.
- Safety population: ITT analyzed according to treatment received rather than randomized.
- Pharmacokinetic population: mITT population who received study drug through morning of day 28 and underwent PK sample collection through day 28.

The mITT population was the one the Applicant used for their analysis

Missing data was to be handled via imputation using the median value for the subject's assigned dose group.

To assess effectiveness, the Applicant proposed the primary analysis would be change from baseline (Visit 3, Day 14) to Day 54. However, the Applicant did not prespecify which endpoint would be the primary measure of effectiveness. The Applicant planned to look at change from baseline to day 54 compared between placebo and treatment groups for all the questionnaires administered and the ECG for heart rate and heart rate variability. There was no plan for a primary effectiveness outcome analysis or for any multiplicity controls. The Applicant did prespecify that the variables of treatment baseline and placebo response obtained from the first 14-day placebo run-in would be included as covariates in the general linear model analysis if $p<0.1$. The Applicant also prespecified that data that violated assumptions of normality would have non-parametric methods substituted.

Reviewer Comment: Overall this study was not designed to assess effectiveness as a primary outcome and therefore it is subject to the biases inherent in exploratory studies, which are appropriate for Phase 2 studies that are primarily geared at assessing safety and dose-range-finding. Additionally, imputation of missing data using the median value of the subject's assigned dose group reduces standard error and biases towards Type I error. While only one subject in the 200 mg/kg BID group withdrew and would have missing data based on study withdrawal, the Applicant did not provide data on whether and how much data was missing from the multiple effectiveness endpoints evaluated and how much data was imputed.

Protocol Amendments

The major amendment for this protocol was to allow for recruitment of another cohort of patients randomized 1:1 placebo:trofinetide-200 mg/kg BID.

6.2.2. Study Results

Compliance with Good Clinical Practices

The Applicant CSR for Neu-2566-RETT-002 included an attestation that the study was conducted in accordance with GCP guidance.

Financial Disclosure

Financial disclosures provided indicate that none of the investigators had a conflict of interest that would influence the conduct or outcomes of the study.

Protocol Violations/Deviations

The Applicant did not provide a DV domain SDTM file for deviations in Study Neu-2566-RETT-002 so their reports could not be verified. The Applicant reported 1 minor protocol deviation occurred when patient (b) (6) was randomized and started study treatment before it was revealed that she had started occupational, speech, and physical therapies as part of her transition to school within the 4 weeks prior to baseline visit. The Applicant did not consider this to compromise interpretability of the data.

Other minor protocol deviations are listed in 45 pages of Attachment 16.2.1. Those included instances of the fasting recommendations not being followed, spillage or lack of return of doses, minor missed or mis-timed assessments or doses.

Reviewer Comment: This reviewer estimates over 400 minor protocol deviations based on the narrative listings provided by the Applicant. This seems excessive given literature documentation of averages of 100-200 deviations in most clinical trials (Getz, Smith et al. 2022). While likely minor, there is a small risk that the start of other therapies at the beginning of the trial may have biased patient (b) (6) *to improvement in her outcome measures, thereby contributing to Type I error given that this patient was assigned to 100 mg/kg BID of trofinetide.*

Patient Disposition

Ninety patients were screened, 82 were randomized, 58 to one of 3 doses of trofinetide, and 24 to placebo. Of the trofinetide patients, 15 received 50 mg/kg BID, 16 100 mg/kg BID, and 27 200 mg/kg BID. Of the entire population, 1 patient in the 200 mg/kg BID cohort was discontinued due to an AE.

Patient Demographics

Demographic characteristics are represented in the tables below created by the reviewer using the DM dataset and JMP Clinical.

Table 21 Neu-2566-RETT-002 Demographics, Age

		NNZ-2566 200 mg/kg BID	NNZ-2566 100 mg/kg BID	NNZ-2566 50 mg/kg BID	Placebo	All
Age	N	27	16	15	24	82
	Mean	8.85	10.25	9.60	8.92	9.28
	Std Dev	3.87	2.93	3.22	3.27	3.40
	Min	5	6	5	5	5
	Quantiles25	5	9	7	5	6
	Median	7	9	9	9	9
	Quantiles75	13	13	12	13	13
	Max	15	15	15	14	15

Table 22 Neu-2566-RETT-002 Demographics, Race and Ethnicity

	NNZ-2566 200 mg/kg BID		NNZ-2566 100 mg/kg BID		NNZ-2566 50 mg/kg BID		Placebo			
Race	Count	Column %	Count	Column %	Count	Column %	Count	Column %	Count	% of Total
ASIAN	2	7.4%	0	0.0%	0	0.0%	1	4.2%	3	3.66%
BLACK OR AFRICAN AMERICAN	0	0.0%	1	6.3%	0	0.0%	0	0.0%	1	1.22%
WHITE	25	92.6%	15	93.8%	15	100.0%	22	91.7%	77	93.90%

OTHER	0	0.0%	0	0.0%	0	0.0%	1	4.2%	1	1.22%
All	27	100.0%	16	100.0%	15	100.0%	24	100.0%	82	100.00%
Ethnicity	Count	Column %	Count	Column %	Count	Column %	Count	Column %	Count	% of Total
HISPANIC OR LATINO	6	22.2%	1	6.3%	1	6.7%	0	0.0%	8	9.76%
NOT HISPANIC OR LATINO	21	77.8%	14	87.5%	14	93.3%	24	100.0%	73	89.02%
NOT REPORTED	0	0.0%	1	6.3%	0	0.0%	0	0.0%	1	1.22%
All	27	100.0%	16	100.0%	15	100.0%	24	100.0%	82	100.00%

Table 23 NEU-2566-RETT-002 Baseline Demographics –Medical History Terms (Combined) 2x Relative Risk in Trofinetide Than Placebo

	NNZ-2566 200 mg/kg BID (N = 27)		NNZ-2566 100 mg/kg BID (N = 16)		NNZ-2566 50 mg/kg BID (N = 15)		Placebo (N = 24)		
Reported Term for the Medical History	Count	%	Count	%	Count	%	Count	%	Total
Epilepsy	6	22.2%	.	.	1	6.7%	1	4.2%	8
scoliosis	3	11.1%	1	6.3%	3	20.0%	1	4.2%	8
Seasonal Allergies	3	11.1%	1	6.3%	1	6.7%	1	4.2%	6
Anxiety	3	11.1%	.	.	1	6.7%	1	4.2%	5
CONSTIPATION	.	.	2	12.5%	1	6.7%	1	4.2%	4
seasonal allergies	.	.	1	6.3%	2	13.3%	1	4.2%	4
Dysphagia	.	.	2	12.5%	.	.	1	4.2%	3

Source: Reviewer, JMP Clinical, MH Report, Reported Medical Terms were grouped for similar concepts by this reviewer

Reviewer Comment: Similarly to ACP-2566-003, this study had an underrepresentation of African American and other racial and ethnic minorities, making the results harder to generalize. With regards to medical history, while the numbers were overall low, the disproportionate rates of epilepsy and anxiety may indicate that the treatment groups were imbalanced with regards to neurological disability.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

Table 24 Baseline Rett Questionnaire Scores for NEU-2566-RETT-002

Core variable	Placebo (N=24)	50 mg/kg BID (N=15)	100 mg/kg BID (N=16)	200 mg/kg BID (N=27)
MBA Total Score				
N	24	15	16	27
Mean	48.8	46.6	48.6	46.6
SD	7.99	8.77	8.82	13.10
Median	47	49	45.5	44.0
Minimum, Maximum	34, 66	25, 58	37, 65	27, 72
RTT-DSC Total Score				
N	24	15	16	27
Mean	446.2	450.44	444.24	495.04
SD	99.75	80.39	79.91	97.21
Median	473.3	450.0	445.35	516.6
Minimum, Maximum	260.0, 636.6	243.0, 619.4	339.0, 587.6	270.0, 640.2
CGI-I Score				
N	24	15	16	27
Mean	3.8	3.8	3.9	3.9
SD	0.48	0.41	0.44	0.62
Median	4.0	4.0	4.0	4.0
Minimum, Maximum	3, 5	3, 4	3, 5	2, 6
Caregiver Top 3 Concerns Total Score				
N	24	15	16	27
Mean	223.87	237.69	211.55	245.9
SD	54.51	63.97	42.6	49.12
Median	236.85	247.0	204.1	259.1
Minimum, Maximum	97.9, 300.0	64.0, 300.0	154.7, 291.9	90.9, 300.0
RSBQ Total Score				
N	24	15	16	27
Mean	39.5	44.7	40.3	42.2
SD	11.83	13.57	11.26	10.99
Median	40.5	47.0	40.5	42.0
Minimum, Maximum	16, 61	13, 67	20, 59	20, 69

Source: APPLICANT, NEU-2566-RETT-002 CSR Table 14.2.1.1.1, Listing 16.6.3, Listing 16.6.6.7.1, Listing 16.6.6, Listing 16.6.4.1, Listing 16.6.5. MBA=Motor Behavior Assessment; RTT-DSC=Rett syndrome Domain Specific Concerns; CGI-I=Clinical Global Impression of Improvement; RBSQ=Rett syndrome Behavioral Questionnaire; mITT=modified intent-to-treat; n=number of subjects; SD=standard deviation.

Reviewer Comment: Baseline characteristics with regards to Rett syndrome severity were generally well balanced between treatment groups.

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

The Applicant reported that treatment compliance was assessed by determining volumes of study medication returned and reviewing caregiver diaries; however, no analyses were presented. Instead, line listing of volume measurements and diary records was provided. The EX and SUPPEX datasets are listings of dose (mg/kg) and volume (mL) of study drug administered, respectively. The datasets do not include end dates or volume of drug returned. No rescue medications were used as there are no approved or standard of therapy treatments for Rett syndrome itself.

The NEU-2566-RETT-002 CSR does not comment or provide an analysis on concomitant therapies. Using the CM dataset, this reviewer ran a JMP Clinical Analysis on treatment emergent concomitant medications combining all 3 trofinetide dosing groups (Table 25).

Table 25 NEU-2566-RETT-002 Treatment Emergent Concomitant Medications, Combining All Trofinetide Groups

	Trofinetide		Placebo		Total
	(N = 58)		(N = 24)		
Standardized Medication Name	Count	%	Count	%	
IBUPROFEN	2	3%	3	13%	5
INFLUENZA VACCINE	2	3%	1	4%	3
PARACETAMOL	3	5%	.	.	3
TUSSEX COUGH	1	2%	1	4%	2
BISACODYL	1	2%	.	.	1
CEFDINIR	.	.	1	4%	1
CLINDAMYCIN	.	.	1	4%	1
CO-ADVIL	1	2%	.	.	1
DEXTROMETHORPHAN HYDROBROMIDE	1	2%	.	.	1
DIPHENHYDRAMINE HYDROCHLORIDE	1	2%	.	.	1
FLEET /01605601/	1	2%	.	.	1
GUAIFENESIN	1	2%	.	.	1
LANSOPRAZOLE	1	2%	.	.	1
LOPERAMIDE HYDROCHLORIDE	1	2%	.	.	1
LORATADINE	1	2%	.	.	1
MACROGOL	1	2%	.	.	1

MAGNESIUM HYDROXIDE	1	2%	.	.	1
MELATONIN	1	2%	.	.	1
MENINGOCOCCAL VACCINE	1	2%	.	.	1
MOMETASONE FUROATE	1	2%	.	.	1
MUPIROCIN	1	2%	.	.	1
NYSTATIN	1	2%	.	.	1
ONDANSETRON	1	2%	.	.	1
PANADEINE CO	1	2%	.	.	1
PEDIACARE COUGH-COLD	.	.	1	4%	1
PSEUDOEPHEDRINE HYDROCHLORIDE	1	2%	.	.	1
SODIUM CHLORIDE	1	2%	.	.	1
Source: Clinical Reviewer, JMP Clinical, Concomitant Medication Analysis					

Reviewer Comment: The Applicant has not provided, and this reviewer cannot, with the datasets provided, make any useful comment regarding treatment compliance in NEU-2566-RETT-002. Given that compliance analysis in ACP-2566-003 was also problematic, this finding is concerning that patients on drug were potentially vomiting or otherwise altering dosing in ways that were not captured adequately.

With regards to concomitant medication use, there was no obvious finding of a dose-dependent increase in concomitant medication use in the trofinetide treated groups, however this reviewer does note that loperamide was only used in the 200 mg/kg BID group, reflective of what was seen at the higher doses and longer duration exposure in ACP-2566-003.

Efficacy Results – Primary Endpoint

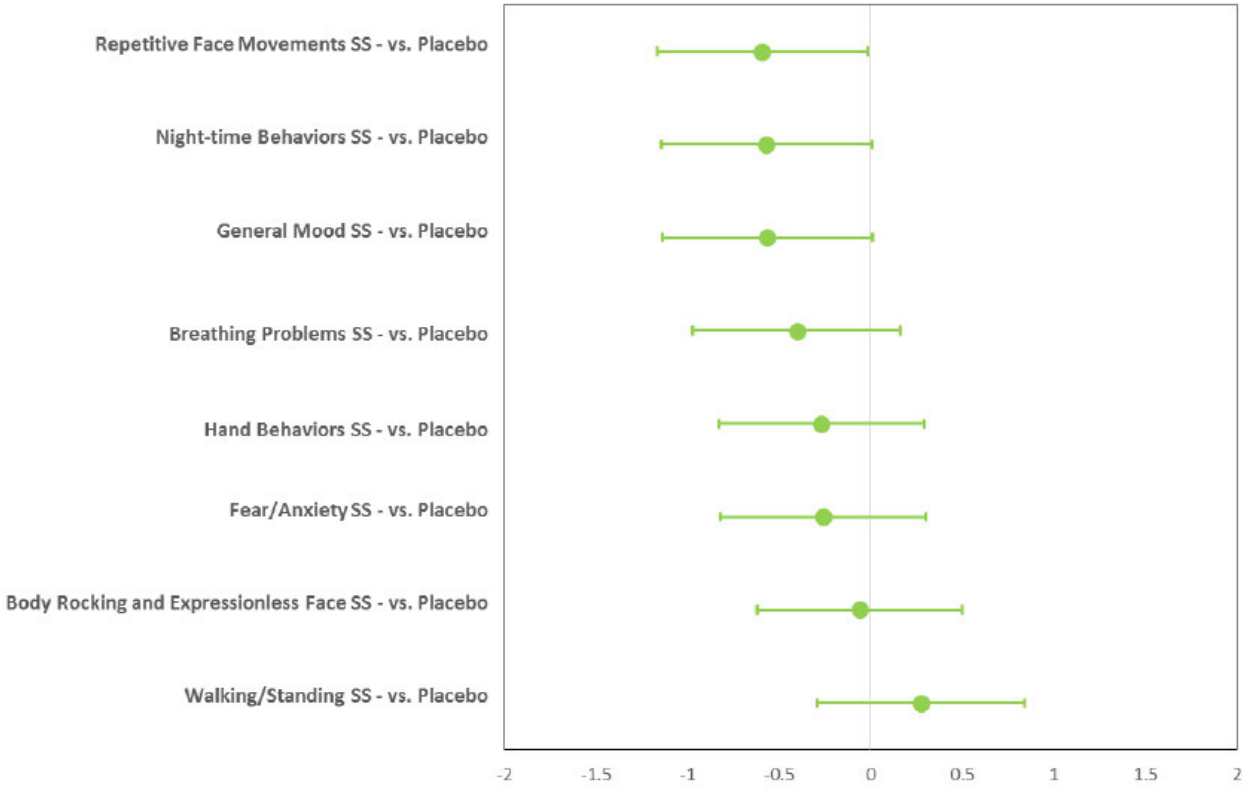
As described in Review section 6.2.1 Study Design, this protocol was designed as an exploratory study with multiple effectiveness endpoints and no single prespecified analysis method or control for multiplicity was planned. As noted in the Agency biostatistical review, the statistical analysis plan had not been originally reviewed by the FDA as this study had been intended as an exploratory study.

As confirmed by the Agency biostatistical reviewer, the primary analysis of the RSBQ and CGI-I (considered most important by the Agency as those were the endpoints used in the pivotal trial ACP-2566-003), used a linear model. The linear model included the Day 14 measure as baseline, treatment baseline by arm interaction, placebo response as the change from Day 1 to Day 14,

and placebo response by arm for terms that were significant at p≤0.10. The RSBQ CFB from Day 14 to Day 54 in the 200 mg/kg trofinetide arm showed evidence of effectiveness with 6.7-point greater improvement (nominal p = 0.035) compared to placebo. CGI-I at Day 54 showed a -0.5-unit difference from placebo (nominal 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.

With regards to other endpoints measured, the RTT-DSC distribution was non-normal in distribution and consequently group medians were used for analysis as specified in the SAP. The CGI-S scores did not change (LS means of 0 for CFB at all time points for both placebo and trofinetide groups. The Applicant presented multiple analyses of effect size examining Cohen's D of trofinetide versus placebo both of the outcome endpoints and the subscales of the various endpoints. While the CGI-I effect size for the 200 mg/kg BID dose group was -0.645 (95% CI -1.219 to -.07), the other 4 main endpoints had effect sizes mainly around -0.2 with CIs that crossed 0. The Applicant also presented CFB at the day 66 follow-up visit, demonstrating that there continued to be a gap between the 200 mg/kg and placebo groups (CFB trofinetide -5.6, placebo -0.9), though both groups had a reduction in benefit compared to Day 54 (trofinetide -6.7, placebo -2.3). The Applicant analyzed 8 subscales of the RSBQ and demonstrated a numerical trend for 7 out of 8 in favor of trofinetide at 200 mg/kg BID. Trofinetide performed worse than placebo in the walking/standing subdomain (trofinetide 0.1, placebo 0.0). Though unplanned, the post hoc MMRM analysis had a nominally significant value for 2 out of the 8 subscales, and favored placebo specifically for the walking/standing subscale (Figure 3)

Figure 3 NEU-2566-RETT-002 Forest Plot of Trofinetide-Placebo Cohen's *d* Effect Sizes and 95% Confidence Intervals for RSBQ Subscales



Source: Applicant NEU-2566-RETT-002 CSR Figure 11-4

The Applicant reported that the clinician rated RTT-DSC showed a trofinetide favoring CFB in the ambulation subscale (-14.14 vs -2.92 compared to placebo, p<0.04) which is in contrast to the caregiver rated RSBQ where the ambulation CFB subscale was in the opposite direction (trofinetide -0.1, placebo 0).

REVIEWER COMMENT:
The main analysis presented for NEU-2566-RETT-002, confirmed by the FDA biostatistical reviewer, was the nominally significant differences favoring trofinetide over placebo in 3 of the 5 endpoint measures (RSBQ, CGI-I, and RTT-DSC) prespecified in the SAP in only the 200 mg/kg group versus placebo, without a particular order or plan for control for multiplicity.

It was also noted that there was no dose-response relationship found for any endpoint. At the 100 mg/kg dose, RSBQ and RTT-DSC favored placebo. Finally, with regards to ambulation, the RSBQ favored trofinetide while the RTT-DSC favored placebo.

Also notable is that subscale analysis of effect sizes did not demonstrate similar patterns as those observed in ACP-2566-003. Whereas in the larger study, the main effects whose lower 95% CI did not cross zero were body rocking/expressionless face subscales, in NEU-2566-RETT-002, only repetitive face movements subscale had a 95% CI that did not cross 0, and in fact the walking/standing subscale favored placebo.

As previously reviewed, the Agency did offer advice regarding the various clinical endpoints being used, as none of them were considered fit for purpose as a standalone instrument. This is best demonstrated in review of these results. For instance, the subject specific visit to visit variability in the RSBQ is quite high. This is also evident in the change in the mean score from week 54, end of treatment, to week 66, end of study follow-up, in both groups. This variability was also present in the Top 3 Caregiver Concerns, and MBA Total Score. There is also clearly a susceptibility to the placebo effect in all of these measures.

Also, both ACP-2566-003 and NEU-2566-RETT-002 were carried out on a population that does not represent US racial diversity and the numbers of racial and ethnic minority patients is not large enough to provide a meaningful analysis. However, there is no reason to anticipate that efficacy or safety would be different in these populations based on the hypothesized mechanisms of the drug. Since Rett syndrome is by far a disorder affecting female patients, these studies were all carried out in females. Both studies took place in the US exclusively, so no regional analysis can be done.

The Applicant presents NEU-2566-RETT-002 as providing confirmatory evidence of effectiveness based on these non-prespecified analyses. Section IV.B. of the 2019 draft FDA guidance, Demonstrating Substantial Evidence of Effectiveness, regarding meeting the substantial evidence standard based on one adequate and well-controlled clinical investigation plus confirmatory evidence, does not define what can be considered confirmatory evidence but does provide examples of types of data or information that could potentially be considered confirmatory evidence. The character of confirmatory evidence can depend on the strength and robustness of the single adequate and well-controlled study and factors such as seriousness of the disease and unmet medical need. Rett syndrome is a rare and serious disorder with dire unmet medical need given that there are no treatments indicated for it. The post hoc analysis of NEU-2566-RETT-002 did demonstrate nominally significant improvement in RSBQ and CGI-I favoring trofinetide at the 200 mg/kg dose, confirming the findings of the pivotal trial, ACP-2566-003. Therefore, it is appropriate to consider the results in the 200 mg/kg group of NEU-2566-RETT-002 as confirmatory evidence.

Data Quality and Integrity

No data quality issues were identified during this review.

Efficacy Results – Secondary and other relevant endpoints

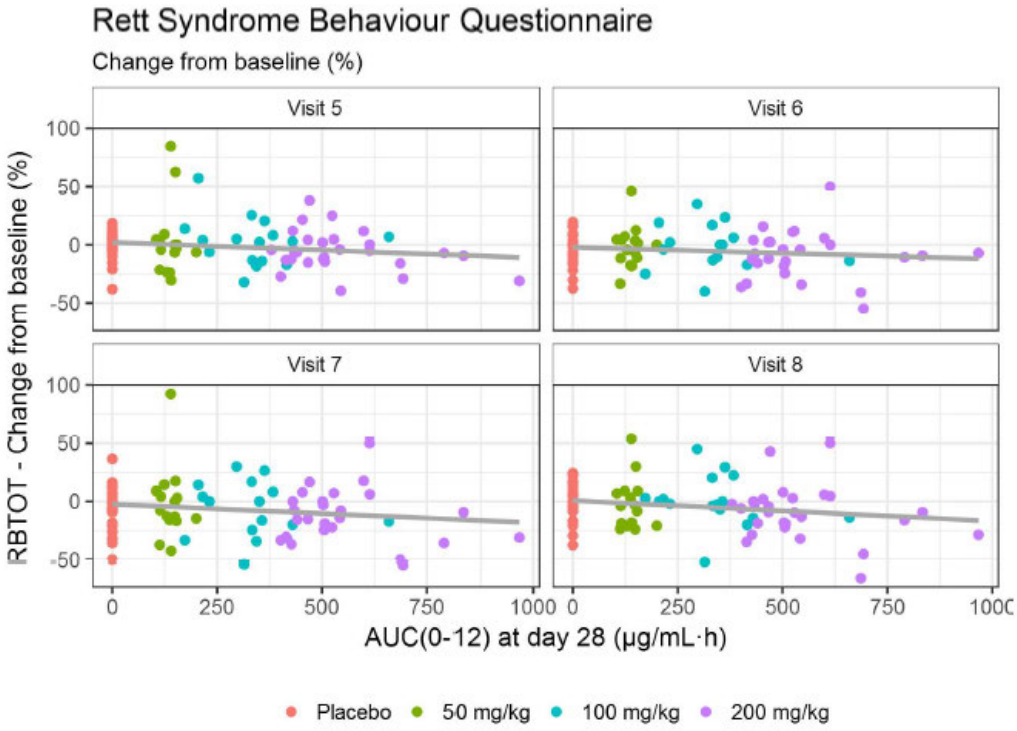
With regard to what the Applicant considered secondary or other effectiveness endpoints, the Rett syndrome Caregiver Burden Inventory demonstrated a nominal CFB favoring trofinetide, but not in its "optimism index" subscale which minimally favored placebo. For the Caregiver Top 3 Concerns, total CFB favored trofinetide (-18.54) compared to placebo (-12.52). However, breaking down the Caregiver Top 3 Concerns into categories demonstrated CFB favoring placebo for autonomic features, hand use and language/communication and trofinetide for ambulation, behavior, seizures, social Interaction and other, though the sample for each of these categories ranged from 0 to maximum of 16. The hand use concern had the largest sample sizes at 13 of 24 for placebo and 16 of 27 in the 200 mg/kg trofinetide groups. No analyses were presented by the Applicant regarding seizures and respiratory events that were recorded in the caregiver diary.

> *REVIEWER COMMENT: It is worth noting that the Caregiver Top 3 Concerns demonstrated a CFB favoring placebo in the language/communication concern. This is in contrast to the key secondary endpoint in ACP-2566-003, the CSBS-DP-IT-SCS. This, along with a similar placebo favoring change in the RTT-DSC language/communication subscale, raise doubts as to the consistency of the finding for CSBS-DP-IT-SCS in ACP-2566-003.*

Dose/Dose Response

For NEU-2566-RETT-002, the Applicant chose weight-based dosing that would increase exposure compared to NEU-2566-RETT-001. Since NEU-2566-RETT-002 was a dose-range-finding study, 50, 100, and 200 mg/kg BID were tested. The Applicant reported a relationship with AUC(0-12) and change from baseline in the RSBQ, displayed in the Applicant's Figure 11-14 from the CSR.

Figure 4 NEU-2566-RETT-002 Relationship Between Percentage Change from Treatment Baseline in RSBQ and Trofinetide Exposure



Follow-up visits during the double-blind treatment period are at Visit 5 (Day 28), Visit 6 (Day 42) and Visit 7 (Day 54). Visit 7 is the end of treatment evaluation. Visit 8 is the post-treatment follow up visit. Solid lines are obtained by linear regression. Where applicable, placebo data (AUC=0) from different visits are pooled together.
Source: NEU-2566-RETT-002 CSR Figure 11-4

REVIEWER COMMENT: There does not appear to be a clear relationship between AUC and the CFB for RSBQ to this reviewer.

6.3. ACP-2566-009

6.3.1. Study Design

Overview and Objective

ACP-2566-009 was designed as a PK bridging study to support the effectiveness of trofinetide in subjects with Rett syndrome ages 2 through 4 years. At the time of NDA submission, ACP-2566-

009 was still ongoing, but an interim synoptic clinical study report was submitted with PK data and safety data for 12 months of treatment in 10 subjects. Please see the Agency clinical pharmacology review for detailed analysis of the submitted data. Here the clinical review will summarize the major design and interim results supporting effectiveness and safety in trofinetide in ages 2 through 4 years.

Trial Design

ACP-2566-009 is an ongoing open label study occurring at seven US sites of twice-daily banded-weight-based dosing of trofinetide in subjects with Rett syndrome ages 2 through 4 years. Subjects were screened for approximately 4 weeks followed by a primary analysis treatment period of 12 weeks (Period A), followed by a up to 21 months of ongoing treatment (Period B). Subjects were scheduled for a 30 day follow-up after completion of Period B.

The Applicant intended to enroll up to 15 subjects with at least 4 subjects being less than 4 years of age at the time of screening. Inclusion and exclusion criteria were similar to those for ACP-2566-003 other than the age restriction.

The dosing of trofinetide was slightly different than ACP-2566-003 as it allowed for the lower weights expected in younger subjects and because there was a titration over three weeks (Table 26). Similarly to the pivotal trial, in this study investigators could instruct caregivers to skip up to four doses in the first 6 weeks of treatment, or to reduce the total dose to as low as 1 g BID if the subject was intolerant of the prescribed dose. The goal was to return the dosing to the highest tolerated dose.

Table 26 ACP-2566-009 Titration Schedule for Banded-Weight-Based Dosing

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

SOURCE: ACP-2566-009 Interim Synoptic Clinical Study Report Table 1

Study Endpoints

The primary endpoints for this study were safety endpoints such as TEAEs and PK endpoints. With regards to TEAE, based on the results of ACP-2566-003, diarrhea was an anticipated

adverse drug reaction (ADR) so a diarrhea management plan was distributed to all sites to consider discontinuing osmotic laxatives at enrollment and to initiate loperamide and fiber as needed for the management of diarrhea. The Applicant did also explore effectiveness by measuring CGI-I, Clinical Global Impression- Severity, Caregiver Global Impression – Improvement, and the Overall Quality of Life Rating of the Impact of Childhood Neurological Disability.

Statistical Analysis Plan

There was no effectiveness statistical analysis plan for this study. The PK analysis plan is reviewed in detail in the Agency clinical pharmacology review.

Protocol Amendments

There was one protocol amendment at the time of NDA submission which included the dosing schedule in Table 26.

6.3.1. Study Results

Compliance with Good Clinical Practices

The Applicant provided a GCP compliance statement.

Financial Disclosure

As only an interim synoptic clinical study report was submitted, a separate financial disclosure report was not submitted.

Subject Disposition

At the time of data cut-off, March 14, 2022, 17 subject had been screened, 14 received 1 dose of study drug, and 1 subject was in screening. One subject had been discontinued due to TEAE of diarrhea. Ten of 13 subjects had completed the 12 week treatment Period A.

Protocol Violations/Deviations

No major protocol deviations occurred.

Table of Demographic Characteristics

Table 27 ACP-2566-009 Demographics

Demographic parameter	**Total (N=14)**
Sex, n (%)	

Female	14 (100.0)
Age at Screening (years)	
Mean (SE)	3.1 (0.22)
SD	0.83
Median (min, max)	3.0 (2, 4)
Age at Screening categories, n (%)	
<4 Years	9 (64.3)
≥4 Years	5 (35.7)
Race	
Non-White	1 (7.1)
White	13 (92.9)
Ethnicity, n (%)	
Hispanic or Latino	1 (7.1)
Not Hispanic or Latino	13 (92.9)
Height at Baseline (cm)	
Mean (SE)	96.4 (1.96)
SD	7.34
Median (min, max)	96.6 (84, 107)
Weight at Baseline (kg)	
Mean (SE)	13.6 (0.61)
SD	2.27
Median (min, max)	14.0 (10, 18)
BMI at Baseline (kg/m²)	
Mean (SE)	14.7 (0.40)
SD	1.50
Median (min, max)	14.2 (12, 17)

SOURCE: Applicant ACP-2566-009 interim synoptic clinical study report Table 3.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

The mean age at Rett syndrome diagnosis was 1.9 years, with mean age at first symptoms noticed of 0.85 years. Similar to the mutation distribution in ACP-2566-003, the most common MeCP2 mutations in ACP-2566-009 (R255, R270, and R230) are amongst those identified as being responsible for more than 60% of Rett syndrome diagnoses in the United States (Ehrhart, Jacobsen et al. 2021).

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

Similarly to ACP-2566-003, loperamide was the most commonly started concomitant medication in this study as well (Table 28).

Table 28 ACP-2566-009 Treatment Emergent Concomitant Medications with Incidence **≥10%**

Standardized Medication Name	Count	%	Total
LOPERAMIDE	6	42.9%	6
AMOXICILLIN	2	14.3%	2
Missing	2	14.3%	2
PLANTAGO OVATA	2	14.3%	2

Primary Endpoint – PK Bridging

The primary objective in ACP-2566-009, besides assessment of safety in the younger cohort of subjects, was assessment of PK of trofinetide at banded-weight-based dosing in smaller subjects for the extrapolation of efficacy. Extrapolation of efficacy is appropriate as there is no reason to consider the pathophysiology of Rett syndrome to be different between ages two through four than it is for ages five and above. It is also appropriate to assume that the exposure-response relationship is the same.

As noted and discussed in the Agency clinical pharmacology review, the PK data from 13 subjects in ACP-2566-009 indicated that median steady state $AUC_{0\text{-}12h}$ maintained exposure of 800-1200 µg*h/mL, similarly to what was seen in ACP-2566-003.

*REVIEWER COMMENT: ACP-2566-009 was a successful PK bridging study to demonstrate effectiveness of trofinetide for treating subjects with Rett syndrome ages 2 through 4 years. Specifically, extrapolation of efficacy for this age group is appropriate given the similar pathophysiology of the disease in this only slightly younger age group and the expected similar exposure-response profile. The exposures achieved in the younger/smaller cohort of ACP-2566-009, which were within the target range of 800-1200 µg*h/mL support extrapolation of effectiveness to ages 2 through 4 years. See the Agency clinical pharmacology review for details.*

6.4. NEU-2566-RETT-001

The Applicant presented results of NEU-2566-RETT-001 as confirmatory evidence of effectiveness of trofinetide for Rett syndrome. NEU-2566-002 was a Phase 1/2 study and first-in-patient study enrolling Rett syndrome patients aged 16-45 to explore the safety of trofinetide, with a dose finding component. It is not reviewed here as a pivotal study.

In brief, NEU-2566-RETT-001 consisted of 3 cohorts. Cohort 0 studied 9 patients who received trofinetide or placebo in 5:4 ratio with trofinetide dosed at 35 mg/kg BID for 14 days. Cohort 1 studied 18 patients randomized 13:5 to trofinetide 35 mg/kg BID or placebo for 28 days. Cohort

2 enrolled 29 subjects randomized 18:11 to trofinetide 70 mg/kg BID or placebo. The Applicant presented a benefit of trofinetide 70 mg/kg BID over placebo at in least square mean change from baseline values at Day 26 in Motor Behavioral Assessment, CGI-I, and Caregiver Top 3 Concerns, none of which were nominally significant.

REVIEWER COMMENT: NEU-2566-RETT-001 was a phase 1 study and not intended to assess effectiveness, therefore the lack of effectiveness in the study results does not contribute positively or negatively to the assessment of effectiveness for trofinetide. The low doses of trofinetide tested and short duration of treatment do not allow for this data to contribute meaningfully to the safety assessment.

7. Integrated Review of Effectiveness

7.1. Assessment of Efficacy Across Trials

The assessment of effectiveness of trofinetide for the treatment of Rett syndrome is based on one adequate and well-controlled clinical trial, ACP-2566-003, with confirmatory evidence from the exploratory trial NEU-2566-RETT-002. There were substantial differences in dosing, treatment duration, and lack of prespecified effectiveness analysis plan for NEU-2566-RETT-002. This review will attempt to compare and contrast findings between the two studies.

7.1.1. Primary Endpoints

Table 29, Table 30 and Figure 5 allow direct visual comparison of the primary endpoints RSBQ CFB and CGI-I for study ACP-2566-003 with those same endpoints in NEU-2566-RETT-002. Figure 5 specifically displays the RSBQ subscale CFB as trofinetide-placebo forest plots for the two studies. It should be noted that a subscale analysis was not part of a prespecified analysis plan to contribute to the evaluation of effectiveness and is only displayed to help with assessment of the strength of the primary outcome.

Table 29 ACP-2566-003 and NEU-2566-RETT-002 Primary Analysis Results of RSBQ Change from Baseline

ACP-2566-003	Placebo	Trofinetide (200 mg/kg BID for NEU-2566-RETT-002)
n	85	76
Baseline Mean (SE)	44.5 (1.26)	43.7 (1.21)
LS Mean Change from Baseline to Week 12 (SE)	-1.7 (0.90)	-4.9 (0.94)
LS Mean Difference (SE)		-3.1 (1.30)
95% CI		(-5.7, -0.6)
Two-sided p-value		0.0175
Effect size (Cohen's d)		0.37
NEU-2566-RETT-002		
n	24	27
Baseline Mean	39.5	42.2
LSMean Change from Day 14 (Week 2) Baseline to Day 54 (Week 7)	-2.3	-6.7
LS mean difference		-4.4
P-Value vs Placebo		0.042

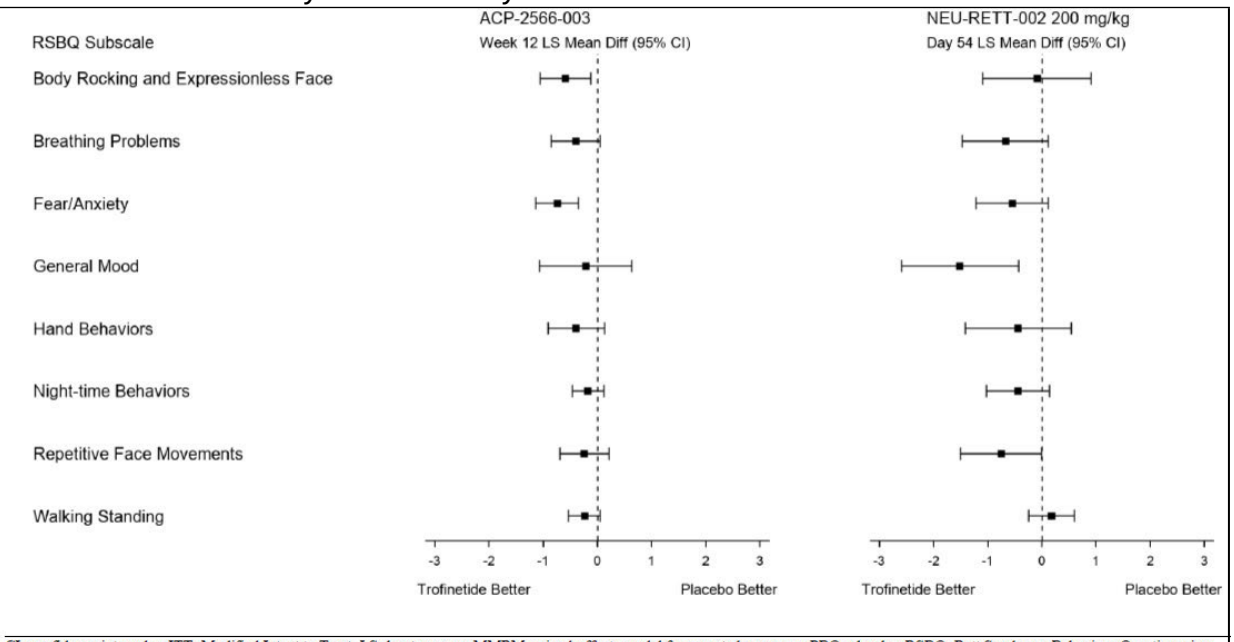
Source: Reviewer, based on results previously reported in Sections 6.1.2 and 6.2.2

Table 30 ACP-2566-003 and NEU-2566-RETT-002 Primary Analysis of CGI-I

ACP-2566-003	Placebo	Trofinetide
Week 12		
n	86	77
LS mean (SE)	3.8 (0.07)	3.5 (0.07)
Difference from placebo		
LS mean difference (SE)		-0.3 (0.10)
95% CI		(-0.5, -0.1)
Two-sided p-value		0.0030
Effect size (Cohen's d)		0.47
NEU-2566-RETT-002		
Week 14 Baseline		
n	24	27
LSMean Change from Day 14 (Week 2) Baseline to Day 54 (Week 7)	3.5	3.0
LS mean difference		-0.5
P value vs Placebo		0.025

Source: Reviewer extracted from Table 12 and Sections 6.1.2 and 6.2.2

Figure 5 Forest Plot of Treatment Difference in LS Mean Change from Baseline to End of Treatment Visit in RSBQ Subscores and Corresponding 95% CI Full Analysis Set in Study ACP-2566-003 and mITT Analysis Set in Study NEU-RETT-002



CI=confidence interval; mITT=Modified Intent to Treat; LS=least squares; MMRM=mixed-effects model for repeated measures; PBO=placebo; RSBQ=Rett Syndrome Behaviour Questionnaire; TROF=trofinetide.
For Study ACP-2566-003, LS mean difference in change from Baseline to Week 12 value between TROF and PBO is based on a Mixed Model Repeated Measure (MMRM) model with treatment, visit, age group, RSBQ Group, treatment*visit Interaction, Baseline value (for the corresponding subscale), Baseline value*visit interaction as fixed factors/covariates.
For Study NEU-RETT-002, LS mean difference in change from Baseline (Day 14; end of placebo run-in) to Day 54 value between TROF and PBO is based on general linear models with following factors/covariates: for General Mood and Walking Standing -- treatment, PBO response (change from Day 1 to Day 14 value), Baseline (Day 14) value; for Breathing Problems, Hand Behavior, Bo Rocking and Expressionless Face, Fear/Anxiety -- treatment, PBO response; for Repetitive Face Movements, Night-time Behaviors -- treatment, Baseline value.
Program: K:¥Statistics¥ACP-2566-CrossStudy¥Rett¥ISS¥Data¥2022_07_12_120dayUpdate¥TLF-FDARFI¥f-forest-rsbq-002-003 [05DEC2022 13:49] Source data: a_rb2, adqrsbq

Sources: Applicant Figure AH.FOREST.RSBQ.002.003 created as response to IR sent by Division. NB: Different subscale groupings were used to each study.

REVIEWER COMMENT:
The primary evidence of effectiveness is dependent on ACP-2566-003, as it was the only study designed to assess effectiveness. While the two primary endpoints of ACP-2566-003 were measured in NEU-2566-002, they were exploratory analyses of effectiveness.

While RSBQ as a whole favored trofinetide in both ACP-2566-003 and NEU-2566-RETT-002, inconsistencies in the strength and direction of treatment effect were evident in the subscale scores of the RSBQ when comparing the two studies. As noted in Section 6.1.1, the construct of the RSBQ subscales were different (based on distribution of three of the 45 items). However, this does not preclude comparing the subscale results of the two studies to determine if there were common trends. For instance, in ACP-2566-003, the RSBQ finding seems to be heavily driven by "body rocking and expressionless face" and "fear/anxiety" subscales, with the 95% confidence intervals for all other subscales crossing 0. Whereas in NEU-2566-RETT-002, the "body rocking and expressionless face" subscale favors trofinetide only slightly. In NEU-2566-RETT-002, the walking/standing

subscale actually favors placebo; a completely opposite finding from ACP-2566-003 (Figure 5). This weakens slightly the conclusion on the strength of the benefit of trofinetide on the RSBQ.

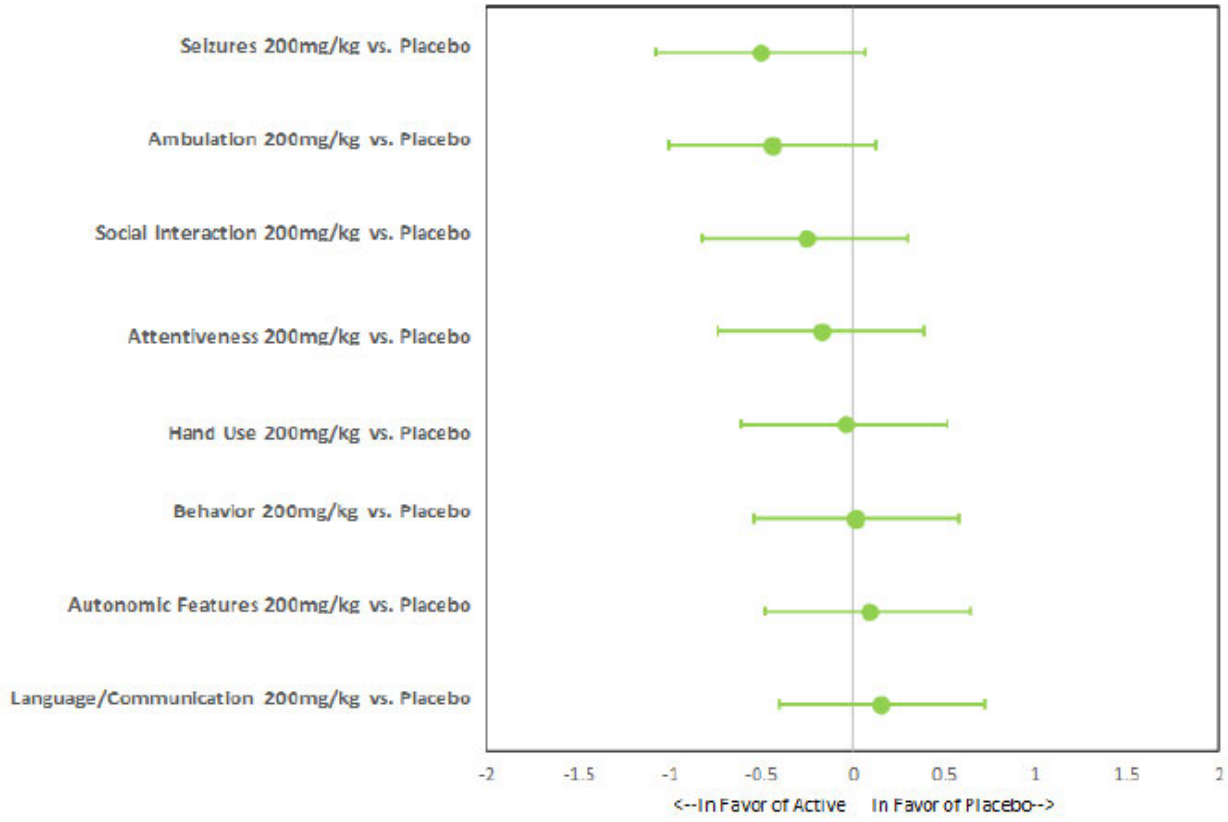
Despite these contrasts and inconsistencies in method of analysis, taken at face value, both studies did favor trofinetide in total scores for RSBQ and CGI-I, the primary endpoints for ACP-2566-003.

7.1.2. Secondary and Other Endpoints

In ACP-2566-003, the CSBS-DP-IT-SCS was used to assess communication beyond the single communication related question in the RSBQ. In study 003, the Applicant demonstrated a statistically significant difference between the trofinetide and placebo groups, controlled for multiplicity (LS mean difference (SE) 1.0 (0.37), 95% CI (0.3, 1.7); p= 0.0064) with the trofinetide group maintaining its baseline score compared to a reduction in the CSBS-DP-IT-SCS for the placebo treated group. The Applicant presented as supportive evidence that the Rett syndrome Clinician Rating of Ability to Communicate Choices (RTT-COMC), an exploratory endpoint included in ACP-2566-003 also nominally favored trofinetide in a non-prespecified analysis unadjusted for multiplicity.

The RTT-COMC is a derivative of the Rett syndrome Clinician Domain Specific Concerns (RTT-DSC), which was used in NEU-2566-RETT-002. That tool is a visual analogue scale (VAS) based assessment asking the clinician to rate severity in the past week from “not at all severe” to “very severe” over the last week, in 8 domains: hand use, ambulation, seizures, autonomic features, behavior, attentiveness, social interaction, and language/communication. Of note, in NEU-2566-RETT-002, placebo was nominally favored in the language/communication subdomain (along with the behavior and autonomic features subdomains).

Figure 6 NEU-2566-RETT-002 RTT-DSC Subdomain Analysis at Day 54 in 200 mg/kg BID Group (Source: Applicant NEU-2566-RETT-002 CSR Figure 11-5)



7.1.3. Subpopulations

REVIEWER COMMENT: Males with Rett syndrome were not studied in the trofinetide clinical development program. Given the predominance of Rett syndrome in females, it is not likely to be feasible to assess the effectiveness of trofinetide in male. However, there is also no scientific reason to believe that males with Rett syndrome would not benefit from the use of trofinetide. Therefore, it is appropriate that the Applicant is claiming an indication for adults and pediatric patients 2 years of age and older, without regard to biological sex. Due to the rarity of Rett syndrome and the demographic distribution of patients enrolled in ACP-2566-003 and NEU-2566-RETT-002, the sample sizes are inadequate to comment regarding effectiveness in minority ethnic or racial populations.

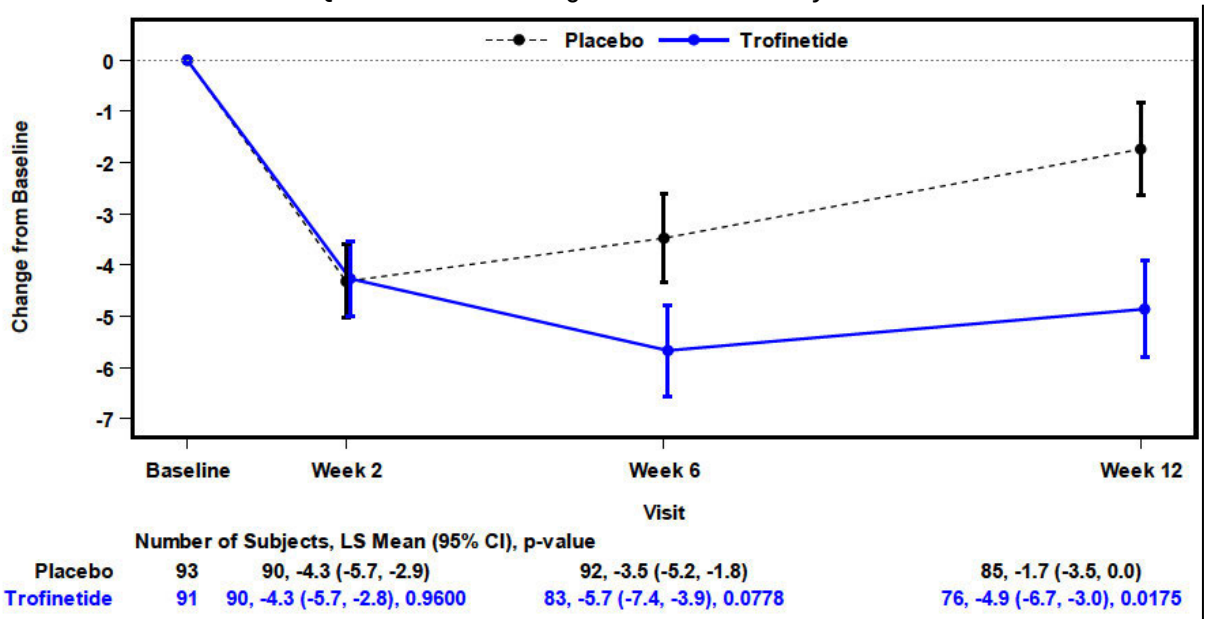
7.1.4. Dose and Dose-Response

In NEU-2566-RETT-002 trofinetide area under the curve increased in a relatively linear fashion for the doses of 50, 100, 200 mg/kg BID. The 50 mg/kg and 100 mg/kg doses did not show any effects on the exploratory effectiveness endpoints. However, at 200 mg/kg BID, there was a nominally significant reduction in RSBQ and CGI-I. However, it was noted that body weight was influential on exposure, with lower weight patients experiencing lower exposures at the same weight-based dosing. Hence for study ACP-2566-003, the weight-based dosing was banded to achieve exposures across weights similar to the highest exposures achieved by the 200 mg/kg BID cohort. Since study ACP-2566-003 enrolled patients ages 5 and above, while Rett syndrome is usually diagnosed as early as age 2, the Applicant conducted ACP-2566-009, an open-label study in patients ages 2 through 4. ACP-2566-009 was ongoing at the time of NDA submission with 10 subjects having completed 12 weeks of treatment. The interim results of ACP-2566-009 demonstrated that effective exposures could be achieved in younger/smaller patients, allowing for extrapolation of effectiveness to the younger age group.

7.1.5. Onset, Duration, and Durability of Efficacy Effects

With regards to onset of effect, the Applicant demonstrated in ACP-2566-003 a separation in RSBQ scores at the week 6 evaluation (Error! Reference source not found.). However, it is notable that at week 2, both treatment groups demonstrated an improvement in RSBQ, indicating a likely placebo effect. After week 2, the separation between trofinetide and placebo groups in RSBQ primarily resulted from loss of the placebo effect in the placebo group (return to baseline RSBQ) and maintenance of the affect in an increasingly smaller group of trofinetide patients (due to study withdrawals). This same effect at week 6 was not evident in the CGI-I where the LS Mean (95% CI) were 3.7 (3.6, 3.9) for placebo and 3.7 (3.5, 3.8) for trofinetide. The key secondary endpoint, CSBS-DP-IT-SCS also did not demonstrate a separation until the Week 12 measurement.

Figure 7 ACP-2566-003 RSBQ Total Score Change from Baseline by Visit



	Number of Subjects, LS Mean (95% CI), p-value			
Placebo	93	90, -4.3 (-5.7, -2.9)	92, -3.5 (-5.2, -1.8)	85, -1.7 (-3.5, 0.0)
Trofinetide	91	90, -4.3 (-5.7, -2.8), 0.9600	83, -5.7 (-7.4, -3.9), 0.0778	76, -4.9 (-6.7, -3.0), 0.0175

Source: Figure AH1.1.1

Abbreviations: CI=confidence interval; LS=least squares; MMRM=mixed-effects model for repeated measures; OC=observed cases; RSBQ=Rett Syndrome Behaviour Questionnaire; SE=standard error

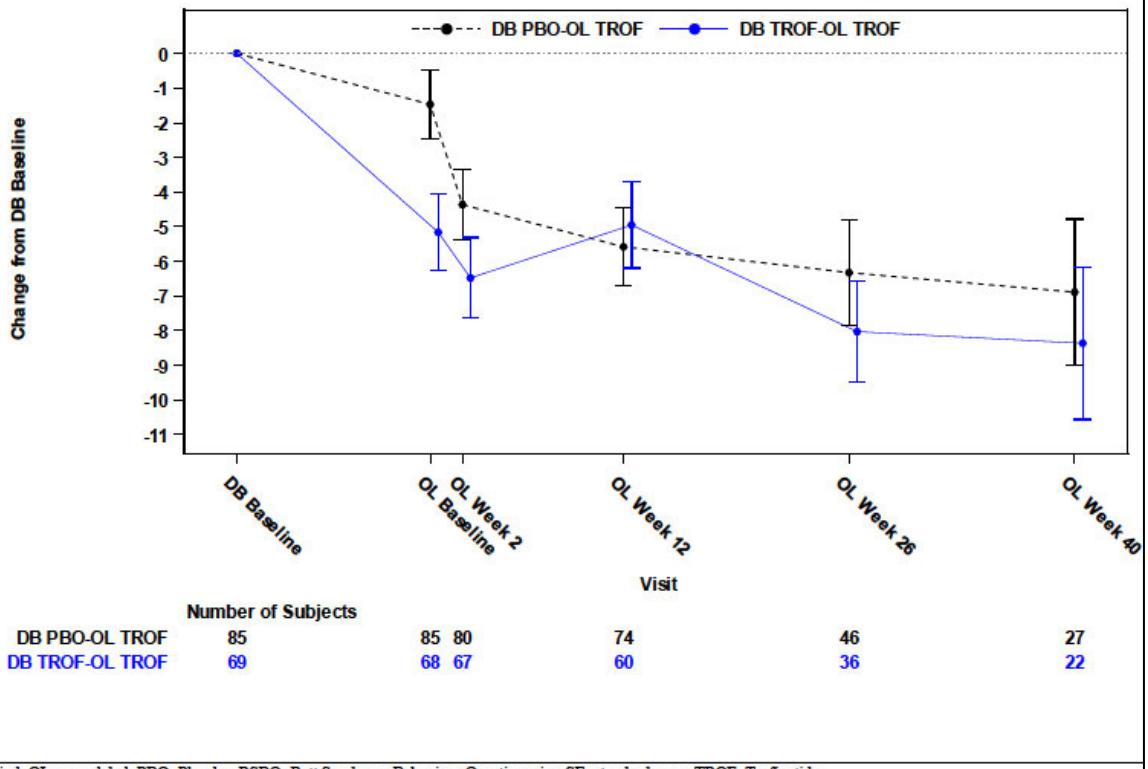
Note: The MMRM included age group, Baseline RSBQ severity, planned treatment, study visit, treatment-by-visit interaction, Baseline-by-visit interaction, and Baseline total score as fixed effects. An unstructured matrix was used to model within-subject errors. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

Source: Applicant ACP-2566-003 CSR

With regards to persistence of clinical effectiveness with continuous treatment, the Applicant submitted results of continued assessments in ACP-2566-004, an ongoing 40-week open-label extension study of trofinetide in Rett syndrome. The data cutoff date for ACP-2566-004 was February 15, 2022. Of the 187 patients first enrolled in ACP-2566-003, 161 patients completed the double-blind study with data at Week 12 (76 trofinetide, 85 placebo). Of those 161 patients, 7 patients did not continue into ACP-2566-004, with a total of 154 patients enrolled in ACP-2566-004; 85 transitioning from blinded placebo to open-label trofinetide, 69 transitioning from blinded trofinetide to open-label trofinetide. Of the 154 patients enrolled in the open-label extension, 63 withdrew from the study early, 51 of whom withdrew due to adverse event, 5 due to lack of effectiveness, and 3 each withdrew due to noncompliance or subject withdrew consent. Of the remaining 92 patients, 45 completed 40 weeks of open-label treatment and 46 were ongoing at the time of data cutoff.

Amongst the patients who were able to tolerate trofinetide, there was overall a 5- to 8-point improvement in RSBQ that seemed to persist throughout the 40-week treatment period. Patients who were on placebo during ACP-2566-003 after some remnant placebo effect, had a further drop to settle near the same 5- to 8-point improvement in the RSBQ after they started on trofinetide in the open-label extension (Figure 8).

Figure 8 RSBQ Total Score Change from ACP-2566-003 DB Baseline into ACP-2566-004 OL



DB=double-blind; OL=open-label; PBO=Placebo; RSBQ=Rett Syndrome Behaviour Questionnaire; SE=standard error; TROF=Trofinetide.

Source: Applicant ACP-2566-004 CSR (Synoptic)

REVIEWER COMMENT: For ACP-2566-004, narratives were not provided. Given the method of coding was likely unchanged from ACP-2566-003, this reviewer anticipates that the 11 patients coded as withdrawing due to lack of effectiveness, non-compliance, or withdrew consent also experienced adverse events, which would bring the total number of withdrawals due to adverse events to 62 of the 63 early withdrawals.

The Applicant did not provide data on what happens to the effect when patients discontinue treatment. With regards to persistence of effect while on treatment, the 12-week data from ACP-2566-003 is the most convincing as the effect in patients who could tolerate the treatment was maintained as compared to placebo.

7.2. Additional Efficacy Considerations

7.2.1. Considerations on Benefit in the Postmarket Setting

The frequency of the diarrhea AE was so high that a protocol amendment was made during ACP-2566-003 to plan for the occurrence of diarrhea and to institute mitigating strategies even before the start of the study drug. It will be important in labeling to address the frequency of diarrhea and to offer mitigating strategies for prescribing clinicians.

7.2.2. Other Relevant Benefits

REVIEWER COMMENT: There are no other relevant benefits.

7.3. Integrated Assessment of Effectiveness

The placebo-controlled ACP-2566-003 was an adequate and well-controlled effectiveness study that supports approval of trofinetide for the treatment of children and adults with Rett syndrome. ACP-2566-003 was a multicenter study that provided reliable and statistically significant evidence that treatment with trofinetide for 3 months improved caregiver scoring on the co-primary endpoints of the Rett syndrome Behavior Questionnaire (RSBQ) and clinician rating of the Clinician Global Impression of Improvement (CGI-I). The RSBQ is a caregiver completed questionnaire of 45 common Rett syndrome behavioral symptom items with available responses of 0 to 2 assessing the frequency or severity of that behavior. For 44 out of the 45 items, a higher number indicates an increased frequency of a Rett syndrome impaired behavior. For the 45th item, the Applicant reversed the scoring for consistency. The total score for the RSBQ is 90 points, which would indicate the most severe or frequent occurrence of Rett syndrome features. In ACP-2566-003, the trofinetide treated group had a LS mean decrease in RSBQ at 12 weeks of 4.9 points compared to 1.7 points for the placebo group. The co-primary endpoint of CGI-I also favored trofinetide, with subjects receiving trofinetide achieving a score of 3.4 at 12 weeks (rating of 3 indicates “Minimal Improvement”) compared to subjects receiving placebo who achieved a rating of 3.7 (rating of 4 indicates “No Change”). Supportive evidence of effectiveness is provided by a prespecified statistically significant difference favoring trofinetide on the secondary endpoint, the Communication and Symbolic Behavior Scales Developmental Profile – Infant and Toddler – Social Composite Score (CSBS-DP-IT-SCS). This scale, developed as a screening tool to alert clinicians to potential communication deficits in infants and toddlers, is not fit for purpose for determining a treatment related benefit in the complex concept of social reciprocity and communication; however, the finding of a benefit in trofinetide patients is still supportive of overall effectiveness.

Further confirmatory evidence of effectiveness was obtained in NEU-2566-RETT-002, a multisite

Phase 2 single-blind placebo run-in, randomized double-blind, placebo-controlled dose ranging clinical trial. The original objective of NEU-2566-RETT-002 was to investigate the safety, tolerability and pharmacokinetics of treatment with 3 different doses of oral trofinetide in girls ages 5 to 15 with Rett syndrome. A total of 82 patients were enrolled and five outcome measures explored, but the primary support for effectiveness came from comparison of RSBQ and CGI-I in 24 placebo-treated patients and 27 patients receiving 200 mg/kg twice daily of trofinetide. The least square means difference between trofinetide and placebo in RSBQ met nominal significance (p=0.042) as did CGI-I (P=0.029). Of note, the lower doses tested in NEU-2566-RETT-002 did not demonstrate a dose response effect in the RSBQ. The demonstration of a trofinetide favoring CGI-I outcome in both trials indicates that the improvements in the RSBQ are likely to be clinically meaningful. And, as demonstrated in the Voice of the Patient Report (Coenraads, Hehn et al. 2022), for this devastating genetic condition with unmet medical need, even small incremental improvements would be considered worthwhile.

The evidence for effectiveness in patients with Rett syndrome aged 2 to 4 was provided by the bridging PK study, ACP-2566-009 in which 10 patients completed 12 weeks of treatment with trofinetide at banded-weight-based-dosing. The study achieved exposure comparable to that required to achieve response in ACP-2566-003. Study 009 was an open-label study not designed to assess effectiveness; however, CGI-I mean score at the end of 12 weeks was 3.3 (SE 0.24) indicating clinician impression of improvement.

This evidence, together, meets the statutory requirement for evidence of effectiveness of trofinetide for the treatment of Rett syndrome in adults and pediatric patients 2 years of age and older. Although the RSBQ assesses primarily behavioral aspects of Rett syndrome with one question regarding mobility, the CGI-I did assess a global impression of improvement. While it is impossible to comment on trofinetide's effects other aspects of Rett syndrome, such as growth impairment, epilepsy, language development, dexterity or other motor skills, or the systemic effects of Rett syndrome, there is no evidence that trofinetide is only efficacious for one particular aspect of Rett syndrome, therefore the general indication is appropriate. Although no male patients with Rett syndrome were included in the clinical trials due to the rarity of this disorder in males, the benefit conferred by the use of trofinetide should not differ in the few males with Rett syndrome as compared to females.

The limitations of the evidence are the 1) reliance on one single adequate and well controlled study with confirmatory evidence, 2) the limitations of the RSBQ as a tool to measure functional improvement, 3) the disproportionate study withdrawal rate (16 trofinetide subjects versus 8 placebo subjects), 4) the disproportionate and rapid onset of diarrhea in the trofinetide arm along with the disproportionate use of loperamide in the trofinetide arm, risking functional unblinding, and 5) confirmatory evidence coming from a post-hoc statistical analysis.

The reliance on one pivotal controlled study and confirmatory evidence is appropriate in this

rare and life-threatening genetic disorder of childhood which leaves patients severely disabled. As expressed in the Voice of the Patient Report, caregivers of Rett patients are willing to try anything that may reduce the suffering of their loved ones (Coenraads, Hehn et al. 2022). As previously noted, Section IV.B. of the 2019 draft FDA guidance, Demonstrating Substantial Evidence of Effectiveness, regarding meeting the substantial evidence standard based on one adequate and well-controlled clinical investigation plus confirmatory evidence, does not define what can be considered confirmatory evidence but does provide examples of types of data or information that could potentially be considered confirmatory evidence. The character of confirmatory evidence can depend on the strength and robustness of the single adequate and well-controlled study and factors such as seriousness of the disease and unmet medical. Rett syndrome is a rare and serious disorder with dire unmet medical need given that there are no treatments indicated for it. Although there are some limitations to ACP-2566-003 that are noted below, it was an adequate and well-controlled study with persuasive and robust results on the co-primary and secondary endpoints. The strength of the primary study allows for flexibility on the character of the confirmatory evidence from the exploratory dose-finding study, NEU-2566-RETT-002. The post hoc analysis of NEU-2566-RETT-002 did demonstrate nominally significant improvement in RSBQ and CGI-I favoring trofinetide at the 200 mg/kg dose, confirming the findings of the pivotal trial, ACP-2566-003. Therefore, it is appropriate to consider the results in the 200 mg/kg group of NEU-2566-RETT-002 as confirmatory evidence.

The RSBQ as an outcome measure, is not ideal in a number of ways. The questions asked of caregivers are subjective and depend on the caregiver impression of the patient's status (e.g., 'has difficulty in breaking/stopping hand stereotypies'). They also are somewhat vague with regards to quantitation (e.g., makes repetitive movements involving fingers around tongue'). Despite these limitations, the RSBQ at this time is the most well studied outcome measure in Rett syndrome.

With regards to study completion, the primary analysis was based on data from any randomized patients who received treatment and had at least 1 post-baseline RSBQ and CGI-I score. This full analysis set consisted of 91 trofinetide and 93 placebo patients. However, 12-week outcome data was available for only 77 trofinetide and 86 placebo patients, as the remainder had both discontinued study drug and withdrawn from the trial. At the 12-week time point, 70 patients were still using trofinetide and 85 were still using placebo, the rest having discontinued the medication. The disproportionate withdrawal of trofinetide patients from the study could have led to informational censoring which limits the strength of the findings. This weakness is mitigated by the sensitivity analyses performed which demonstrated benefit for trofinetide (See the Office of Biostatistics Review).

Regarding the extremely common occurrence of diarrhea and the large percentage of trofinetide patients placed on loperamide, there are two concerns. First, the rapid onset of diarrhea with trofinetide created a chance of unblinding the study and biasing the results, given

that the outcome measures were survey based. The subsequent start of loperamide only in trofinetide patients (only 3 placebo-treated patients received loperamide) further increased the chances of unblinding. This imbalance suggests that changes seen in RSBQ or CGI-I were due to the combination of loperamide and trofinetide rather than trofinetide alone. The Office of Biostatistics reviewer completed a mediation analysis that determined that for the CGI-I, loperamide was unlikely to have contributed to the change, while for RSBQ there was likely a small effect of loperamide, but not large enough to explain the main effect (See Office of Biostatics Review).

Altogether, despite limitations, the finding of improved RSBQ and CGI-I scores in trofinetide treated patients in ACP-2566-003, with confirmatory evidence from study NEU-2566-RETT-002 are compelling and clinically meaningful. Application of regulatory flexibility allows for determining that trofinetide has met substantial evidence of effectiveness given the unmet need in this rare, severe, and life-threatening disorder.

8. Review of Safety

8.1. Safety Review Approach

This safety evaluation is specific to the indication of Rett syndrome in females, ages 2 and above. The Applicant created the RTTDB pool, consisting of the populations of studies NEU-2566-RETT-001, NEU-2566-RETT-002, and ACP-2566-003. Because the two phase 2 studies in Rett syndrome NEU-2566-RETT-001 and NEU-2566-RETT-002 were double-blind placebo-controlled studies, they could contribute to the overall safety database especially for looking for small differences from placebo. However, given the lower dosing and shorter durations of those trials, a comparison was made to the single ACP-2566-003 pivotal trial before presenting the RTTDB pooled safety data here. In order to assess long-term safety, pooled safety data from trofinetide treated patients in ACP-2566-003, ACP-2566-004, and ACP-2566-005 were pooled into the RTTLT pool. The RTTLT pool is made of 178 patients including the original 93 patients who received trofinetide as part of ACP-2566-003 (whether or not they completed that study) and the 85 patients who received placebo in ACP-2566-003, completed the study, and continued into the open-label extensions where they started receiving trofinetide. The Applicant also created an RTTOL pool that captured safety data from the two consecutive open-label extension studies ACP-2566-004 and ACP-2566-005; however, this was not used for this reviewer's analysis.

Safety in ages 2-5 was examined via the open-label PK study ACP-2566-009.

Because ACP-2566-004, ACP-2566-005, and ACP-2566-009 continue to be ongoing, the primary data cutoff used was February 15, 2022, for ACP-2566-004, March 4, 2022, for ACP-2566-005,

and March 14, 2022, for ACP-2566-009. The 120-day-safety-update cutoff date was July 12, 2022.

The available narratives for deaths, serious adverse events, laboratory studies, and vital signs were reviewed. Reviewer and clinical data scientist analyses were conducted on the submitted datasets for NEU-2566-RETT-001, NEU-2566-RETT-002, ACP-2566-003, ACP-2566-004, and ACP-2566-005, including adverse events, serious adverse events, deaths, and laboratory value assessments.

The majority of safety data presented is from the time of NDA submission, with new or additional information provided by the 120-day-safety-upate included with an indication of its source as needed.

8.2. Review of the Safety Database

8.2.1. Overall Exposure

Overall Exposure

Total Unique Human Subjects Exposure

In response to an information request during review of this NDA, the Applicant provided the following Table 31 to indicate the total number of human subjects exposed to trofinetide or placebo in this and other development programs.

Table 31: Number of Unique Human Subjects Exposed to Trofinetide

Clinical Trial Groups (Unique Counts Across All Groups)	**Trofinetide (n=673)**	**Placebo (n=307)**
Healthy volunteers in any and all trials[a]	183	55
Controlled trials conducted for this indication[b]	176	133
All other trials conducted for this indication[c]	83	--
Controlled trials conducted for other indications	231	119

Source: Applicant Table AH.Exposure.3.1
Note: includes subjects from Studies Neu-2566-HV-001, -002, -003, -004, -005, -TBI-001/002, TBI-003, FXS-001, RETT-001, RETT-002; ACP-2566-003, -004, -005, -006, -007, -008, -009, (b) (4) Study Summaries available in NDA Section 2.7.4.9

[a] Subject counts in this row reflect the number of unique subjects treated in the Phase 1 studies including 10 subjects (b) (4)

[b] Subject counts in this row reflect the number of unique subjects treated in Phase 2 and Phase 3 controlled trials for this indication. There were 39 subjects who enrolled in both Study Neu-2566-RETT-002 and Study ACP-2566-003; of these, 10 subjects received trofinetide in both studies and 5 subjects received placebo in both studies. These

repeated subjects who received the same treatment assignments in both studies were counted only once in the corresponding cells.

[c] Subject counts in this row reflect the number unique subjects treated with trofinetide in open-label extension studies who previously received placebo in double-blind studies

As Rett syndrome is a rare disease, the safety exposure database was agreed to at the pre-NDA meeting as documented in the minutes dated March 24, 2022. The Applicant indicated that 35 patients would be exposed to trofinetide for at least 12 months, 58 patients for at least 9 months, and 87 patients for at least 6 months. The Agency indicated that the minimum number of patients exposed for a minimum of 12 months should be at least 35.

It should be noted that patient exposure numbers are taken from the placebo-controlled clinical trials that involved patients with Rett syndrome, or those in which patients participating initially in a placebo-controlled study rolled over into an open-label extension. This includes the Phase 2 studies Neu-2566-001, Neu-2566-002, and the Phase 3 and open-label studies ACP-2566-003 and its consecutive open-label extensions -004, and -005. There were 39 patients who participated in Neu-2566-002 who went on to participate in ACP-2566-003. These patients may have received placebo in one study and/or trofinetide in the other (See Table 32). Total exposure was counted, deducting any interruptions in trofinetide exposure lasting 30 days or more.

Table 32: Treatment Assignments of 39 Patients Participating in Study RETT-002 followed by Study RETT-003

Placebo -> Placebo	Placebo -> Trofinetide	Trofinetide-> Placebo	Trofinetide- >Trofinetide
5	7	17	10

Source: Applicant 2.7.4 Summary of Clinical Safety, narrative, P31/239

The Applicant did meet the exposure durations as agreed to in the Pre-NDA Meeting (Table 33). In addition, at the 120-day safety update, the Applicant provided an update on exposure duration (Table 34). At the time of the 120-day safety update, 100 subjects had ≥6 months of exposure, 86 had ≥9 months, and 69 had ≥ 12 months.

Table 33: Trofinetide Exposure Duration in Rett syndrome at the Time of NDA Submission

Exposure Duration	**≥12** months	**≥9** month	**≥6** months
Number of Patients	40	61	92

Source: Applicant Integrated Summary of Safety Table RTTLT.4

Table 34 Trofinetide Exposure Duration at 120-Day Safety Update

	All TROFINETIDE (N=178)
Total Duration of Exposure (days)[1]	
n	178
Mean (SE)	285.2 (16.50)
SD	220.18
Median	261.0
Min, Max	8, 896
Total Duration of Exposure - Categorical, n (%)	
<6 weeks	23 (12.9)
≥6 weeks to <12 weeks	21 (11.8)
≥12 weeks to <6 months	34 (19.1)
≥6 months to <9 months	14 (7.9)
≥9 months to <12 months	17 (9.6)
≥12 months to <18 months	45 (25.3)
≥18 months to <24 months	20 (11.2)
≥24 months	4 (2.2)

SOURCE: Applicant Integrated Summary of Safety at 120 day safety update Table RTTLT.120d.4 TROF=trofinetide. SD=standard deviation; SE=standard error; Min=minimum; Max=maximum. N is used as the denominator for calculating percentages within each column. [1] Duration of exposure to trofinetide within each study is calculated as last trofinetide dose date - first trofinetide dose date + 1. If a subject experienced continuous study drug interruption for more than 30 days, the duration of exposure will be calculated as last trofinetide dose date – first trofinetide dose date + 1 - duration of interruption. For a given subject, the total duration of trofinetide exposure is calculated as the sum of trofinetide exposure durations from all studies in which the subject received trofinetide. For studies ACP-2566-004 or ACP-2566-005, if a subject is still alive and ongoing at the time of data cut, the last dose date will be imputed as the data cut date. Program: K:\Statistics\ACP-2566-CrossStudy\Rett\ISS\Data\2022_07_12_120dayUpdate\TLF-CSR\t-ex-rttlt [29AUG2022 10:27] Source data: ADSL

REVIEWER COMMENT: Given the prevalence of the disease, the exposure is deemed adequate to support a reasonable assessment of safety.

8.2.2. Relevant characteristics of the safety population:

The safety and effectiveness populations are nearly the same, with the majority of safety data coming from pivotal trial ACP-2566-003 and supportive data from NEU-2566-RETT-002. The demographics of these studies are discussed in sections 6.1.2 Study Results and 6.2.2 Study Results. Since NEU-2566-001 also provides a minimal amount of safety data in patients with Rett syndrome, its demographics are presented in Table 35, Table 36, and Table 37. Overall, the only area of concern is the lack of racial and ethnic diversity in these studies. Otherwise, they are well representative of the Rett syndrome population for whom the drug is indicated.

Table 35: NEU-2566-001 AGE DISTRIBUTION

Age Group	Count	% of Total
11 ≤ AGE<16	1	1.79%

16 ≤ AGE<21	20	35.71%
21 ≤ AGE	35	62.50%
All	56	100.00%

Source: Reviewer Analysis, JMP Clinical Demographics Report NEU-2566-001

Table 36: NEU-2566-001 RACE DISTRIBUTION

Race	Count	% of Total
ASIAN	1	1.79%
BLACK OR AFRICAN AMERICAN	5	8.93%
WHITE	50	89.29%
All	56	100.00%

Source: Reviewer Analysis, JMP Clinical Demographics Report NEU-2566-001

Table 37: NEU-2566-001 ETHNICITY DISTRIBUTION

Ethnicity	Count	% of Total
HISPANIC OR LATINO	5	8.93%
NOT HISPANIC OR LATINO	51	91.07%
All	56	100.00%

Source: Reviewer Analysis, JMP Clinical Demographics Report NEU-2566-001

8.2.3. Adequacy of the safety database:

For chronically administered drugs for non-life-threatening diseases, the International Conference on Harmonisation (ICH) E1 guidelines recommend having studied drug exposure in 1500 patients overall, 300-600 patients for six months, and 100 patients for one year at the dose or dose range believed to be efficacious. Because Rett syndrome is a rare disease, there is no specific minimum number of patients that should be studied to establish clinical safety. The overall Rett syndrome patient exposure in the clinical development program is adequate, and the Applicant did achieve the agreed to sample of at least 35 subjects who were exposed for at least 12 months.

8.3. Adequacy of Applicant's Clinical Safety Assessments

8.3.1. Issues Regarding Data Integrity and Submission Quality

Overall, the NDA submission was complete and relatively well organized. This reviewer had difficulty conducting pooled analyses of AEs for patients who took part in some combination of NEU-2566-RETT-002 and ACP-2566-003 and its subsequent open-label extensions since the Applicant organized the data in such a way that the different studies were not treated as separate treatment periods. Despite that, because the pooled analyses were not the primary source of the safety assessment, given the differences between the studies, this did not affect the conclusions made. The submission quality with respect to the Applicant's clinical safety

assessments was acceptable.

An information request was made to the Applicant to attempt to clarify findings regarding weight and growth during ACP-2566-003 as there was some initial indication that patients receiving trofinetide did not maintain the same level of weight gain as patients on placebo.

8.3.2. Categorization of Adverse Events

The Applicant's process for capturing, recording, categorizing, and coding AEs was appropriate. The Applicant's definition of treatment emergent as occurring after the first dose of treatment and continuing up to 30 days after treatment end for ACP-2566-003 was appropriate. The Applicant coded AE to MedDRA 24 Preferred Terms and FDA review of this coding did not result in clinically significant errors or need for modifications. The Applicant categorized AE severity as mild, moderate, or severe. Serious adverse events fit the FDA definition.

8.3.3. Routine Clinical Tests

The schedules of procedures, including routine safety evaluations, are available in Sections 6.1.1 and 6.2.1 in Table 4 and Table 20. The testing schedules included general physical and neurological examinations, vital sign assessments, ECG, hematology, chemistry, urinalysis, thyroid, and hemoglobin A1C evaluations, along with various clinical outcome assessments conducted by questionnaire.

8.4. Safety Results

8.4.1. Deaths

Deaths in RETT Development Program

ACP-2566-003 (b) (6)
One death (Subject ACP-2566-003 (b) (6)) occurred in the ISS Safety Population by the time of NDA submission in a subject that had enrolled originally in the pivotal trial ACP-2566-003 and then subsequently rolled over into OLE studies 004 and 005.

The subject was first enrolled and randomized to daily placebo in ACP-2566-003 on (b) (6) At the time, the subject was 14 years old and had a past medical history of vomiting since (b) (6) persistent aversion to oral nutrition, weight loss, failure to thrive, and gastroesophageal reflux disease. The subject was noted to have a moderate severity episode of vomiting (b) (6) On (b) (6) she had an event of vomiting and dehydration. Study drug was interrupted. On (b) (6), she had multiple episodes of vomiting and temperature of 103.9 and was sent to the emergency room where she was admitted for IV fluids and broad-spectrum IV antibiotics. Her symptoms resolved on (b) (6), and the placebo was restarted. She had an AE of headache on (b) (6) The subject completed 12 weeks of

placebo treatment in ACP-2566-003 on (b) (6)

ACP-2566-003 (b) (6) was then enrolled in ACP-2566-004 on (b) (6) and began receiving trofinetide 40 mL (8 g) BID. She had mild episodes of vomiting (b) (6) (b) (6) The subject had an episode of fever with temperature 101.5 on (b) (6) treated empirically for a short time with clindamycin. The subject's condition was considered resolved by (b) (6) The subject completed ACP-2566-004 on (b) (6)

The subject was immediately enrolled in ACP-2566-005 on (b) (6) and continued on trofinetide 40 mL (8 g) BID. The subject had an AE of dental caries, ongoing, recurrent with onset of (b) (6) The subject had a planned multiple tooth extraction scheduled for (b) (6) She was admitted to Phoenix Children's Hospital (b) (6) for elective laparoscopic gastrostomy tube placement in anticipation of the tooth extraction. The subject had 1 seizure post-operatively. The subject was discharged on (b) (6) after having tolerated gastrostomy tube feedings and medications. At approximately 02:20 on (b) (6) the subject's camera monitoring system recorded her to be vomiting dark colored fluid; no alarms (set for seizure activity) were alarmed. The mother found the subject at 06:30 on (b) (6) unresponsive in bed with 'old blood' around her mouth. She was moved to the floor and CPR was initiated. Paramedics performed ACLS for 28 minutes with the subject remaining in asystole and confirmed time of death at (b) (6). Autopsy found that mucosal surfaces of the airways were coated by a thick film of dark brown emesis material throughout the trachea and into the mainstem bronchi bilaterally consistent with aspiration of gastric contents. Cause of death was listed as complications of Rett syndrome.

ACP-2566-003 (b) (6)
A second death occurred 1 month after the 120-day-safety-update cutoff date of (b) (6) This subject started in ACP-2566-003 on (b) (6) receiving double-blind trofinetide 8 g BID. The subject subsequently rolled over into ACP-2566-004 on (b) (6) and then ACP-2566-005 on (b) (6) The subject received an elective spinal fusion surgery in (b) (6) The subject was started on scheduled ibuprofen for post-surgical pain management. Approximately two weeks after this surgery, she began having "gastric issues". The subject had stopped trofinetide pre-operatively. When the subject attempted to restart trofinetide post-operatively, she vomited after each of two doses – this was on (b) (6) Because of that reaction, (b) (6) was the subject's last dose of trofinetide which was not restarted again. Abdominal pain was noted approximately (b) (6) In a follow-up appointment with the surgeon (b) (6) (Day 25 of ACP-2566-005) and found to have free air in abdomen. Exploratory surgery was performed on (b) (6) and diagnosed gastric perforation thought to be most likely perforated peptic ulcer from prolonged ibuprofen use, repaired with graham patch.

After nutrition with total parenteral nutrition (TPN), the subject was able to return to an alimental diet and on (b) (6) the serious adverse event (SAE) of gastric ulcer with perforation was considered resolved.

The subject had emergency surgery on (b) (6), due to bleeding ulcer with 3 episodes of asystole. Per report from home nurse, the subject died on (b) (6), with cause of death being bleeding ulcer.

REVIEWER COMMENT: Patient ACP-2566-003- (b) (6) appears to have had a prior history of chronic emesis and poor oral intake. Therefore, it is highly likely that her premorbid condition could lead to the occurrence of such severe emesis as to lead to aspiration and pulmonary arrest.

Patient ACP-2566-003 (b) (6) had no AEs with trofinetide for approximately 1 year while participating in ACP-2566-003 and ACP-2566-004. When her gastric perforation was discovered at the start of ACP-2566-005, trofinetide was stopped. The patient, after recovering from that perforation with surgical treatment and continued gastric ulcer treatment, had gastric ulcer hemorrhaging and death 2 months later. It is unlikely that trofinetide contributed to this death.

However, there is concern that a disproportionate number of subjects experienced vomiting as a TEAE on trofinetide than placebo. Given the tendency of subjects with Rett syndrome to have various gastrointestinal comorbidities, gastrointestinal AE from treatment may need to be more carefully considered.

Death in Non-Rett Development Program

Trofinetide was studied in moderate to severe traumatic brain injury (TBI) in studies TBI-001/002. Subjects were treated emergently with IV trofinetide or placebo randomized 2:1. This patient population is severely injured and has a high risk of death. This manifested as 6 deaths in the placebo group (n=84) and 17 in the trofinetide group (n=167) documented as TEAE.

REVIEWER COMMENT: Based on review of the Applicant's Table 5.3.5.3-6 "List of Subjects with Narratives in All Trofinetide Clinical Studies" in the ISS and corresponding Table 5.3.5.3-5 in the 120-day-safety-update, none of the preferred terms (PT) associated with the TBI subjects who died in TBI-001/002 coincide with the most frequent PT for TEAE in pivotal study ACP-2566-003. It appears that these deaths, though disproportionately affecting the trofinetide group compared to placebo, are unlikely to be related to the drug.

8.4.2. Serious Adverse Events

While serious adverse events were relatively rare, total counts were higher for trofinetide than placebo when all double-blind studies were combined. Most notably, 2 cases of seizure (1 coded to the term tonic convulsion) were serious in nature and as will be noted later, there was a slightly disproportionate occurrence of seizures in trofinetide-treated subjects compared to placebo (Table 38).

Table 38 Serious Adverse Events in NEU-2566-RETT-001, NEU-2566-RETT-002, and ACP-2566-003

Preferred Terms	All Trofinetide (N = 176)	Placebo (N = 133)
Total Subjects with any Adverse Events	8 (4.5%)	4 (3.0%)
Seizure, Tonic Convulsion	2 (1.1%)	0 (0.0%)
Altered state of consciousness	1 (0.6%)	0 (0.0%)
Bacteremia	1 (0.6%)	0 (0.0%)
Bronchiolitis	1 (0.6%)	0 (0.0%)
COVID-19 pneumonia	1 (0.6%)	0 (0.0%)
Ludwig angina	1 (0.6%)	0 (0.0%)
Oral candidiasis	1 (0.6%)	0 (0.0%)
Pneumonia	1 (0.6%)	1 (0.8%)
Pulmonary embolism	1 (0.6%)	0 (0.0%)
Streptococcal infection	1 (0.6%)	0 (0.0%)
Urinary tract infection	1 (0.6%)	0 (0.0%)
Constipation	0 (0.0%)	1 (0.8%)
Pneumatosis intestinalis	0 (0.0%)	1 (0.8%)
Respiratory distress	0 (0.0%)	1 (0.8%)

Source: CDS analyst, reviewer confirmed and edited

The majority of serious adverse events that occurred in controlled trials, occurred in the pivotal trial ACP-2566-003 (Table 39), occurring in only a few subjects.

Table 39 Serious Adverse Events in ACP-2566-003

Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Total Subjects with any Adverse Events	3 (3.2%)	3 (3.2%)
Bacteremia	1 (1.1%)	0 (0.0%)
Bronchiolitis	1 (1.1%)	0 (0.0%)
COVID-19 pneumonia	1 (1.1%)	0 (0.0%)
Seizure	1 (1.1%)	0 (0.0%)
Urinary tract infection	1 (1.1%)	0 (0.0%)

Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Constipation	0 (0.0%)	1 (1.1%)
Pneumatosis intestinalis	0 (0.0%)	1 (1.1%)
Respiratory distress	0 (0.0%)	1 (1.1%)

Source: CDS analyst, reviewer confirmed and edited

Looking at long-term safety of trofinetide, 10 additional subjects experienced an SAE between the NDA submission in July 2022 and 120-day safety update submitted in November 2022 (Table 40).

Table 40 Serious Adverse Events in the RTTLT Pool at 120-Day Safety Update

MedDRA preferred term	Number (%) of subjects
	Overall (N=178)
Any serious TEAE	31 (17.4)
Seizure	8 (4.5)
Pneumonia	4 (2.2)
Urinary tract infection	4 (2.2)
Acute respiratory failure	3 (1.7)
Rhinovirus infection	3 (1.7)
Status epilepticus	3 (1.7)
Dehydration	3 (1.7)
Aspiration	2 (1.1)
COVID-19 pneumonia	2 (1.1)
Vomiting	2 (1.1)
Enterovirus infection	2 (1.1)
Viral infection	2 (1.1)
COVID-19	2 (1.1)
Pyrexia	2 (1.1)

Source: Applicant 120-Day Safety Update

In ACP-2566-009, by the time of the 120-day safety update, there were 3 subjects who had experienced an SAE: two seizure events and one gastroenteritis sapovirus event.

REVIEWER COMMENT: Overall, many serious adverse events reported in the clinical trials appear compatible with chronic conditions and complications of Rett syndrome. As

patients with Rett syndrome often do have oral intake impairments and require feeding tubes, aspiration, aspiration pneumonia and co-occurring morbidities are not uncommon as are other infectious complications that can arise from a relatively immobile dependent state. However, it should be noted that the rates of vomiting that occurred with trofinetide likely raised the risk of aspiration and subsequent adverse consequences. The case of urinary tract infection that led to bacteremia, ACP-2566-003 (b) (6) was considered related by the study investigator. The investigator concluded that frequent and unpredictable diarrhea from trofinetide required frequent diaper changes that could not always be accommodated. Immobility for prolonged periods with a diaper full of diarrhea placed the subject at risk. Since the subject had never previously experienced a urinary tract infection, the investigator concluded that this was a related SAE.

At the 120-day safety update, two more subjects had experienced an SAE of seizure and two cases of status epilepticus occurred in the RTTLT pool. In addition, two of the younger subjects in ACP-2566-009 experienced seizures requiring brief hospitalization, and hence counting as SAE. Though infrequent, this continues to raise concern that treatment with trofinetide may reduce the threshold to seize in this epilepsy prone population.

8.4.3. Dropouts and/or Discontinuations Due to Adverse Effects

Withdrawals from treatment and from the study were fairly frequent in ACP-2566-003 (Table 41). There were fewer withdrawals in the prior phase 2 studies, but the ones that occurred were relevant to the most common AEs encountered in ACP-2566-003, therefore they are combined for Table 42. Of note, investigators withdrew 5 subjects for "consent withdrawal" or "non-compliance with study medication". On review of the narratives, 4 out of the 5 subjects had AE recorded in their narratives and two of the subjects (ACP-2566-003- (b) (6) and ACP-2566-003 (b) (6)) required treatment interruption for their diarrhea before they were withdrawn for non-compliance. Those two subjects were added to the count of subjects experiencing diarrhea as a reason for their discontinuation.

Table 41 TEAE Leading to Treatment Withdrawal in ACP-2566-003

Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Total Subjects with any Adverse Events	18 (19.4%)	2 (2.1%)
Diarrhea	14 (15.1%)	0 (0.0%)
Decreased appetite	3 (3.2%)	0 (0.0%)
Lethargy	2 (2.2%)	0 (0.0%)
Seizure	2 (2.2%)	0 (0.0%)
Frequent bowel movements	1 (1.1%)	0 (0.0%)

Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Gastroesophageal reflux disease	1 (1.1%)	0 (0.0%)
Vomiting	1 (1.1%)	0 (0.0%)
Weight decreased	1 (1.1%)	0 (0.0%)
Arthralgia	0 (0.0%)	1 (1.1%)
Pneumatosis intestinalis	0 (0.0%)	1 (1.1%)

Source: CDS Analysis, edited by Clinical Reviewer. Narratives for subjects ACP-2566-003 (b) (6) and ACP-2566-003- (b) (6) indicate that they suffered frequent and recurrent diarrhea prior to being withdrawn due to non-compliance. Their withdrawal should be accounted for as an AE, so withdrawal incidence was updated to reflect these AEs

Table 42 TEAE Leading to Treatment Withdrawal in NEU-2566-RETT-001, NEU-2566-RETT-002, and ACP-2566-003

Preferred Terms	All Trofinetide (N = 176)	Placebo (N = 133)
Total Subjects with any Adverse Events	20 (11.4%)	2 (1.5%)
Diarrhea, Frequent Bowel Movements, or Non-Compliance + Diarrhea	16 (9.1%)	0 (0.0%)
Decreased appetite	3 (1.7%)	0 (0.0%)
Lethargy	2 (1.1%)	0 (0.0%)
Seizure	2 (1.1%)	0 (0.0%)
Vomiting	2 (1.1%)	0 (0.0%)
Altered state of consciousness	1 (0.6%)	0 (0.0%)
Gastroesophageal reflux disease	1 (0.6%)	0 (0.0%)
Pulmonary embolism	1 (0.6%)	0 (0.0%)
Weight decreased	1 (0.6%)	0 (0.0%)
Arthralgia	0 (0.0%)	1 (0.8%)
Pneumatosis intestinalis	0 (0.0%)	1 (0.8%)

Preferred Terms	All Trofinetide (N = 176)	Placebo (N = 133)

Source: CDS Analyst, Reviewer Confirmed, subjects ACP-2566-003-(b) (6) and ACP-2566-003 (b) (6) were withdrawn due to non-compliance but review of narratives indicated an AE of diarrhea occurring prior to withdrawal so they were added to the total and row for Diarrhea.

Within the RTTLT pool, further treatment and study withdrawals occurred over time, both to subjects who had previously received trofinetide in ACP-2566-003 and those who had received placebo. Another 50 subjects experienced TEAEs that led to withdrawal (Table 43). While gastrointestinal TEAEs continued to be the predominant organ class of AE, a notable number of nervous system TEAEs also led to withdrawal.

Table 43 TEAE Leading to Treatment/Study Withdrawal in RTTLT

MedDRA v24 preferred term	**120-Day-Update Number (%) of subjects**
	Overall (N=178)
Any TEAE leading to study drug discontinuation or study termination	**72 (40.4)**
Diarrhea, Frequent Bowel Movements	45 (25.3)
Vomiting	12 (6.7)
Seizure, Seizure Cluster	7 (3.9)
Decreased appetite	4 (2.2)
Weight decreased	4 (2.2)
Agitation, Breath Holding, Screaming	3 (1.7)
Lethargy	2 (1.1)
Aspiration	2 (1.1)
Acute respiratory failure	1 (0.6)
Enterovirus infection	1 (0.6)
Feeding disorder	1 (0.6)
Gastroesophageal reflux disease	1 (0.6)
Alanine aminotransferase increased	1 (0.6)
Gastroenteritis	1 (0.6)

Gross motor delay	1 (0.6)
Oropharyngeal pain	1 (0.6)
Tremor	1 (0.6)

Source: Adapted from Applicant 120-day-safety-update Table 2.7.4-34

It is also important to note the concomitant medications that were added to subject regimens to manage adverse effects. The most prominent being those added for diarrhea. An information request was sent to the Applicant to request that they specifically break out treatment-emergent concomitant medications for the trofinetide-treated subjects who were part of the RTTLT pool. In order to reduce splitting by preferred terms, the Applicant was asked to group therapies together based on their active ingredient as it related to the indication for which it was prescribed. Below is an extract of the information provided by the Applicant, chosen for those classes of medications that were started in ≥10% of subjects in RTTLT after trofinetide was initiated. As expected, antipropulsives and intestinal absorbents made up the largest percentage of therapies needed while on trofinetide. Concerningly, new antiepileptic drugs were being started after trofinetide, despite the fact that the majority of subjects had already been on antiepileptic drugs before treatment – raising the concern that seizure frequency occurrence increased after trofinetide was started. (Table 44 Treatment Emergent Concomitant Medication Use in RTTLT

Table 44 Treatment Emergent Concomitant Medication Use in RTTLT

TREATMENT EMERGENT CONCOMITANT MEDICATION CLASS N= number of subjects (%)	156 (87.6)
ANTIPROPULSIVES	109 (61.2)
INTESTINAL ADSORBENTS	61 (34.3)
ANTIEPILEPTICS	41 (23.0)
OTHER BETA-LACTAM ANTIBACTERIALS	36 (20.2)
OTHER ANALGESICS AND ANTIPYRETICS	31 (17.4)
DRUGS FOR CONSTIPATION	26 (14.6)
ANTIEMETICS AND ANTINAUSEANTS	24 (13.5)
ANTIINFLAMMATORY AND ANTIRHEUMATIC PRODUCTS, NON-STEROIDS	23 (12.9)
BETA-LACTAM ANTIBACTERIALS, PENICILLINS	18 (10.1)
VIRAL VACCINES	18 (10.1)

8.4.4. Significant Adverse Events

Diarrhea

As is noted in Table 50, 84.4% of trofinetide-treated subjects from the RTTLT pool experienced the TEAE of diarrhea (Broad FMQ). While this AE only led to withdrawal of study drug in 25.3%,

it did require concomitant therapy with loperamide in about 50%. With regards to other interventions and their effects on the diarrhea that subjects experienced, an information request was sent to the Applicant on December 8, 2022, and a response was received on December 20, 2022. The purpose of this information request was to determine how persistent diarrhea was and whether it resolved with dose interruptions, reductions, or concomitant therapy. The Applicant provided Table 45. This reviewer notes that in the 22.5% of subjects in whom trofinetide was stopped (either withdrawn during the study or stopped at study end), diarrhea did resolve. However, it is also notable that only 13.5% of subjects experiencing diarrhea had complete resolution after an average of 64 days, despite 9% experiencing dose reduction or interruption, with dose reductions from 47.2-60.9% of prescribed dose for a mean of 110 days. Forty-nine percent of subjects receiving long-term trofinetide experienced diarrhea without resolution or with recurrence. Dose interruptions or reduction occurred for 42.2% of subjects. Of those who had their doses reduced, the reduction and subsequent re-escalation was on average to 51.5% and 74.5%, respectively, of the weight-based recommended dose. Concomitant antidiarrheal therapy was used in a total of 52.2% of subjects treated long-term with trofinetide. The larger proportion of use was in the group that did not experience diarrhea resolution. The mean duration of concomitant antidiarrheal therapy was over 240 days in both those whose diarrhea resolved and those that did not, indicating a chronic need for dual therapy.

In Study ACP-2566-009, the ongoing long-term PK study in Rett syndrome subjects 2-5 years of age, 12 of 15 subjects have reported any diarrhea TEAE with 1 subject withdrawing due to the AE. Eight subjects were recorded as unresolved at the time of reporting with four experiencing dose interruption and seven each counted as having dose reductions and/or antidiarrheal therapy.

Table 45 Summary of Diarrhea Resolution Status, Trofinetide Dose Reductions/Interruptions, and Concomitant Antidiarrheal Therapy in RTTLT Pool. Denominator for % based on N=178, not on nesting.

Parameter	Number (%) subjects on Trofinetide (N=178)
Any Diarrhea TEAEs (Diarrhea, Feces soft, Frequent bowel movements), n (%)	151 (84.8)
Diarrhea resolved after last dose of trofinetide and did not recur, n (%)	13 (7.3)
Diarrhea resulted with drug withdrawn and did not recur, n (%)	27 (15.2)
Diarrhea completely resolved while on trofinetide and did not recur, n (%)	24 (13.5)
Duration of diarrhea (days), Mean	64.2
Dose interrupted for any reason [1], n (%)	6 (3.4)
Duration dose interrupted (days), Mean	7.8
Dose reduced for any reason [1], n (%)	10 (5.6)
Duration dose reduced (days), Mean	110.6

Amount of dose reduction (%), Mean	47.2
Dose returned to (%), Mean	60.9
Concomitant antidiarrheal treatment [2], n (%)	18 (10.1)
Duration of concomitant antidiarrheal treatment (days), Mean	244.5
Diarrhea did not resolve while on trofinetide or recurred after resolution, n (%)	87 (48.9)
Diarrhea recurred after resolution, n(%)	60 (33.7)
Duration of diarrhea (days), Mean	110.6
Dose interrupted for any reason [1], n (%)	17 (9.6)
Duration dose interrupted (days), Mean	11.5
Dose reduced for any reason [1], n (%)	58 (32.6)
Duration dose reduced (days), Mean	137.9
Amount of dose reduction (%), Mean	51.5
Dose returned to (%), Mean	74.5
Concomitant antidiarrheal treatment [2], n (%)	75 (42.1)
Duration of concomitant antidiarrheal treatment (days), Mean	245.5

Source: Applicant Table AH.DIARRHEA.RTTLT
[1] If morning and evening doses in the dose modification log were both 0 or HELD, it was considered as a dose interruption; else if either morning or evening doses were less than the initial dose, it was considered as a dose reduction. A subject can be counted as both a dose reduction and a dose interruption.
[2] The concomitant antidiarrheal treatment includes the following values from ADCM.CMDECOD: 'LOPERAMIDE', 'LOPERAMIDE HYDROCHLORIDE', 'LOPERAMIDE HYDROCHLORIDE;SIMETICONE', 'PLANTAGO OVATA', 'FIBRE, DIETARY'.
Ongoing adverse events and concomitant antidiarrheal treatment end dates were imputed as the date of last trofinetide for analysis. Missing dates for exposure were not imputed; omitted from duration calculation.

With regards to severity, the vast majority of cases of diarrhea that received a rating were evenly divided between mild and moderate in severity (Table 46)

Table 46 Severity Rating of Diarrhea Reported in RTTLT at 120 Day Safety Update

Treatment-emergent diarrhea Maximum severity, n(%)[a]	All trofinetide (N=178)
n	149
Mild	67 (45.0)
Moderate	76 (51.0)
Severe	6 (4.0)

Source: Applicant Integrated Summary of Safety 120 Day Safety Update Table 2.7.4-39

Weight Loss

It was noted by the ACP-2566-003/ACP-2566-004 DSMB that diarrhea and weight loss could be related after 75% of subjects had been recruited into the study:

> *"weight loss was greater in subjects with diarrhea, and weight gain was less. For -004, the numbers were worse: in subjects with diarrhea, 32% had weight loss from baseline of ≥ 3%, 24% had weight loss from baseline of ≥ 5%, and 20% had weight loss from baseline of ≥ 7% raising the issue that duration of treatment has an effect of diarrhea-induced weight loss."* (Source: DSMB Closed Meeting Minutes of July 15, 2021).

The Applicant, in their 120-day safety update argued the following:

> "The significant majority of TEAEs of diarrhea were not associated with other morbidities such as weight loss or dehydration, as shown by the very small number of subjects with these concurrent AEs."

In ACP-2566-003, 12% of trofinetide-treated subjects experienced weight loss of >7% compared to 4% of placebo patients. Four subjects (2.2%) in RTTLT pool withdrew specifically for the TEAE of weight loss (Table 43).

At baseline Rett syndrome is associated with oral intake abnormalities, poor weight gain, and reduced growth. To further explore the effects of trofinetide on weight, an information request was sent to the Applicant on October 20, 2022. The information request referenced the Rett syndrome adjusted growth curve (Tarquinio, Motil et al. 2012) (Figure 9). The Rett syndrome adjusted growth curve (Figure 9) shows that compared to healthy children, children with Rett syndrome gain weight at a slower rate and remain lower weight on average compared to their healthy peers.

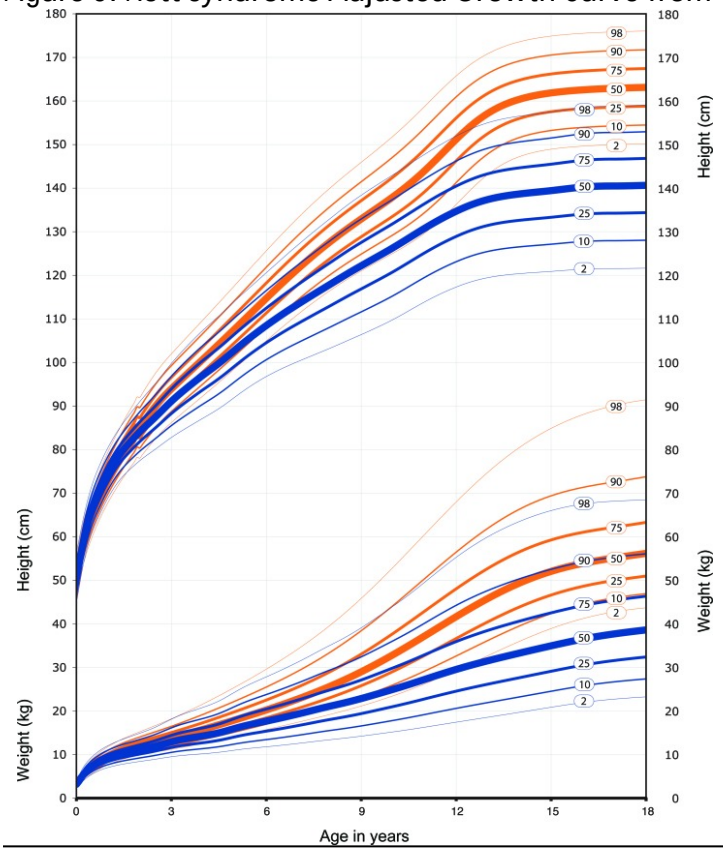
The Applicant also provide a dataset of subject weights referenced to where they would fall on the Rett syndrome adjusted growth curve for weight. Since the Rett syndrome adjusted growth curve only specified 2nd, 10th, 25th, 50th, 75th, 90th, and 98th percentiles, the Applicant coded each subject's weight as falling between an upper and lower percentile boundary. In Figure 10, we plotted individual subject and the RTTLT population mean weight as described by the Rett syndrome adjusted growth curve. The X axis of Figure 11 consists of visits across consecutive studies NEU-2566-002, ACP-2566-003, -004, and -005 (not proportional to time). The Y axis at any visit shows the upper (A) and lower (B) age-adjusted-growth-curve percentile bounds that the patient's weight fell between. Since these percentiles are based on the Rett syndrome adjusted growth curve, they account for the slower rate of weight gain in this population. Despite this, the mean weight percentile bounds indicate a small decrease in weight or inadequate weight gain in the trofinetide treated population over time.

REVIEWER COMMENT:

Diarrhea, loose stools, and frequent stools are all clearly adverse drug reactions of trofinetide. The cases of diarrhea were generally rated as mild or moderate in severity. One case of an SAE of UTI with bacteremia was categorized as related to trofinetide because of the baseline of diaper use with newly occurring chronic diarrhea. Given the degree of neurological disability and need for ADL support in this population, the AE of diarrhea has the potential for serious outcomes. Constipation is the background gastrointestinal complication most associated with Rett syndrome. Constipation may be somewhat easier to deal with in subjects with reduced mobility. Management strategies aside from the use of various daily laxatives, are timed bowel training involving the use of post-prandial suppositories and enemas which can allow subjects to time their bowel movements at the convenience of their care team and lifestyle (Chong 2001). Uncontrolled and unpredictable diarrhea; however, may be more difficult to manage for a severely neurologically impaired child or adult who may be dependent on caregivers for bowel hygiene and care. This potentially increases the risk for skin and infectious disorders related to maceration and frequent skin and genitourinary exposure to loose or watery stool. Therefore, it is notable that nearly 50% of subjects receiving trofinetide in the long-term experienced diarrhea that never completely resolved despite dose interruptions, reductions, and use of concomitant antidiarrheals (Table 45).

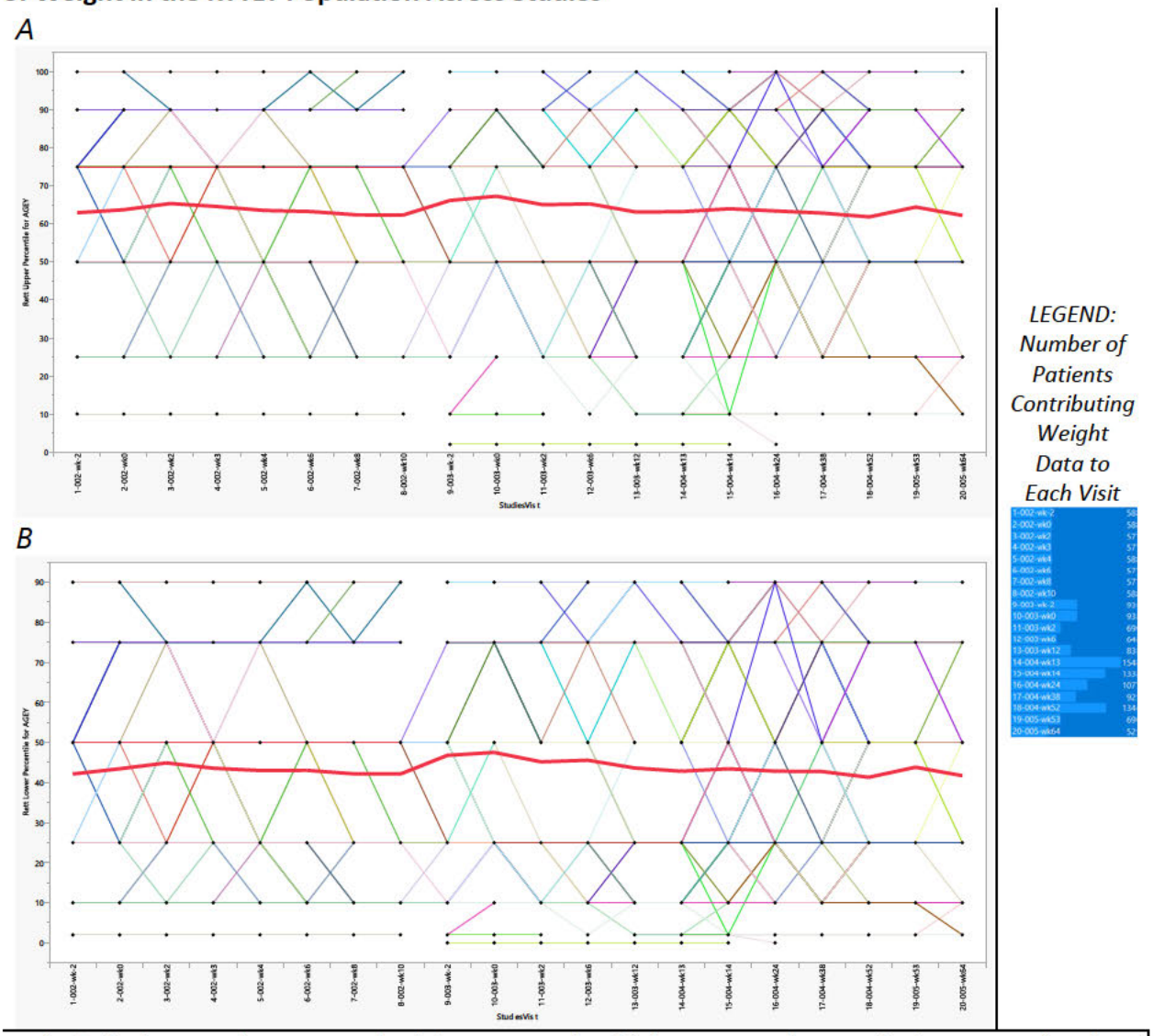
Overall, trofinetide seems to be associated with weight loss or at least inadequate weight gain. In a population that already experiences stunted growth, this risk needs to be considered by the family and prescriber and weight should be monitored accurately and regularly during treatment.

Figure 9: Rett syndrome Adjusted Growth Curve from Tarquinio et al



Height and Weight growth curves in unaffected children (orange) and children with classic Rett syndrome (blue) Source: (Tarquinio, Motil et al. 2012)

Figure 10: Individual and Mean Age-Adjusted Upper (A) and Lower (B) Percentile Boundaries of Weight in the RTTLT Population Across Studies



Individual subject weights expressed as (A) Upper Bound and (B) Lower Bound Percentiles by Age Based on the Rett syndrome Adjusted Growth Curve of Tarquinio et al. Each thin line in the spaghetti plots records an individual subject participating in NEU-2566-002 (StudiesVisit 1-8), ACP-2566-003 (StudiesVisit 9-13), ACP-2566-004 (StudiesVisit 14-18), and the first two visits of ACP-2566-005 (StudiesVisit 19-20). The remaining two visits of ACP-2566-005 were not included as data was only available for 5 subjects each). The Legend indicates the number of subjects contributing data to each visit time point. It should be noted that there was a large gap (3 years) between NEU-2566-002 and ACP-2566-003, while ACP-2566-003, -004, and -005 occurred consecutively. The thick red line represents the mean of all subjects. Note the flat/slight downtrend in both means.
Source: Reviewer analysis of Applicant provided dataset adwtpctb.xpt

8.4.5. Treatment Emergent Adverse Events and Adverse Reactions

A number of factors influenced analysis of treatment emergent adverse events (TEAE) and determination of whether a TEAE was an adverse drug reaction due to trofinetide. First, Rett syndrome is a complex disorder with many neurologically mediated symptoms that affect, not only behavior and epilepsy, but also cardiopulmonary and gastrointestinal systems, leading to a variety of complications. Therefore, it was expected that there would be an underlying large number of adverse events throughout the population in any study. Second, ACP-2566-003 was the only double-blind placebo-controlled study in which the indicated dose of trofinetide was used; therefore, it would be the primary study on which to base an understanding of adverse drug reactions. However, this study only enrolled 187 subjects in the safety population. Therefore, looking at the RTTDB pool had the potential of both revealing some rarer but associated adverse drug reactions, but also of diluting the safety signal given the notably lower doses and reduced temporal exposure to trofinetide in studies NEU-2566-RETT-001 and NEU-2566-RETT-002. Finally, using MedDRA v 24.0 PT alone, risked splitting associated symptoms. Therefore, this reviewer started the analysis by looking at broad FDA medical queries (FMQ) of TEAEs that occurred in ACP-2566-003 with a risk difference of ≥2% in trofinetide compared to placebo (Table 47). For PTs that were subsumed within related FMQ PTs (such as irritability being subsumed within the anxiety FMQ PT, this reviewer used the FMQ PT that captured the most AEs). The RTTDB pool, containing data from studies NEU-2566-RETT-001, NEU-2566-RETT-002, and ACP-2566-003, was then used for the same analysis. It was presumed that pooling the smaller, shorter duration, lower dose trofinetide studies along with the pivotal Phase 3 study could potentially lead to dilution of the safety signals for trofinetide, but they also had the potential of reinforcing trofinetide related adverse drug reactions and providing a larger placebo group with which to compare. The results of this analysis are in Table 48. While the same criteria were used (broad FMQ PTs with ≥2% risk difference of trofinetide over placebo), TEAEs that did not meet this criterion were included if they had met the criteria in ACP-2566-003 alone, in order to compare. Taken together, these analyses indicate that the following TEAEs are associated with trofinetide: diarrhea, vomiting, dyspepsia, decreased appetite, anxiety, fatigue, somnolence, confusional state, parasomnia, and nasopharyngitis. Of note, four concerning AEs of pyrexia, seizure, hepatic injury, and pneumonia did disproportionately affect trofinetide subjects in ACP-2566-003 but not in the larger RTTDB pool. Finally, the terms mania and insomnia were present with ≥2% risk difference in the RTTDB pool but not ACP-2566-003. Of note, vomiting and nausea, and fatigue and somnolence are subsumed within each other in the FMQ PTs and hence vomiting, and fatigue were used for these tables.

*Table 47 TEAE by Broad FMQ PT in Trofinetide **≥2%** Over Placebo in ACP-2566-003*

FMQ Preferred Terms	Trofinetide (N = 93)	Placebo (N = 94)
Total Subjects with any Adverse Events	85 (91.4%)	50 (53.2%)
Diarrhea	76 (81.7%)	19 (20.2%)
Vomiting	27 (29.0%)	11 (11.7%)
Pyrexia	8 (8.6%)	4 (4.3%)
Seizure	8 (8.6%)	6 (6.4%)
Anxiety	7 (7.5%)	1 (1.1%)
Decreased appetite	7 (7.5%)	2 (2.1%)
Fatigue	7 (7.5%)	2 (2.1%)
Somnolence	6 (6.5%)	2 (2.1%)
Nasopharyngitis	5 (5.4%)	1 (1.1%)
Dyspepsia	4 (4.3%)	2 (2.1%)
Confusional state	3 (3.2%)	1 (1.1%)
Hepatic injury	3 (3.2%)	0 (0.0%)
Parasomnia	3 (3.2%)	0 (0.0%)
Pneumonia	3 (3.2%)	1 (1.1%)

Source: CDS Analysis, Reviewer Edited. Blue highlighted terms appeared in both ACP-2566-003 and the RTTDB Pool with a risk difference of trofinetide ≥2% over placebo

Table 48 TEAE by Broad FMQ PT in RTTDB Pool

FMQ Preferred Terms	All Trofinetide (N = 176)	Placebo (N = 133)
Total Subjects with any Adverse Events	143 (81.3%)	78 (58.6%)
Diarrhea	102 (58.0%)	24 (18.0%)
Vomiting	38 (21.6%)	15 (11.3%)
Anxiety	21 (11.9%)	8 (6.0%)
Pyrexia	17 (9.7%)	13 (9.8%)
Somnolence	14 (8.0%)	5 (3.8%)
Fatigue	12 (6.8%)	4 (3.0%)
Seizure	12 (6.8%)	9 (6.8%)
Nasopharyngitis	11 (6.3%)	5 (3.8%)
Decreased appetite	10 (5.7%)	3 (2.3%)
Confusional state	9 (5.1%)	2 (1.5%)
Mania	9 (5.1%)	1 (0.8%)
Dyspepsia	8 (4.5%)	2 (1.5%)
Insomnia	5 (2.8%)	0 (0.0%)
Pneumonia	5 (2.8%)	3 (2.3%)
Parasomnia	4 (2.3%)	0 (0.0%)
Hepatic injury	3 (1.7%)	1 (0.8%)

Source: CDS Analysis, Reviewer Edited. Red highlighted terms appear in the RTTDB Pool but NOT in ACP-2566-003 with a risk difference of trofinetide ≥2% over placebo. Terms highlighted in yellow were found to occur with the ≥2% risk difference in ACP-2566-003 but NOT in the RTTDB Pool.

Since the broad FMQ queries subsume certain PTs together, the rates of various AEs shown in Table 47 are generally higher than the Applicant's AE table. In order to determine where the additions occurred, this reviewer and the CDS analyzed each broad FMQ term that rated at higher than 5% incidence and found the contributing PTs (Table 49).

Table 49 ACP-2566-003 TEAE by FMQ and Subsumed PTs

FMQ Term Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Diarrhea	76 (81.7%)	19 (20.2%)

FMQ Term Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Diarrhea	75 (80.6%)	18 (19.1%)
Fecal volume increased	1 (1.1%)	0 (0.0%)
Frequent bowel movements	1 (1.1%)	0 (0.0%)
Gastroenteritis	1 (1.1%)	1 (1.1%)
Vomiting	27 (29.0%)	11 (11.7%)
Gastroenteritis viral	0 (0.0%)	1 (1.1%)
Nausea	1 (1.1%)	0 (0.0%)
Regurgitation	0 (0.0%)	2 (2.1%)
Retching	4 (4.3%)	1 (1.1%)
Vomiting	25 (26.9%)	9 (9.6%)
Pyrexia	8 (8.6%)	4 (4.3%)
Seizure	8 (8.6%)	6 (6.4%)
Partial seizures	0 (0.0%)	1 (1.1%)
Seizure	8 (8.6%)	5 (5.3%)
Anxiety	7 (7.5%)	1 (1.1%)
Agitation	1 (1.1%)	0 (0.0%)
Emotional disorder	0 (0.0%)	1 (1.1%)
Irritability	6 (6.5%)	0 (0.0%)
Decreased Appetite	7 (7.5%)	2 (2.1%)
Decreased appetite	5 (5.4%)	2 (2.1%)
Feeding disorder	1 (1.1%)	0 (0.0%)
Hypophagia	1 (1.1%)	0 (0.0%)
Fatigue	7 (7.5%)	2 (2.1%)

FMQ Term Preferred Terms	All Trofinetide (N = 93)	Placebo (N = 94)
Lethargy	3 (3.2%)	1 (1.1%)
Listless	1 (1.1%)	0 (0.0%)
Somnolence	3 (3.2%)	1 (1.1%)
Nasopharyngitis	5 (5.4%)	1 (1.1%)
Nasopharyngitis	2 (2.2%)	1 (1.1%)
Pharyngitis streptococcal	3 (3.2%)	0 (0.0%)

Source: CDS, Safety population and TRTEMFL = Y; [taeptsub1.rtf] [taeptsub1.sas] 22FEB2023, 20:04

To determine if these identified TEAEs continued to affect subjects, this reviewer examined the TEAE FMQ PTs reported for the RTTLT pool at a rate of ≥5% in the sample, which examined subjects treated with trofinetide from the start of ACP-2566-003 through and continuing into the two open-label studies, ACP-2566-004 and ACP-2566-005 (Table 41). Compared to TEAEs found with a ≥2% risk difference in ACP-2566-003 (Table 47), diarrhea, vomiting, decreased appetite, anxiety, fatigue, and somnolence continue to occur in the open-label studies, but not dyspepsia, confusional state, parasomnia, or nasopharyngitis. In addition, seizure, pyrexia, pneumonia, and hepatic injury, had been identified in ACP-2566-003 but did not reveal a risk difference in the RTTDB pool.

Table 50 TEAE by Broad FMQ PT in RTTLT Pool

Preferred Terms	All Trofinetide (N = 178)
Total Subjects with any Adverse Events	**166 (93.3%)**
Diarrhea	151 (84.8%)
Vomiting	67 (37.6%)
Seizure	21 (11.8%)
Decreased appetite	19 (10.7%)
Pyrexia	19 (10.7%)
Anxiety	18 (10.1%)

Preferred Terms	All Trofinetide (N = 178)
Fatigue	16 (9.0%)
Somnolence	15 (8.4%)
Pneumonia	12 (6.7%)
Hepatic injury	9 (5.1%)
Rash	9 (5.1%)

Source: CDS Analysis, Reviewer Confirmed and Edited

Based on Table 49, and using more consumer friendly language, the final table of adverse reactions placed in the trofinetide label is Table 51:

Table 51 Adverse Reactions in at Least 5% of Patients Treated With DAYBUE and at Least 2% Greater than Placebo in ACP-2566-003

Adverse Reaction	DAYBUE (N=93) %	Placebo (N=94) %
Diarrhea	82	20
Vomiting	29	12
Fever	9	4
Seizure	9	6
Anxiety	8	1
Decreased appetite	8	2
Fatigue	8	2
Nasopharyngitis	5	1

*REVIEWER COMMENT: Overall, the listing of TEAE occurring in **≥5%** from the RTTLT pool coincides well with TEAE that occurred more frequently in trofinetide compared to placebo in the controlled trials. The final table of adverse drug reactions listed in the label are a fair representation of symptoms that caregivers and clinicians should be monitoring.*

8.4.6. Laboratory Findings

There were no laboratory TEAEs reported in greater than 5% of subjects exposed to trofinetide in either the RTTDB or RTTLT pools. Alanine aminotransferase (ALT) increased was reported in 6

subject (3.4%) of the 178 subjects in RTTLT and 2 subjects (1.1%) of the 176 in the RTTDB pool (both from ACP-2566-003). Aspartate aminotransferase (AST) increased was reported in 3 (1.7%) of the RTTLT pool. It is likely these TEAE combined contributed to the FMQ of hepatic injury being identified in the subjects treated long-term with trofinetide. Abnormal blood thyroid stimulating hormone and increased eosinophil count was reported in 2 and 1 patient in RTTDB and RTTLT respectively. Thereafter, any other laboratory TEAE were reported in only 1 patient in either cohort (including placebo patients in RTTDB). Shift tables were created for each serum measurement for the RTTDB pool to examine for differences between trofinetide and placebo. The only laboratory value that demonstrated a greater than 2% shift in the trofinetide group that was not mirrored in the placebo group was alanine aminotransferase (Table 52). Those 7 cases that had a change from <3x the upper limit of normal (ULN) to 3 to <5x the ULN likely contributed to the 9 cases of TEAEs that were categorized under the broad FMQ term of hepatic injury. There were no notable differences in alkaline phosphatase, gamma-glutamyl transferase, bilirubin, or lactate dehydrogenase, and no patients met the criteria for Hy's Law. Subject ACP-2566 (b) (6) was withdrawn from ACP-2566-004 due to increased alanine aminotransferase increased from study day 1 to 58, with a mildly elevated level of 77 U/L that resolved to normal 24 U/L on follow-up. This patient had also experienced a TEAE of diarrhea and vomiting through much of ACP-2566-003 and ACP-2566-004.

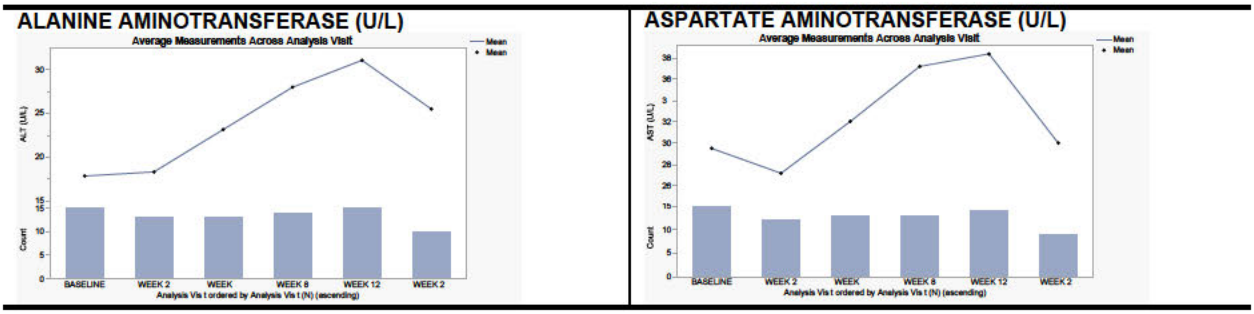
Table 52 Shift Table for Alanine Aminotransferase in RTTLT Pool

Treatment	Baseline	<3 ULN	3-<5 ULN
Alanine Aminotransferase (IU/L)			
Placebo (N = 133)	<3 ULN	129 (97.7%)	2 (1.5%)
	5-<10 ULN	0 (0.0%)	1 (0.8%)
All Trofinetide (N = 176)	<3 ULN	167 (96.0%)	7 (4.0%)
	5-<10 ULN	0 (0.0%)	0 (0.0%)

Subjects had baseline and at least one postline lab test results.
[tshiftdb rtf] [tshiftdb.sas] 07OCT2022, 22:08

Since Study ACP-2566-009 was studying an especially vulnerable population, ages 2-5 without a placebo control, this reviewer chose to also look into laboratory time trends for these patients (Figure 11).

Figure 11 ALT and AST Time Trends in ACP-2566-009 at 120 Day Safety Update



REVIEWER COMMENT: There may have been a mild increase in ALT with long term use of trofinetide (Table 52) and in both ALT and AST in the youngest patients receiving trofinetide (Figure 11). However, neither of these reached a level of potential clinical significance. Despite the levels of diarrhea found with trofinetide, it was notable that BUN and creatinine did not seem to indicate laboratory findings of dehydration, though it was notable in ACP-2566-009 that levels of serum sodium and other measures may have indicated hemoconcentration. The sample size and number of visits in ACP-2566-009 was relatively low.

8.4.7. Vital Signs

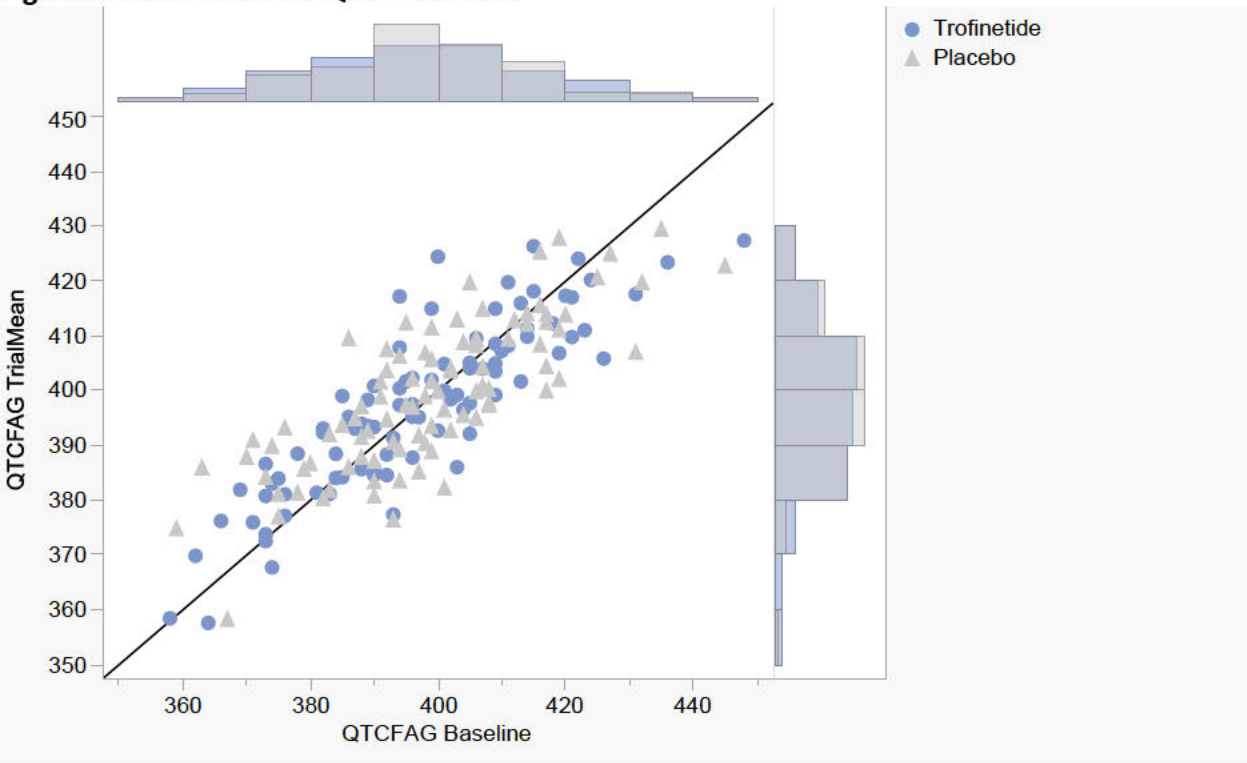
Aside from weight, discussed in Section 8.4.4, no other substantial changes were noted with exposure to trofinetide. Evaluation of vital signs in the RTTLT pool for subjects receiving trofinetide was done with time trend plots. Due to the Applicant labeling of visits and recording vitals differently across trials and not coding the data structure such that one could follow a single subject through the subsequent trials correctly, there was some discrepancy in the time scale these graphs represent. However, overall vital sign time trends were reassuring for no major effects of trofinetide.

8.4.8. Electrocardiograms (ECGs)

In ACP-2566-003, twelve-lead ECGs were complete, standardized recordings, whenever possible. Electrocardiograms were completed in triplicate at Visit 1 (Screening); at Visit 2 (baseline), both before dosing and 2 to 3 hours after dosing; and at Visit 5 (Week 12/EOT/ET). A single ECG was completed at Visit 3 (Week 2) and Visit 4 (Week 6). For visits at which more than one ECG was completed, the average QTcF interval of all legible ECGs was used to determine the QTcF interval for that visit. While the overall mean post-baseline QTcF was not elevated in either trofinetide or placebo groups (Figure 12 ACP-2566-003 QTcF Shift Plot), there were some individual prolonged QTcF intervals reported. At any postbaseline visit, QTcF >450 ms was reported in 3.2% of subjects in the placebo group and 1.1% of subjects in the trofinetide group. None of the values were >480 ms. No subjects in either treatment group had QTcF >500 ms. No

subjects in the trofinetide group had QTcF change from baseline >60 ms compared with 2.1% of subjects in the placebo group.

Figure 12 ACP-2566-003 QTcF Shift Plot



8.4.9. QT

The Applicant conducted ACP-2566-008 as a thorough QT study. It was a Phase 1, randomized, DB, placebo-controlled, ascending dose, nested crossover study. Forty subjects were enrolled and randomized to receive a single dose of 12, 18, and 24 g of trofinetide or matching placebo, as well as a single dose 400 mg of moxifloxacin and a single-dose placebo matching moxifloxacin. All subjects received a total of five doses of study medication with at least 4 days between each administration. The primary ECG endpoint was the time-matched change from baseline in corrected QT interval using Fridericia's correction method (ΔQTcF), at all timepoints, where QTc was derived using Fridericia's formula. The relationship between ΔQTcF and trofinetide blood concentrations was investigated by linear mixed-effects modeling. The placebo-adjusted ΔQTcF value was calculated at each dose level as the placebo-corrected ΔQTcF estimated from the model and its 1-sided 95% CI was then constructed.

The interdisciplinary review team for QT studies was consulted for this application. The summarized results of their review of this thorough QT study (TQT) and the totality of evidence presented by the Applicant are as follows:

> "The totality of evidence from the TQT study suggests an absence of QTc prolongation at the clinical exposure; however, the data does not permit excluding QTc prolongation at the high clinical exposure scenario (renal impairment)."

8.4.10. Immunogenicity

Not applicable as the drug is a small molecule.

8.5. Analysis of Submission-Specific Safety Issues

Not applicable.

8.6. Safety Analyses by Demographic Subgroups

Because of the low recruitment of subjects of racial and ethnic minorities, an analysis by those demographic subgroups is not feasible. Due to the wide age range that is affected by Rett syndrome, and with stratification by age used as part of the enrollment process for ACP-2566-003, it is possible to observe TEAE as related to the three age groups studied throughout ACP-2566-003 and its open-label extensions (RTTLT pool). The original age stratification was 5 to 10, 11 to 15, and 16 to 20. To conform to ranges more standardly applied to childhood, adolescence, and adulthood, the Applicant presented Table 53 using ages 5 to 11, 12 to 16 and 17 and older. This table demonstrates that there was relatively similar distribution of TEAE across age groups, though serious TEAEs seemed to occur less in adult subjects (≥17 years of age).

The Applicant reported the most common (≥10% incidence) TEAEs in the RTTLT Pool were diarrhea, vomiting, COVID-19, pyrexia, and seizure. For trofinetide-treated subjects aged 5 to <12, 12 to <17, and ≥17 years, the incidence of diarrhea was 86.5%, 72.1%, and 90.3%, respectively; the incidence of vomiting was 41.3%, 27.9%, and 22.6%, respectively; and the incidence of COVID-19 was 16.3%, 11.6%, and 12.9%, respectively. The incidence of seizure was higher in the 5 to <12 age group (17.3%) than in the 12 to <17 and ≥17 years age groups (2.3% and 6.5%, respectively).

Table 53 RTTLT Pool TEAE by Age Range

Age Ranges with N (%)

	5-<12 (N=104)	12-<17 (N=43)	≥17 (N=31)	Overall (N=178)
Any TEAE	99 (95.2)	41 (95.3)	30 (96.8)	170 (95.5)
Any serious TEAE	20 (19.2)	8 (18.6)	3 (9.7)	31 (17.4)
Any TEAE leading to discontinuation of study drug or study termination	45 (43.3)	14 (32.6)	13 (41.9)	72 (40.4)

Source: Applicant Summary of Clinical Safety 120 Day Update Table 2.7.4-56 adapted

REVIEWER COMMENT: The disproportionate occurrence of vomiting and seizures in the younger subjects is notable and may have contributed to the greater frequency of hospitalizations contributing to the serious categorization.

8.7. Specific Safety Studies/Clinical Trials

8.7.1. ACP-2566-009

ACP-2566-009 Study Design

Study ACP-2566-009 is an ongoing study of trofinetide in patients with Rett syndrome ages 2 through 4 years of age with at least moderate severity disability (CGI-S ≥4). The status at time of NDA submission was submitted as an interim synoptic clinical study report. The study was designed as a multicenter, open-label study of treatment for 12 weeks (Period A) followed by continuing treatment for 21 weeks (Period B). For Period A, patients were evaluated at screening (-28 d), baseline (Day 0), and weeks 2, 4, 8, and 12. As of the NDA submission, the data cutoff date was March 14, 2022. At that time, 15 patients had been randomized, 14 patients were included in the safety analysis population, given that they had received at least 1 dose of trofinetide, 1 subject was discontinued for an AE and 10 patients had completed the 12-week treatment cycle of Period A.

For safety in this younger age group, trofinetide was titrated over 3 weeks with the week 4 and onward treatment being weight-based (Table 54Error! Reference source not found.). The goal was to achieve at least 5 g BID, which was modeled as being equivalent to the exposure of older/larger patients in ACP-2566-003. However, investigators were allowed to assess tolerance before increasing the dose and to use the maximally tolerated dose for each patient.

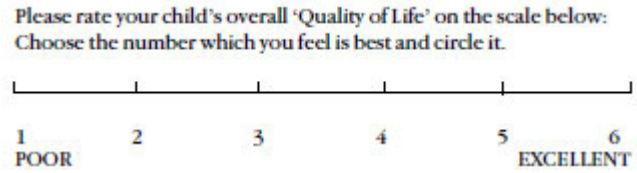
Table 54 ACP-2566-009 Trofinetide Titration and Weight Based Dosing in Ages 2-5

Week	Dose	Total Daily Dose
Week 1[a] (all subjects)	10 mL (2 g) BID	20 mL (4 g)
Week 2 (all subjects)	20 mL (4 g) BID	40 mL (8 g)
Week 4 (subjects ≥9 to <12 kg)	25 mL (5 g) BID	50 mL (10 g)
Week 4 (subjects 12 to <20 kg)	30 mL (6 g) BID	60 mL (12 g)

Source: Applicant Study 009 Interim Synoptic CSR Table 1

ACP-2566-009 was not designed to assess effectiveness in a statistically rigorous manner; however, the CGI-I, CGI-S, the Caregiver Global Impression Improvement (CaGI-I) and Impact of Childhood Neurologic Disability Scale Quality of Life Score (ICND-QOL) were measured. The ICND-QOL measure is a simple 6-point scale that asks caregivers to rate the child's quality of life, and can be used separately from the remainder of the ICND (Figure 13Error! Reference source not found.). The majority of the ICND is a Likert-like-scale that asks caregivers to rate how much various impairments affect a child's overall health, relationships, social life, academics, self-esteem, hopes, and family activities. The scale includes five response options: a Lot, some, a little, not at all, or does not apply. The impairments are divided into inattentiveness/impulsivity/mood, ability to think and remember, other neurological or physical limitations, and epilepsy.

Figure 13 ICND Quality of Life Question



Source: (Camfield, Breau et al. 2003)

ACP-2566-009 Results

At the time of NDA submission, 14 subjects were part of the safety analysis set (enrolled and having received at least 1 dose of study drug) consisted of 14 subjects. The demographics (Table 55), TEAE incidence (Table 56) and TEAE distribution (Table 57) are presented below. For safety overall, there were no deaths, one serious TEAE, and otherwise the types of TEAE were similar in scope and proportion to those in ACP-2566-003. The one serious TEAE was in ACP-2566-009-(b) (6), a 3-year-old subject in Treatment Period B (Day 146) had a new onset of seizure with no prior medical history of seizure. The event was moderate in severity and resolved without sequalae and was not considered related to trofinetide, which was continued

without alteration. There was one study withdrawal due to TEAE, ACP-2566-009-[redacted] (b) (6), who experienced diarrhea between days 43 and 71, with study draw withdrawn at day 65 during Treatment Period A.

Table 55 ACP-2566-009 Demographics

Demographic parameter	Total (N=14)
Sex, n (%)	
Female	14 (100.0)
Age at Screening (years)	
Mean (SE)	3.1 (0.22)
SD	0.83
Median (min, max)	3.0 (2, 4)
Age at Screening categories, n (%)	
<4 Years	9 (64.3)
≥4 Years	5 (35.7)
Race	
Non-White	1 (7.1)
White	13 (92.9)
Ethnicity, n (%)	
Hispanic or Latino	1 (7.1)
Not Hispanic or Latino	13 (92.9)

Table 56 ACP-2566-003 TEAE Incidence

Category	Treatment Period A		Overall (Treatment Periods A and B)	
	Subjects (N=14) n (%)	Events n	Subjects (N=14) n (%)	Events n
Any TEAE	12 (85.7)	55	13 (92.9)	60
Any related TEAE [a]	8 (57.1)	18	9 (64.3)	19
Any severe TEAE [b]	- -	- -	- -	- -
Any fatal TEAE	- -	- -	- -	- -
Any serious TEAE	- -	- -	1 (7.1)	1
Any related serious TEAE [a]	- -	- -	- -	- -
Any TEAE leading to drug withdrawn	1 (7.1)	1	1 (7.1)	1

Source: Table 14.3.1.2.1 and Table 14.3.1.2.2 Abbreviations:

TEAE=treatment-emergent adverse event

Notes: A TEAE is an adverse event with onset date on or after the first study dose date and no later than last study dose date plus 30 days. A subject may have more than 1 TEAE per category. In the "Subjects" column, a subject is counted at most once per category. In the "Events" column, all occurrences of TEAEs are counted per category. The number of subjects in the Safety Analysis Set is used as the denominator for calculating percentages.

[a] Events with missing relationship were counted as related.

[b] Events with missing severity were counted as severe.

Table 57 ACP-2566-009 TEAE Distribution

	Treatment Period A Total (N=14)		Overall (Treatment Periods A and B) Total (N=14)	
Preferred Term	**Subjects n (%)**	**Events n**	**Subjects n (%)**	**Events n**
Any TEAE	12 (85.5)	55	13 (92.9)	60
Diarrhoea	9 (64.3)	14	10 (71.4)	15
Vomiting	5 (35.7)	6	5 (35.7)	6
COVID-19	4 (28.6)	4	4 (28.6)	4
Pyrexia	4 (28.6)	4	4 (28.6)	4
Seizure	2 (14.3)	2	3 (21.4)	4
Cough	2 (14.3)	3	2 (14.3)	3
Dermatitis diaper	2 (14.3)	2	2 (14.3)	2
Epilepsy	1 (7.1)	1	2 (14.3)	2
Somnolence	2 (14.3)	2	2 (14.3)	2

Source: Applicant ACP-2566-009 Interim Synoptic CSR Table 14.3.1.2.1, Table 14.3.1.2.2, Table 14 3.1.4.1 and Table 14.3.1.4.2
Abbreviations: COVID-19=coronavirus disease 2019; MedDRA=Medical Dictionary for Regulatory Activities; N=number of subjects in the Safety Analysis Set; PT=preferred term; TEAE=treatment-emergent adverse event Notes: Adverse events were coded using MedDRA version 24.0. A TEAE is an adverse event with onset date on or after the first study dose date and no later than the last study dose date plus 30 days. A subject may have more than 1 TEAE per PT. In the "Subjects" column, a subject is counted at most once per PT. In the "Events" column, all occurrences of TEAE are counted per PT. The number of subjects in the Safety Analysis Set is used as the denominator for calculating percentages for the subject counts.

The primary purpose of the study was to act as a PK bridging study to ACP-2566-009, to demonstrate that exposure levels correlated with improvement in the pivotal trial could be achieved safely in younger subjects. Please see the clinical pharmacology review for details, but this goal was achieved. In addition, an exploratory analysis of efficacy was conducted by the Applicant and reported that at the conclusion of 12 weeks, the CGI-I mean score was 3.3 (SE=0.24) while the CaGI-I was 2.2 (SE=0.13). The ICND-QOL score improved on average by 0.4 (SE=0.27) points from a mean of 3.9 (SE=0.25) to a mean of 4.2 (SE=0.44).

REVIEWER COMMENT: ACP-2566-009 interim results are indicative of adequate and safe achievement of potentially beneficial exposure of trofinetide in subjects ages 2 through 4 with similar safety profile as seen in older subjects in ACP-2566-003.

(b) (4)

(b) (4)

the Applicant has proposed that trofinetide be labeled to not be administered to subjects with moderate or severe renal impairment.

8.8. Additional Safety Explorations

8.8.1. Human Carcinogenicity or Tumor Development

The Applicant is planning to conduct carcinogenicity studies of trofinetide in two rodent species for up to two years as a PMR.

8.8.2. Human Reproduction and Pregnancy

Because of the severe neurological impairment in Rett patients, pregnancy is not expected, and no clinical data is available regarding trofinetide in pregnant women. Developmental and reproductive toxicology studies with trofinetide in male and female rats, embryo-fetal toxicity in rats and rabbits, pre- and post-natal toxicity study in rats, have all been completed. No effects on male or female fertility were noted. The rat study revealed no embryofetal developmental toxicities. Maternal reductions in body weight and food consumption were noted in the rabbit study at 150 mg/kg/d, however no malformations were noted at 600 mg/kg/d. No toxicities related to development, growth, behavior, reproductive performance, and fertility of the F1 generation in rats

8.8.3. Pediatrics and Assessment of Effects on Growth

This application is for a pediatric indication and hence the entire review covers the pediatric development for trofinetide. On June 14, 2021, Acadia Pharmaceuticals, Inc submitted a proposed pediatric study request (PPSR) outlining 3 studies. These studies were further discussed and refined via an end-of-phase 2 meeting held on October 12, 2017, and via a Type C Written Response Only on January 23, 2019. The Agency issued a Pediatric Written Request (PWR) October 8, 2021. Acadia responded in agreement with the PWR on March 22, 2022. The three studies outlined in the PWR were ACP-2566-003, ACP-2566-004, and ACP-2566-009.

Special emphasis on the effects of trofinetide on weight are discussed in section 8.4.4, Significant Adverse Events.

8.8.4. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Accidental overdose was reported for six subjects amongst all clinical trials (two in ACP-2256-003, two in ACP-2566-004, and one in ACP-2566-009). One of the subjects with overdose in ACP-2566-003 reported AE of vomiting associated with the overdose. There is no recommended course of action for an overdose. If an overdose occurs or is suspected, the

subject should be monitored closely.

A consult was obtained from the FDA Controlled Substance Staff (CSS). The CSS consult reviewed nonclinical safety and toxicology data, and adverse event assessments on trofinetide that were submitted by the Applicant and determined:

> "Trofinetide was shown to bind significantly to NMDA and AMPA receptors at micromolar concentrations. When activated or blocked, NMDA and AMPA receptors are known to induce central nervous system (CNS) -mediated adverse events (AEs) which may be drug abuse-related. However, for trofinetide, CNS-related AEs associated with the drug's administration were found not to be drug abuse-related and there were no AEs related to a withdrawal syndrome upon discontinuation of the drug. Based on the information submitted, CSS concluded and conveyed to the Applicant that nonclinical and clinical abuse-related studies were not necessary"

8.9. Safety in the Postmarket Setting

8.9.1. Safety Concerns Identified Through Postmarket Experience

Not applicable as trofinetide is not yet marketed.

8.9.2. Expectations on Safety in the Postmarket Setting

The types and frequencies of adverse drug reaction in the postmarketing setting are likely to be similar to those in the clinical trials. Prescribing clinicians and caregivers will need to be aware of the need to aggressively manage diarrhea and vomiting associated with trofinetide. Seizure frequency will also need to be monitored. The long-term effects on weight and development will be matters for post-marketing safety monitoring.

8.9.3. Additional Safety Issues From Other Disciplines

Not applicable.

8.10. Integrated Assessment of Safety

Rett syndrome is a serious chronic condition with morbidity that has substantial impact on day-to-day functioning. Nearly all Rett syndrome patients suffer from enough disability that they are dependent on a caregiver for most of their lives. There is significant unmet medical need in Rett syndrome as there are no FDA approved medications specifically for the behavioral symptoms of the disorder, though many treatments are used to treat the associated epilepsy, and many treatments are used off-label to assist with the multitude of symptoms. Therefore, the overall conclusion of this review is that the safety profile of trofinetide is acceptable given the evidence of effectiveness and unmet medical need.

There were two deaths in the clinical development of trofinetide for Rett syndrome. One death was due to severe vomiting, aspiration, and respiratory arrest the night after placement of a percutaneous gastrostomy tube. The second was in a subject who suffered multiple gastrointestinal hemorrhages and perforation after chronic treatment with ibuprofen for post-spinal surgery pain. The narratives were reviewed for both deaths, and it is unlikely that trofinetide directly contributed to either, given the natural history of comorbidities in both subjects.

Serious adverse effects were relatively rare in the double-blind placebo-controlled trials of trofinetide (RTTDB), with a slightly greater occurrence in trofinetide compared to placebo driven by seizures, pneumonia, and UTI leading to bacteremia. In the long-term pool of trofinetide treated subjects (RTTLT), 11 cases of serious seizure or status epilepticus, 4 serious pneumonias, and 4 urinary tract infections were reported.

Treatment emergent adverse effects led to withdrawal of study drug in 40.4% of the RTTLT pool. This is a large dropout rate, consistent with the difference in the pivotal study ACP-2566-003, where at least 17.2% of trofinetide treated subjects discontinued study drug due to adverse effects, compared to only 2.1% of placebo-treated subjects.

Along with this, the frequency of diarrhea with trofinetide led to the initiation of loperamide in greater than 50% of long term treated subjects. This is also a complication that prescribing clinicians and families should be aware of.

No QT, Hy's Law cases, or hematological, or carcinogenicity safety signals have been found.

A notable concern with trofinetide that will need to be monitored post-marketing is the incidence of inadequate weight gain and alteration of growth curves in children receiving the treatment.

This reviewer concludes that while there are number of safety signals that need to be monitored in this vulnerable population, that the safety profile of trofinetide for the treatment of Rett syndrome is acceptable given the unmet medical need in this serious condition.

9. Advisory Committee Meeting and Other External Consultations

Not applicable

10. Labeling Recommendations

10.1. Prescription Drug Labeling

The Applicant has proposed the indication of "for the treatment of Rett syndrome in adults and pediatric patients 2 year of age and older." The Applicant has proposed no information for the Warnings and Precautions section of the trofinetide label. For the adverse reactions section, the Applicant proposes to label TEAEs that occurred in ≥10% of subjects and twice the rate of placebo, and list diarrhea and vomiting.

REVIEWER COMMENT: Given the frequency of TEAE, the vulnerability of the population involved, the substantial comorbidities, and the indication for chronic long-term use, this reviewer recommends:
Inclusion of a Warnings and Precautions Section to list diarrhea and weight loss given the frequency of the former and potential for serious consequences of the latter in a pediatric population that already has growth deficiency.
*A listing of most common adverse drug reactions as being those that occurred in **≥5%** of trofinetide-treated subjects and **≥2%** risk difference of placebo based on ACP-2566-003, to include diarrhea, vomiting, fever, seizure, anxiety, decreased appetite, fatigue, and nasopharyngitis.*
Given the basis of the effectiveness measure being the coprimary endpoint of RSBQ change from baseline, it may be more specific to list the indication as "for the treatment of behavioral impairments in Rett syndrome." However, given the finding of the CGI-I favoring trofinetide, this reviewer can accept the Applicant's indication statement.

10.2. Nonprescription Drug Labeling

Not applicable.

11. Risk Evaluation and Mitigation Strategies (REMS)

A REMS is not necessary for oral trofinetide for the treatment of behavioral impairments of Rett syndrome. While there were numerous and frequent adverse drug reactions identified in the clinical trials, most were mild to moderate at most in severity. There were relatively few serious adverse events. Since patients with Rett syndrome are severely neurologically impaired, their medication administration and monitoring will be dependent on adult caregivers who will be able to be informed and understand the risks. Adequate labeling will alert caregivers to be prepared for the gastrointestinal adverse effects most common with trofinetide. Since Rett syndrome patients already suffer from epilepsy, monitoring seizure frequency for increases can be managed by caregivers and prescribers. Finally, monitoring of weight and growth is also feasible.

12. Postmarketing Requirements and Commitments

The following postmarketing requirements (PMR) have been proposed by the Applicant and FDA:
PMR 1:

A carcinogenicity study of trofinetide in mouse.

Draft Protocol Submission:	06/2023
Final Protocol Submission:	08/2023
Study Completion:	08/2025
Final Report Submission:	08/2026

PMR 2:

A 2-year carcinogenicity study of trofinetide in rat.

Draft Protocol Submission:	06/2023
Final Protocol Submission:	08/2023
Study Completion:	08/2025
Final Report Submission:	08/2026

PMR 3:

Conduct a clinical trial to evaluate the effect of moderate renal impairment on the exposure of trofinetide relative to that in subjects with normal renal function after oral administration of trofinetide. Please refer to the Guidance for Industry Pharmacokinetics in Patients with Impaired Renal Function: Study Design, Data Analysis, and Impact on Dosing and Labeling (https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM204959.pdf

Draft Protocol Submission:	04/2021
Final Protocol Submission:	10/2021
Study Completion:	12/2022
Final Report Submission:	09/2023

PMR 4:

In vitro drug interaction study to evaluate the time-dependent inhibition of CYP 2B6 enzyme by trofinetide based on the Guidance for Industry In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (Jan 2020

https://www.fda.gov/media/134582/download).

Draft Protocol Submission:	04/2023
Final Protocol Submission:	08/2023
Study Completion:	02/2024
Final Report Submission:	05/2024

PMR 5:

In vivo pharmacokinetic drug interaction study in healthy subjects to evaluate the effect of trofinetide on inhibiting OATP1B1 and OATP1B3 transporters using an appropriate probe substrate for each transporter. Please refer to the Guidance for Industry Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (Jan 2020 https://www.fda.gov/media/134581/download).

Draft Protocol Submission:	03/2024
Final Protocol Submission:	06/2024
Study Completion:	12/2025
Final Report Submission:	09/2026

13. Appendices

13.1. References

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13.2. Financial Disclosure

Covered Clinical Study (Name and/or Number): ACP-2566-003

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 21		
Number of investigators who are Applicant employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455):		

1		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 1 Significant payments of other sorts: 0 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator in S Applicant of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 21		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

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/s/

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