CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

217026Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

<table>
<tr><td>NDA Number</td><td>217026</td></tr>
<tr><td>Link to EDR</td><td>\\CDSESUB1\EVSPROD\nda217026\</td></tr>
<tr><td>Submission Date</td><td>07/12/2022</td></tr>
<tr><td>Submission Type</td><td>505(b)(1); Priority Review</td></tr>
<tr><td>Brand Name</td><td>DAYBUE</td></tr>
<tr><td>Generic Name</td><td>Trofinetide</td></tr>
<tr><td>Dosage Form and Strength</td><td>Oral solution, 200 mg/mL</td></tr>
<tr><td>Dosing Regimen and Route of Administration</td><td>Recommended dose is twice daily, morning and evening, according to patient weight
<table>
<tr><th>Patient Weight</th><th>DAYBUE Dose</th></tr>
<tr><td>9 kg to <12 kg</td><td>5g (25 mL) twice daily</td></tr>
<tr><td>≥12 kg to <20 kg</td><td>6g (30 mL) twice daily</td></tr>
<tr><td>≥20 kg to <35 kg</td><td>8g (40 mL) twice daily</td></tr>
<tr><td>≥35 kg to <50 kg</td><td>10g (50 mL) twice daily</td></tr>
<tr><td>≥50 kg</td><td>12g (60 mL) twice daily</td></tr>
</table></td></tr>
<tr><td>Proposed Indication</td><td>For the treatment of Rett syndrome in adults and pediatric patients 2 years of age and older</td></tr>
<tr><td>Applicant</td><td>Acadia Pharmaceuticals Inc.</td></tr>
<tr><td>Associated IND</td><td>114319</td></tr>
<tr><td>OCP Review Team</td><td>Min Li, Ph.D.; Jie Liu, Ph.D.; Ying-Hong, Wang, Ph.D.; Atul Bhattaram, Ph.D.; Yuching Yang, Ph.D., Bilal AbuAsal, Ph.D.</td></tr>
<tr><td>OCP Final Signatory</td><td>Mehul Mehta, Ph.D. Division Director</td></tr>
</table>

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1. EXECUTIVE SUMMARY

Acadia Pharmaceuticals Inc. submitted this New Drug Application (NDA #217026) for trofinetide under the 505(b)(1) pathway for the treatment of Rett syndrome (RTT) in adults and pediatric patients 2 years of age and older. RTT is a rare, seriously debilitating neurodevelopmental disorder, which manifests in early childhood mainly in females. The main clinical features of RTT include loss of verbal and nonverbal communication and voluntary motor function, characteristic repetitive hand stereotypies, and gait problems, etc. RTT is often associated with mutations in the X-linked methyl CpG binding protein 2 (MeCP2 in humans) gene, which is important for the function of nerve cells including both neurons and astrocytes. The activity of the MeCP2 protein is diminished in RTT. There are no approved therapies for RTT currently and the treatment focuses mainly on management of symptoms with limited effect on functional improvement.

Trofinetide is a synthetic analog of glycine-proline-glutamate (GPE), a peptide that occurs naturally in the brain. The proposed mechanism of action is to enhance neuronal synaptic function and morphology. The ready-to-use oral solution of trofinetide (200 mg/mL) is proposed to be administered twice daily orally or by gastrostomy tube without regard to meals. The proposed dosage is a weigh-band based dosing with individually fixed dose for 5 different weight bands ranging the body weight from 9 kg to ≥ 50 kg.

The applicant is seeking approval of trofinetide primarily based on a phase 3 clinical trial Study APC-2566-003 (Study 003) in 5-20 years old RTT patients. In this randomized, double-blind, placebo-controlled study, the change from baseline in Rett Syndrome Behaviour Questionnaire (RSBQ) total score and the Clinical Global Impression–Improvement (CGI-I) score at Week 12 was significantly greater in patients receiving trofinetide compared to placebo, indicating more clinical improvement with trofinetide. Additionally, supportive evidence of effectiveness was also provided by a Phase 2 dose ranging study (#Neu-2566-RETT-002) which studied trofinetide at three levels (50 mg/kg, 100 mg/kg and 200 mg/kg) for 6 weeks in 76 female children and adolescents aged 5 to 15 years with RTT. Significant evidence of efficacy was found only at the 200 mg/kg BID dose while an exploratory pharmacokinetics/pharmacodynamics (PK/PD) analyses suggested a correlation between trofinetide exposure and the magnitude of clinical response for core clinical measures.

The effectiveness of trofinetide in patients 2 to 4 years of age was established through extrapolation of the efficacy observed in the Study 003 in RTT patients 5 years of age and older, based on the similarity of the disease pathophysiology as well as the assumption of similar exposure response relationship between the young age patients (2-4 years old) and patients 5 years of age and older. An open label Study ACP-2566-009 (Study 009) has been conducted to evaluate the PK and safety in female RTT patients between the age range of 2-4 years (ongoing). The interim pharmacokinetic (PK) analysis based on the data from 13 pediatric patients 2 to 4 years of age treated with trofinetide for 12 weeks demonstrated similar PK exposure of trofinetide and similar safety profiles to those in the pediatric patients ≥5 years of age and adults.

The clinical pharmacology development program includes 5 dose-escalation studies (4 for intravenous administration and 1 for oral administration), a mass balance study, a food effect study and a thorough

QT/QTc study in healthy volunteers. There was a total of 15 clinical studies including 8 studies in healthy subjects, 5 studies in subjects with RTT, 2 studies in subjects with other disease populations. Population PK (popPK) analyses were conducted to characterize the PK characteristics and clinically meaningful covariates using all the PK data collected in the clinical program. The exposure-response (ER) analyses (for efficacy and safety) were conducted mainly based on the two phase 2 studies and the pivotal phase 3 study in the RTT patients to characterize the ER relationships and support proposed dosing regimen. The physiologically based pharmacokinetic (PBPK) analyses were submitted to support renal impairment assessment, as well as drug-drug interaction (DDI) assessment to evaluate trofinetide's inhibition potential on CYP3A4 using midazolam as a sensitive substrate.

The primary objectives of this review are (1) to assess the adequacy of dosing recommendations in the general patient population; (2) to evaluate the appropriateness for the extrapolation of efficacy to pediatric patients population aged 2-4 years; (2) to verify the in vitro findings as well PBPK analyses to support DDI evaluation; 3) to evaluate the PBPK analyses for renal impairment assessment; and (4) to evaluate the dose modification recommendation for management of diarrhea (most frequently reported adverse reaction).

1.1 Recommendations

The Office of Clinical Pharmacology reviewed this NDA and recommends approval of this application. The specific recommendations and comments are summarized below:

<table>
<tr><th>Review Issue</th><th>Recommendations and Comments</th></tr>
<tr><td>Pivotal or supportive evidence of effectiveness</td><td>The evidence of effectiveness is primarily based on results from Study RETT-003 in 187 RTT female patients at age between 5-50 years. The co-primary endpoints to support approval is the change from baseline at week 12 in the RSBQ total score and CGI-I score. The phase 2 dose ranging Study Neu-2566-002 and exposure response analyses provided supportive evidence of effectiveness. Extrapolation of efficacy for patients 2-4 years of age was supported by the results from Study ACP-2566-009 based on PK exposure matching to that of RTT patients at ages of 5-50 years in the pivotal Study RETT-003.</td></tr>
<tr><td>General dosing instructions</td><td>The recommended dosage is weight-band based dosing administration via oral or via gastrostomy tube (G-tube). The drug should be taken twice daily (BID) in the morning and evening, without regard to food.
<table>
<tr><th>Patient Weight</th><th>DAYBUE Dose</th></tr>
<tr><td>9 kg to <12 kg</td><td>25 mL (5 g) twice daily</td></tr>
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</table></td></tr>
<tr><td>Dosing in patient subgroups (intrinsic and extrinsic factors)</td><td><u>Intrinsic factors:</u>
Trofinetide should be avoided in patients with moderate or severe renal impairment.</td></tr>
</table>

	No dose adjustment is needed for trofinetide for the following intrinsic factors: • Mild renal impairment • Mild, moderate, or severe hepatic impairment Extrinsic factors: No dose adjustment is needed for trofinetide with concomitant medications. Closely monitor when trofinetide is used in combination with orally administered CYP3A4 sensitive substrates for which a small change in substrate plasma concentration may lead to serious toxicities. Avoid the concomitant use of trofinetide with OAT1B1 and OAT1B3 substrates for which a small change in substrate plasma concentration may lead to serious toxicities
Labeling	The proposed labeling concepts are generally acceptable. The DDI labeling in Section 7 (b) (4) was updated according to the in vitro results and PBPK analyses, mainly to clarify the DDI potential for trofinetide as a weak inhibitor on CYP 3A4 enzyme based on PBPK analyses, potential inhibition on UGT enzymes, and the inhibition potential on OAT1B1 and OAT1B3 based on in vitro assays. More details refer to 2.4 Summary of labeling.
Bridge between the to-be-marketed and clinical trial formulations	There is no difference between the pivotal clinical trial formulation and the to be marketed formulation.

1.2 Post-Marketing Requirements and Commitments

The following post-marketing requirements (PMRs) will be issued:

- 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)
- 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).
- 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).

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

The pharmacokinetics (PK) of trofinetide has been characterized in phase 1 clinical studies in healthy subjects as well as using pooled data from studies in patients based on the PopPK approach. Trofinetide exhibits linear kinetics. Systemic exposure to trofinetide was dose proportional across the tested clinical dose range (up to 12 g). Minimal to no accumulation was observed following twice daily (BID) administration.

Mechanism of Action: The mechanism of trofinetide in the treatment of Rett syndrome is unclear. However, trofinetide is thought to enhance neuronal synaptic function and morphology. This hypothesis is supported by findings from studies of GPE and trofinetide in a methyl-CpG-binding protein 2 gene (Mecp2) mouse model of Rett syndrome, in which increased branching of the dendrites that form synapses and synaptic plasticity signals were observed.

Absorption: Trofinetide is rapidly absorbed after oral administration with time to maximum drug concentration (T_{max}) of 2 to 3 hours under both fasted and fed state. Based on the mass balance study, at least 83.8% of the administered dose was absorbed following oral administration of 12 g trofinetide. Trofinetide's exposure parameters (maximum observed drug concentration [C_{max}] and area under the concentration-time curve [AUC]) after administration of trofinetide solution through G-tube were similar to those after oral administration.

Effect of Food: Coadministration of trofinetide with a high-fat meal had no impact on the total exposure ($_{AUC0\text{-}inf}$) of trofinetide and only reduced the peak plasma concentration (C_{max}) by approximately 20%.

Distribution: The apparent volume of distribution of trofinetide in adult healthy subjects was approximately 80 L. Trofinetide exhibits low protein binding in human plasma (less than 6%). The blood-to-plasma ratio (R_{bp}) was consistent ranging between 0.529 and 0.592 over the studied concentration range, indicating that trofinetide is not preferentially distributed into red blood cells.

Metabolism: Trofinetide is not significantly metabolized by CYP450 enzymes. Hepatic metabolism is not a significant route of trofinetide's disposition.

Elimination: Trofinetide is primarily excreted unchanged (approximately 80% of the dose) in urine, with minor excretion in feces (15.3%). The elimination is characterized by an initial rapid elimination phase ($t_{½,\alpha}$ 1.5 hours) followed by a relatively slow elimination phase ($t_{½,\beta}$ 30 hours). The initial elimination half-life is considered the effective half-life. Based on population PK analysis, the trofinetide CL/F is estimated to be 11.8 L/h at steady state.

Specific Populations

Pediatric Patients with RTT 2 to 4 Years of Age: The drug exposure of trofinetide in pediatric patients ages 2 to 4 years of is similar to children older than 4 years and adults when following the recommend dosage.

Renal Impairment: No dedicated clinical study is available to evaluate the pharmacokinetics of trofinetide in subjects with renal impairment. Based on popPK data, no impact on trofinetide's PK exposure in the subjects with mild renal impairment (eGFR ranges 60-89 mL/min/1.73 m^2) compared to subjects with normal renal functions (eGFR ranges 90-120 mL/min/1.73 m^2). (b) (4) Until the data is available, the use of trofinetide is not recommended in patients with moderate or severe renal impairment.

Hepatic Impairment: No dedicated clinical study is available to evaluate the pharmacokinetics of trofinetide in subjects with hepatic impairment. The in vitro studies and the mass balance study indicated that trofinetide is not significantly metabolized by hepatic enzymes and hepatic clearance is not a primary route of trofinetide elimination. Hepatic impairment is not expected to have clinically meaningful effect on trofinetide's exposure.

2.2 Dosing and Therapeutic Individualization

2.2.1 General Dosing

Trofinetide oral solution should be taken twice daily, morning and evening, according to patient weight as shown in Table 1. The oral solution is recommended to be given orally or via gastrostomy tube (G-Tube). The drug should be taken twice daily in the morning and evening, without regard to food.

Table 1 Recommended Dosage for Trofinetide in RTT patients

Patient Weight	DAYBUE Dose
9 kg to <12 kg	25 mL (5 g) twice daily
≥12 kg to <20 kg	30 Ml (6 g) twice daily
≥20 kg to <35 kg	40 mL (8 g) twice daily
≥35 kg to <50 kg	50 mL (10 g) twice daily
≥50 kg	60 mL (12 g) twice daily

As diarrhea is the most frequently seen adverse reaction (80% subjects with trofinetide treatment), strategies for diarrhea management are recommended including dose adjustment as below:

For management of diarrhea (mostly common adverse reaction), advise patients to stop laxatives before starting DAYBUE. If diarrhea occurs, patients should start antidiarrheal treatment, increase oral fluids, and notify their healthcare provider. Interrupt, reduce dose, or discontinue DAYBUE if severe diarrhea occurs or if dehydration is suspected.

2.2.2 Therapeutic Individualization

Drug interaction with major metabolizing enzymes and transporters:

Trofinetide is not a substrate of CYP450 enzymes, uridine diphosphate glucuronosyltransferase (UGT), or major drug transporters. Therefore, coadministration of drugs that are inducers or inhibitors of CYP450, or major drug transporters will not significantly affect the systemic exposure of trofinetide.

No inhibition on CYP450 enzymes, CYP1A2, 2C8 ,2C9, 2C19, and 2D6 is expected at therapeutic systemic concentrations based on the in vitro assays and the static mechanistic models. Time-dependent inhibition on CYP 2B6 was inconclusive based on in vitro data. Using physiologically based pharmacokinetic modeling, coadministration of trofinetide with orally administered midazolam (a sensitive CYP3A4 substrate) was predicted to increase the AUC of midazolam by approximately 1.33-fold, indicating trofinetide is a weak inhibitor of CYP3A4.

No inhibition was observed at therapeutic systemic concentrations on P-gp, BCRP, BSEP, OAT1, OAT3, OCT2, MATE1, and MATE2-K, based on the in vitro assays. Trofinetide inhibits OATP1B1 and OATP1B3 in vitro.

Based on the data, we recommend close monitor of safety when trofinetide is used in combination with sensitive CYP3A4 substrates administered orally and avoiding use of sensitive OAT1B1 and OAT1B3 substrates, for which a small change in substrate plasma concentration may lead to serious toxicities.

Renal Impairment: No dedicate clinical study is available to evaluate the pharmacokinetics of trofinetide in subjects with renal impairment. Based on popPK data, mild renal impairment showed no impact on trofinetide's PK exposure compared to subjects with normal renal functions and hence no dose adjustment is recommended. The use of trofinetide is not recommended in patients with moderate or severe renal impairment.

Hepatic Impairment: Trofinetide is predominantly excreted in urine in the unchanged form. Hepatic metabolism has minimum contribution to the elimination of trofinetide and is not expected to affect the drug exposure. No dose adjustment is needed for patients with hepatic impairment.

Pediatric Use: The safety and effectiveness of trofinetide for the treatment of Rett syndrome in pediatric patients 5 years of age and older was established in a randomized, double-blind, placebo-controlled, 12-week study (Study 003), which included 108 pediatric patients aged 5 to less than 12 years and 47 pediatric patients aged 12 to less than 17 years.

The effectiveness of trofinetide in patients 2 to 4 years of age was established through extrapolation of the efficacy observed in the Study 003 in RTT patients 5 years of age and older, based on the similarity of the disease pathophysiology as well as the assumption of similar exposure response relationship between the young age patients (2-4 years old) and patients at ages of 5 years and older. After administration of the proposed weight-band based dosing, the interim results of the ongoing open label PK and safety study (Study 009) from 13 pediatric patients 2 to 4 years of age treated with trofinetide for 12 weeks demonstrated similar PK exposure of trofinetide and similar safety profiles to that seen in the pediatric patients ≥5 years of age and adults. The interim PK and safety data from Study 009 support the extrapolation of efficacy.

The safety and efficacy of trofinetide for the treatment of Rett syndrome in pediatric patients less than 2 years of age have not been established.

Geriatric Use: Clinical studies of trofinetide did not include patients 65 years of age and older to determine whether or not they respond differently from younger patients. This drug is known to be substantially excreted by the kidney. Because elderly patients are more likely to have decreased renal function, it may be useful to monitor renal function.

2.3 Outstanding Issues

The following clinical pharmacology studies are outstanding and will be conducted under PMR:

- 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)
- 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).
- 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).

2.4 Summary of Labeling Recommendations

The general labeling recommendations are acceptable. The major revisions in the label are presented below:

- Section 2.3 Dose Modification [redacted (b) (4)] Diarrhea-- Advise patients to stop laxatives before starting trofinetide. [redacted (b) (4)] Interrupt, reduce dose, or discontinue trofinetide if severe diarrhea occurs or if dehydration is suspected.
- Section 7--Section 7.1 Effect of Other Drugs on trofinetide is not needed. Section 7.2 Effect of trofinetide on Other Drugs should recommend close monitor of safety when trofinetide is used in combination with sensitive CYP3A4 substrates administered orally and avoiding use of sensitive OAT1B1 and OAT1B3 substrates, for which a small change in substrate plasma concentration may lead to serious toxicities.
- Section 12.3 -- Drug interaction information was updated according to the in vitro results and PBPK analyses, mainly to clarify the DDI potential for trofinetide as a perpetrator, such as a weak inhibition on CYP 3A4 enzymes based on PBPK analyses, potential inhibition on UGT enzymes, and the inhibition risk on OAT1B1 and OAT1B3 based on in vitro assays. No dose adjustment for

trofinetide is recommended regarding DDI as the drug is not a substrate of major metabolizing enzymes and transporters.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Rett syndrome (RTT) is a seriously debilitating neurodevelopmental disorder for which there is currently no approved treatment. Currently available treatment for RTT focuses on the management of each patient's symptoms, which is often unsatisfactory with only a limited effect on functional improvement.

Trofinetide is a new molecular entity (NME) not previously approved. Trofinetide is a synthetic analog of glycine-proline-glutamate (GPE). GPE is a product of the naturally occurring cleavage of IGF-1 protein. Compared to GPE, trofinetide displayed resistance to proteolytic degradation in human plasma and in human colorectal adenocarcinoma-2 cells. The mechanism of trofinetide in the treatment of RTT is unclear. However, trofinetide is thought to enhance neuronal synaptic function and morphology. This hypothesis is supported by findings from studies of GPE and trofinetide in a methyl-CpG-binding protein 2 gene (Mecp2) mouse model of Rett syndrome, in which increased branching of the dendrites that form synapses and synaptic plasticity signals were observed.

The commercial drug product is a ready to use (RTU) oral solution at a concentration of 200 mg/mL for the convenience of weight-based dosing and possible administration by G-tube in pediatric patients. The formulation used in the initial phase 1 and 2 clinical studies for IV administration was presented as lyophilized powder to be reconstituted with bicarbonate buffer. The same formulation was used in the initial Phase 1 and 2 clinical studies for oral administration, which was to be reconstituted into a flavored water-based vehicle. The formulation used in Phase 3 and the most recent Phase 1 clinical studies (i.e., the mass balance study, food effect study, and thorough QT/QTc study), is the RTU oral solution which is also the to-be-marketed formulation.

Investigation of trofinetide for RTT was designated as a Fast Track development program on 03 June 2013. Trofinetide was granted Orphan Drug Designation for the treatment of RTT on 11 February 2015 in recognition that RTT is a rare disease. Trofinetide for the treatment of RTT received Rare Pediatric Disease Designation on 02 March 2020. The Applicant is seeking priority review for trofinetide in this NDA.

The clinical development program for trofinetide has evaluated the PK, intrinsic and extrinsic factors affecting the PK, pharmacodynamics (PD), exposure-response (E-R) relationship, safety, tolerability, and efficacy in clinical studies. There were a total of 15 clinical studies (Table 7 in Appendix 4.2) including eight studies in healthy subjects, five studies in subjects with RTT, one study in subjects with fragile X syndrome (FXS), and one study in subjects with traumatic brain injury (TBI). Three randomized, double-blind, placebo-controlled studies have been completed in subjects with RTT to evaluate the safety and efficacy of trofinetide including two phase 2 studies (Study RETT-001 and 002) and the pivotal phase 3 efficacy study (Study 003) along with the ongoing open label extension studies to Study 003 for long-term safety and efficacy. An open label Study ACP-2566-009 (Study 009) is ongoing to evaluate the PK and safety in

female RTT patients between the age range of 2-4 years, for extrapolation of efficacy from older children and adults to the younger patient population.

3.2 General Pharmacology and Pharmacokinetic Characteristics

The general pharmacology and PK characteristics are summarized in Table 2.

Table 2 Summary of General Pharmacology and Pharmacokinetics Characteristics

Pharmacology	
Mechanism of Action	The mechanism of trofinetide in the treatment of Rett syndrome is unclear. However, trofinetide is thought to enhance neuronal synaptic function and morphology. This hypothesis is supported by findings from studies of GPE and trofinetide in a methyl-CpG-binding protein 2 gene (Mecp2) mouse model of Rett syndrome, in which increased branching of the dendrites that form synapses and synaptic plasticity signals were observed.
Active Moieties	The active moiety is trofinetide, a synthetic analog of the tripeptide glycine-proline-glutamate (GPE) which is a product of the naturally occurring cleavage of insulin-like growth factor 1.
QT Prolongation	A clinical study was conducted to evaluate the potential of trofinetide to prolong QTcF at a single dose of 24g in healthy subjects (Study 008). There was no clinically significant effect of trofinetide on the ECG at the resulted exposure level (similar to that resulted from the maximum approved dose at steady state), as determined by C-E modeling, central tendency modeling, categorical ECG rate and interval analysis, and diagnostic statement analysis. The upper 90% 2-sided confidence bound for ΔQTcF and ΔΔQTcF were below 10 ms (the threshold of concern for cardiac repolarization). Please refer to the QT review documented in DARRTS.
General Information	
Bioanalysis	The bioanalytical methods for trofinetide quantification were developed and validated in human blood and urine. The methods met the acceptance criteria for bioanalytical methods according to the FDA Bioanalytical Method Validation Guidance, and were shown to be accurate, selective and robust. Please refer to Section 4.1 for details.
Dose Proportionality	In RTT patients, trofinetide exposure increased in a dose-proportional manner over the therapeutic dose range (up to 12 g). Increase in exposure appeared to be less than dose proportional at supratherapeutic doses (18 g and 24 g).
Accumulation	No drug accumulation was observed following recommended dosing regimen.
Variability	Inter-subject variability (as %CV) for trofinetide C_{max} and AUC at steady state were less than 25%.
Healthy Volunteers vs. Patients	There was no significant difference in PK between RTT patients and healthy subjects

Absorption	
Bioavailability	The absolute bioavailability (BA) of oral trofinetide has not been determined in a dedicated study. However, the mean urinary recovery of total [^{14}C]trofinetide in 48 hours post-oral dose in Study 007 (mass balance study) represented 83.8% of the administered dose, indicating a minimum 83.8% of oral BA. The oral BA following drug administration via G-tube was similar with that observed with regular oral administration based on pop-PK analysis.
Cmax and AUC	PopPK model predicted steady-state C_{max} and AUC of trofinetide were 139.5-215.8 µg/mL and 839.6-1109.2 h*ng/mL, respectively, in RTT patients at the recommended dosage (12 g BID).
Tmax	Tmax is approximately 2-3 hours.
Food effect (Fed/fasted)	The rate of absorption of trofinetide was affected to a small extent by oral administration with a high-fat meal, with the C_{max} being reduced by approximately 20% and a 0.5 hour delay in T_{max} (from 2 hours [fasted] to 2.5 hours [with a high-fat meal]) when compared with administration after fasting. The extent of absorption, expressed as AUC, was unaffected by administration with food. The time of day of administration (morning versus evening dose) showed no impact on trofinetide PK, indicating the absence of circadian impact.
Distribution	
Volume of Distribution	Trofinetide volume of distribution at steady state was approximately 80 L, which suggests that trofinetide reaches beyond the extracellular fluid with moderate distribution into tissues (intracellularly).
Plasma Protein Binding	Protein binding of trofinetide is low (<6% in human plasma) and not concentration dependent.
Substrate transporter systems	In vitro study indicated that trofinetide is unlikely to be a substrate of major transporters, including P-gp, BCRP, BSEP, OAT1, OAT3, OATP1B1, OATP1B3 OCT2, MATE1, and MATE2-K.
Metabolism	
Primary Metabolic Pathway(s)	Trofinetide is minimally metabolized. Unchanged trofinetide was the major component in all samples. No major or active metabolites were detected in the blood or urine.
Metabolism-based DDI	Trofinetide was not significantly metabolized by human CYP and UGT enzymes based on in vitro assays. Consequently, co-administered drugs that are CYP/UGT inducers or inhibitors would not be expected to affect the exposure of trofinetide. No inhibition on CYP450 enzymes, CYP1A2, 2C8 ,2C9, 2C19, and 2D6 is expected at therapeutic systemic concentrations of trofinetide based on the in vitro assays and the static mechanistic models. Time-dependent inhibition on CYP 2B6 was inconclusive based on in vitro data. Using physiologically based pharmacokinetic modeling, coadministration of trofinetide with midazolam, a sensitive CYP3A4 substrate, was predicted to increase the AUC of

	midazolam by approximately 1.33-fold. In addition, trofinetide inhibits UGT enzymes, UGT1A9, 2B7, and 2B15 in vitro. Accordingly, no dedicated clinical study conducted to evaluate trofinetide's DDI risk associated with CYP enzyme system.
Excretion	
Primary Excretion Pathways	The recovery of trofinetide after oral administration of [^{14}C]trofinetide was complete (99%), with 15% and 84% excreted in feces and urine, respectively. The fraction excreted unchanged in urine was approximately 80% of the administered dose.
Transporter-based DDI	Trofinetide is not a substrate of major transporters based on in vitro assays. Trofinetide showed potential on inhibiting OATP1B1 and OATP1B3 in vitro. However, no clinical DDI studies were conducted to evaluate the interaction with these transporters.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The primary evidence of effectiveness was demonstrated in a pivotal phase 3 study (Study 003), which is a 12-week, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of trofinetide administered orally or via gastrostomy tube (G-tube) twice daily (BID) compared to placebo in 187 female subjects (aged 5 to 20 years) with RTT. The study consisted of an active treatment arm receiving weight-band based dosing of trofinetide (same as the recommended dosage in the label as shown in Table 1), and a placebo treatment arm. Subjects were randomized in a 1:1 ratio with randomization strata for age and Baseline RSBQ[1] severity. The double-blind treatment period was 12 weeks.

The coprimary efficacy endpoints in Study 003 were change from Baseline to Week 12 in the RSBQ total score and the CGI-I score [2]at Week 12. The CSBS-DP-IT Social Composite Score[3] was selected as the key secondary efficacy endpoint in Study 003, an endpoint to assess communication ability. Based on the results, trofinetide treatment demonstrated statistically significant and clinically meaningful improvement over placebo on both the coprimary and key secondary endpoints (see Table 3). The long-term safety and efficacy of trofinetide is being studied in the ongoing Studies ACP-2566-004 (Study 004) and ACP-2566-005 (Study 005) which are open-label (OL) extensions to the Phase 3 study (#003). As of the interim data cutoff, the Applicant claimed that improvements in efficacy measures seen in Study 003

[1] The RSBQ is the most well-known and widely used rating scale for assessment of RTT patients. The RSBQ is a caregiver assessment as individuals with RTT cannot communicate adequately to provide a patient-reported outcome assessment.

[2] The Clinical Global Impression scale, which includes both the Improvement (CGI-I) and Severity (CGI-S) scales, is a well-established research rating tool used widely in clinical studies of CNS disorders, including neurodevelopmental disorders. The CGI-I is completed by the clinician who rates how much the subject's illness improved or worsened relative to the subject's illness at a baseline state using RTT-specific anchors

[3] CSBS-DP-IT Social: Communication and Symbolic Behavior Scales Developmental Profile™ Infant-Toddler Checklist

were maintained with OL trofinetide treatment in Study 004. For more details please refer to Clinical Review by Dr. Michael Dimyan in DARRTS.

Table 3 Coprimary and Key Secondary Endpoints Analysis – Study 003

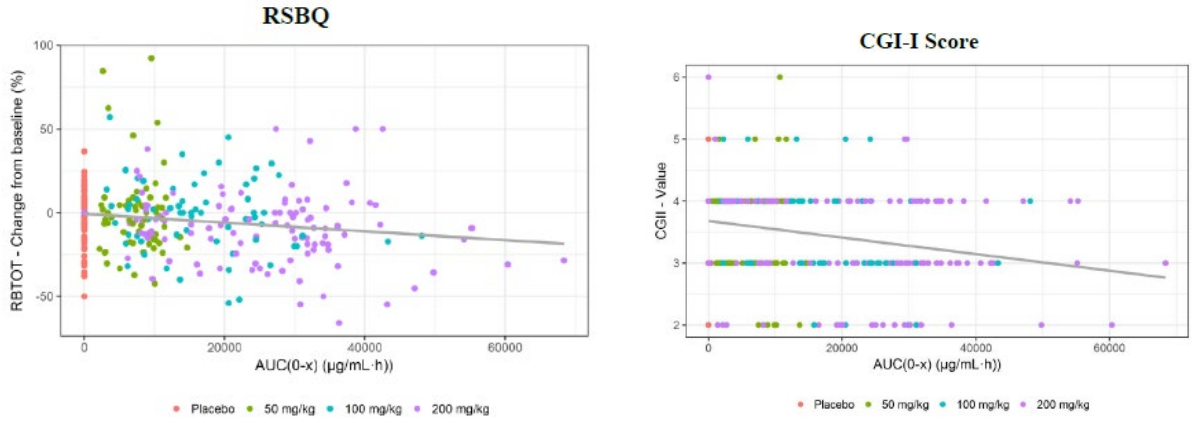
Endpoint/Analyses		LSM (SE)		Treatment group comparison (Trofinetide – placebo)		
		Placebo N=93	Trofinetide N=91	LSM difference (SE)	2-sided p-value	Effect size (Cohen's *d*)
Coprimary endpoints	RSBQ CFB at Week 12	-1.7 (0.90)	-4.9 (0.94)	-3.1 (1.30)	0.0175	0.37
	CGI-I at Week 12	3.8 (0.07)	3.5 (0.07)	-0.3 (0.10)	0.0030	0.47
Key secondary endpoint	CSBS-DP-IT Social Composite Score CFB at Week 12	-1.1 (0.25)	-0.1 (0.26)	1.0 (0.37)	0.0064	0.43

CFB=change from Baseline, LSM=least squares mean.

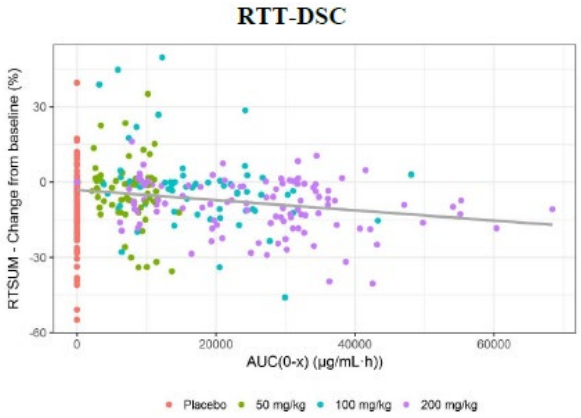
Source: \\CDSESUB1\EVSPROD\nda217026\0001\m2\25-clin-over\25-clinical-overview.pdf Page 28

Additionally, a Phase 2 dose ranging study (#Neu-2566-RETT-002) in RTT subjects with randomized, double-blind, placebo-controlled designs also provided supportive evidence of effectiveness. Study Neu-2566-RETT-002 (Study RETT-002) studied the drug at three higher levels (50 mg/kg, 100 mg/kg and 200 mg/kg) for 6 weeks in 76 female children and adolescents aged 5 to 15 years with RTT. Significant evidence of efficacy was found only at the 200 mg/kg BID dose in three core measures: RSBQ, CGI-I and RTT-DSC[4]. An exploratory PK/PD analysis of the drug exposure and efficacy data suggested a correlation between trofinetide exposure ($AUC_{0\text{-}12h}$ and cumulative AUC [over 42 days]) and the magnitude of response for all of three core clinical measures (see Figure 1). The exploratory analyses were used to guide the dose selection for the pivotal study in subjects with RTT (Study 003), as discussed in Section 3.3.2.

Figure 1 Relationship Between RSBQ, CGI-I and RTT-DSC, Score Change From Baseline and Cumulative Exposure (AUC) During Active Dosing Period (Study RETT-002)



[4] Rett Syndrome Clinician Domain Specific Concerns



Source: Study RETT-002 CSR Figure 11-15, Figure 11-16, and Figure 11-17

The final E-R models updated with the pivotal Phase 3 Study 003 demonstrated a significant trofinetide exposure-related effect on RSBQ total scores, CSBS-DP-IT Social Composite Scores, and RTT-COMC[5] scores, while no E-R relationship was found for CGI-I scores. The E-R analyses confirmed trofinetide is efficacious with the PK exposure resulted from the proposed dosing regimen (same as the used in the pivotal study). For more details please refer to Appendix 4.4.1.3.

To support the extrapolation of efficacy for RTT patients at ages of 2-4 years, Study ACP-2566-009 (Study 009) is being conducted in younger females with RTT (2 to 4 years of age). The extrapolation approach based on PK exposure matching is supported by the similarity of the disease pathophysiology as well as the assumption of similar exposure response relationship between the younger age patients (2-4 years old) and patients at ages of 5 years and older. Study 009 is an open-label study to evaluate safety and tolerability, PK, and efficacy of trofinetide in younger female RTT patients aged 2 to 4 years. The study consists of two treatment periods: Treatment Period A (12 weeks) and Treatment Period B (up to approximately 21 months for long term safety). The clinical study dose levels (weight-band based see Table 4) in this younger patient population have been selected based on popPK simulation targeting similar PK exposure as seen in the older patient population (≥5 years old). While this study is ongoing, as of the interim cut-off date of 14 March 2022, a total of 15 subjects were enrolled, of which 14 subjects were dosed.

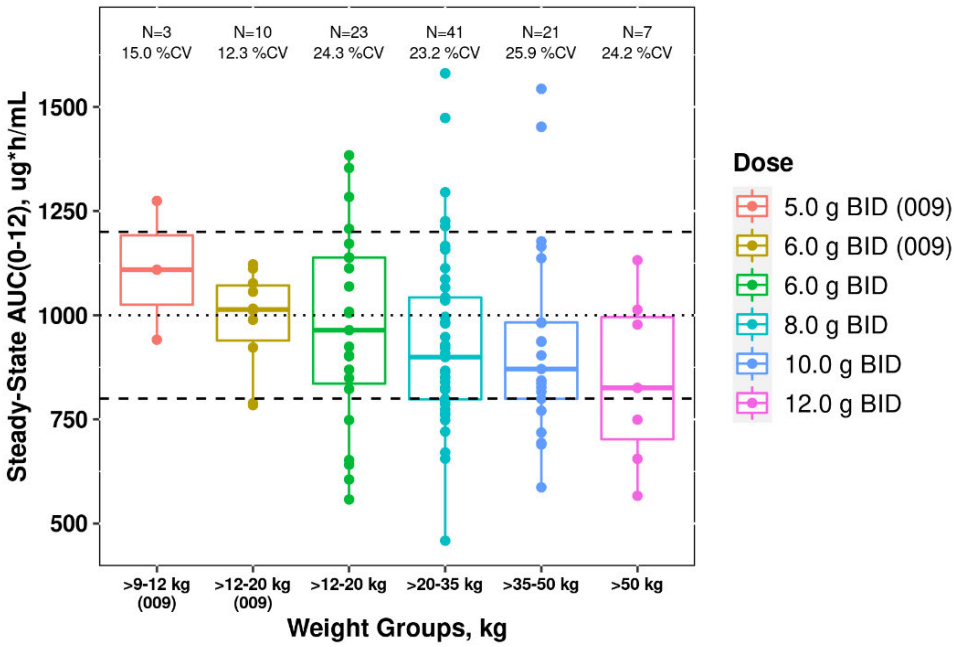
Table 4 Dosing regimen in Study 009

Dose Commences (Visit)	Weight at Baseline	Dose
Day 1	All subjects	10 mL (2 g) BID
Week 2 (Visit 3)	All subjects	20 mL (4 g) BID
Week 4 (Visit 4)	≥9 to <12 kg	25 mL (5 g) BID
	12 to <20 kg	30 mL (6 g) BID

[5] Rett Syndrome Clinician Rating of Ability to Communicate Choices

After administration of trofinetide using the proposed weight-band based dosing, interim evaluation of PK from 13 subjects who completed 12 weeks of treatment with trofinetide in Study 009 indicated that the median $AUC_{0\text{-}12h,ss}$ values at steady state in the younger children (based on popPK analysis) were largely contained within the target exposure range of 800-1200 µg*h/mL, and similar to the exposure levels seen in the 5-25 years old subjects enrolled in the pivotal phase 3 study (see Figure 2). The interim PK results support the extrapolation of the efficacy to these younger ages (2-4 yrs).

Figure 2 Boxplot of Population Pharmacokinetic Model-Predicted $AUC_{0\text{-}12h,ss}$ Values in Studies 003 and 009 Subjects by Body Weight-Based Banded Dosing Regimen (Report MS-010)



Note: The dashed lines indicate the target exposure range (800 to 1200 µg•h/mL), and the middle-dashed line represents the median target exposure (1000 µg•h/mL). The bottom and top of each box represent the 25th and 75th percentiles, respectively; the whiskers represent the 25th/75th percentile+1.5 × IQR; and the line within the box represents the median. The circles represent the values above/below the 25th/75th percentile+1.5 × IQR. IQR=interquartile range.

Source: Report MS-010 Figure 8

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

The proposed dosing regimen for RTT patients in adults and pediatric patients 2 years of age and older is based on body weight as described in Table 1. Specifically, patients would receive a fixed dose according to the body weight band to achieve target exposure. The drug should be administered BID orally or via G-tube and given with or without food. The proposed weight-band based dosing has been studied in the pivotal Phase 3 study (Study 003) in female patients aged 5-25 years and Study 009 (for efficacy

extrapolation) in younger female patients 2 to 4 years of age, as well as the long-term extension studies (Study 004 and Study 005) to Study 003. The available efficacy and safety data (including E-R analyses) support the proposed dosing regimen.

We note that this dosing regimen is different from those used in phase 2 studies (Study RETT-001 and Study RETT-002), which were mg/kg based dosing. The change to weight-band based fixed dose in the Phase 3 study was supported by popPK and PK/PD analyses, primarily based on the phase 2 studies. Phase 2 study results identified only the 200 mg/kg BID dose (highest of the studied doses) manifested a positive clinical outcome, while higher exposure level indicated more improvement in the clinical measures but did not associate with greater safety risk. Therefore, the top 10% quantile of trofinetide exposure observed with the 200 mg/kg dose in Study RETT-002 was chosen as the target exposure (i.e., 790 µg/mL•h and above).

The popPK analyses also showed that subjects with lower body weight had lower exposure compared to the higher body weight subjects with mg/kg dosing. Hence, the dosing regimen was further optimized based on popPK analyses. The simulations indicated that the dosing regimen with different fixed dose for 4 individual weight bands (12-20 kg, 20-35 kg, 35-50kg, and >50 g) was optimal for the phase 3 study, because the target range of exposure could be reached in most subjects and the exposures are more consistent across different weight-bands. This dosing regimen also simplifies dosing implementation and reduces risk of dosing errors. The final pop-PK analysis of Study 003 and Study 009 (Report MS-10) also confirmed that the distribution of $AUC_{0\text{-}12h,ss}$ values were largely contained within the target exposure range (800-1200 µg/mL•h), and that the median $AUC_{0\text{-}12h,ss}$ fell within the target exposure range for all body weight bands following proposed dosing regimen(see Figure 2). For more details please refer to Pharmacometric Review in Appendix 4.4.1.2.

Overall, the proposed dosing regimen based on body weight bands is acceptable for the general RTT patient population.

3.3.3 Is the proposed dose adjustment and management strategy for patients with diarrhea appropriate?

Diarrhea was the most frequently reported adverse reaction recurring in the RTT patients during trofinetide treatment in the clinical trials. In the Phase 3 Study 003, 80% of the patients (75 out of 93 subjects) who received trofinetide treatment reported diarrhea. While the majority of those events (97%) were rated as mild or moderate, 12 subjects were discontinued due to diarrhea in this study. Approaches to manage diarrhea during the 12-week placebo-controlled study included the adjustment or discontinuation of laxative medications, initiating fiber supplements and antidiarrheal medication, e.g., loperamide (used in 51% of trofinetide-treated patients), and dose reduction or interruption of trofinetide if necessary.

The proposed dose modification and management strategy for diarrhea in the United States Prescription Information (USPI section) aligns with the clinical practice taken in the clinical trials. To support the rationale of dose reduction and/or interruption for diarrhea management, exposure-safety analysis with respect to the probability of diarrhea occurrence was conducted by Dr. Jie Liu (see Appendix 4.4

Pharmacometric Review). The results from the phase 2 study (Study Rett-002) showed that higher dose of trofinetide associated with a higher proportion of subjects with diarrhea (see Figure XXII in Appendix 4.4.1.3) and higher trofinetide exposure were predictive of an increase in the probability of occurrence of diarrhea (see Figure XXIII in Appendix 4.4.1.3). Dose reduction or interruption would be helpful to decrease the probability of diarrhea since the PK exposure would be reduced. The final labeling recommendation is provided below:

Advise patients to stop laxatives before starting DAYBUE. If diarrhea occurs, patients should start antidiarrheal treatment, increase oral fluids, and notify their healthcare provider. Interrupt, reduce dose, or discontinue DAYBUE if severe diarrhea occurs or if dehydration is suspected.

Overall, the proposed dose modification and management strategy for patients with diarrhea in the USPI is reasonable from a clinical pharmacology perspective.

3.3.4 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

The effects of various intrinsic factors on trofinetide exposure are described below.

Renal Impairment:

As trofinetide is primarily (80.6%) excreted unchanged in urine, and hence renal impairment is expected to affect trofinetide PK. (b) (4)

Of note, renal impairment is not a condition typically associated with the RTT population. However, considering a few RTT patients might reach the age of 40 years and above, it is not possible to completely rule out the possibility of renal impairment in the patient population. In the clinical trials in RTT patients, no subject was identified with moderate or severe renal impairment and very few subjects had mild renal impairment (i.e., estimated glomerular filtration rate, eGFR, between 60-89mL/min/1.73 m^2). By including all the PK data from the clinical trials conducted for trofinetide including healthy subjects and patients with other diseases, the range of eGFR in the popPK analysis population was 59.7 to 285 mL/min/1.73 m^2. The popPK analysis identified the eGFR as a statistically significant covariate affecting trofinetide's clearance. For subjects with the eGFR range of 59.7-99.4 mL/min/1.73 m^2 (covering the mild renal impairment range 60-89 mL/min/1.73 m^2), the geometric mean ratios (90% Confidence Intervals) of $C_{max,ss}$ and $AUC_{0-12h,ss}$, fell within the bioequivalence boundaries (0.8 to 1.25) when compared to the PK exposure of the reference group with normal renal function (eGFR ranging 117-126 mL/min/1.73 m^2). Thus, the popPK analysis supports no dose adjustment in the patients with mild renal impairment.

The impact of different degrees of renal impairment (mild, moderate, severe impairment, and ESRD) on trofinetide PK was also evaluated using PBPK modeling. The PBPK model predicted that renal impairment would result in a clinically meaningful increase in trofinetide AUC, and the extent of exposure increase was dependent upon the degree of renal impairment. However, the submitted PBPK model was considered inadequate as insufficient data was provided to verify the base model for this context of use (please refer to the PBPK modeling review by Dr. Ying-Hong Wang in Appendix 4.5).

In the currently proposed USPI, the use of trofinetide is not recommended in patients with moderate or severe renal impairment and no dose adjustment recommended for patients with mild renal impairment. A PMR will be issued for a clinical study to evaluate the effect of moderate renal impairment on the PK exposure of trofinetide after oral administration of trofinetide.

Hepatic impairment

No dedicated clinical hepatic impairment study was conducted for trofinetide, since trofinetide is metabolically stable in vitro and almost completely excreted unchanged in the urine. The measures (total bilirubin, alanine aminotransferase [ALT], and aspartate aminotransferase [AST]) indicative of hepatic function were found not to significantly affect trofinetide's PK, supporting the lack of clinical impact of hepatic impairment on trofinetide exposure.

No dose adjustment is recommended for patients with hepatic impairment. The PBPK modeling and simulation was submitted for the evaluation hepatic impairment. Because the contribution of hepatic clearance for trofinetide is not significantly (less than 20%), the PBPK analysis for hepatic impairment is not reviewed.

Age, Gender, Body Weight, eGFR, Disease status, and Diarrhea

The popPK model (Report MS-010) was established based on data from seven phase 1 studies (Studies HV-001, HV-002, HV-003, HV-004, HV-005, HV-006, and HV-007), four phase 2 studies, including subjects with RTT (Studies RETT-001 and RETT-002), FXS (Study FXS-001), and TBI (Study TBI-001/002), the phase 3 study in subjects with RTT (Study 003) and the PK study 009 in RTT patients aged 2-4 years. The key intrinsic factors evaluated as covariates in the popPK model included demographic characteristics (age, gender, body weight, body mass index), laboratory indices of renal (glomerular filtration rate [GFR]) and hepatic function (total bilirubin, ALT, and AST), and disease status (RTT, TBI, and FXS). In addition, the effect of diarrhea, the most frequently reported TEAE, on the PK of trofinetide was assessed.

The statistically significant intrinsic covariates included in the final PK model are body weight, age, GFR, and disease status. However, no clinically relevant impact on systemic exposure parameters (C_{max} and AUC) by these factors was identified and thus, no dose adjustment is needed beyond applying the proposed weight-band based dosing over the studied age range (2 years and above). For more details, please refer to Appendix 4.4 Pharmacometric review.

Although the evaluation of trofinetide for the treatment of RTT was only conducted in female patients, male subjects were enrolled in some of the phase 1 studies (healthy subjects) and clinical trials in subjects with other diseases. Based on popPK analyses, gender was found to not be a significant covariate on any trofinetide PK parameters. The absence of a gender effect on trofinetide PK supports the approval of trofinetide for the treatment of RTT in both female and male RTT patients.

Overall based on the evaluation on intrinsic factors, only renal impairment is considered as a significant factor to impact trofinetide's PK exposure. The use of trofinetide is not recommended in patients with

moderate or severe renal impairment but no dose adjustment is needed for patients with mild renal impairment.

3.3.5 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

Food Effect

A phase 1, open-label, single-dose, crossover study (Study ACP-2566-006) was conducted to evaluate the effect of food and timing of dosing (morning vs evening) on the PK of trofinetide administered orally in healthy subjects (N=36). The study had 3 dosing periods in which subjects received 12 g trofinetide according to the fed and fasted treatment conditions as follows:

- A: fasted in the morning (reference)
- B: in the morning following a high-fat meal
- C: fasted in the evening

Subjects were randomized to treatment conditions A and B for the first and second dosing periods while all subjects were to receive Treatment condition C in the third dosing period. Following a single 12 g dose of trofinetide under morning fasted, morning fed, and evening fasted conditions, the trofinetide PK profiles were similar under all treatment conditions. The AUC values were comparable across treatment conditions (Table 5). The C_{max} and AUC for the morning fasted and evening fasted conditions were comparable across treatment conditions, indicating no diurnal variation. Co-administration of trofinetide at a dose of 12 g with a standard high-fat meal (morning fed vs morning fasted) resulted in reducing the C_{max} approximately by 20% and a half of an hour delay in T_{max} compared to fasted state. Overall, the effects of food and timing of administration on the PK of trofinetide are minimal. This food effect study informed the dosing instruction of efficacy studies including the pivotal phase 3 Study 003, in which trofinetide was administered without regards to meals. The study also supports the dosing of trofinetide without regard to food, as recommended in the proposed label.

Table 5: Relative bioavailability comparisons between evening fasted vs morning fasted and between morning fed vs morning fasted

Parameter	Test (N)	Reference (N)	GMR (%) (test/reference)	Lower 90% CI	Upper 90% CI
Evening fasted vs morning fasted					
Cmax, µg/mL	35	38	99.75	95.09	104.64
AUC0-t, µg•h/mL	35	38	110.19	106.20	114.34
AUC0-inf, µg•h/mL	35	38	109.79	105.86	113.86
Morning fed vs morning fasted					
Cmax, µg/mL	40	38	79.02	75.42	82.80
AUC0-t, µg•h/mL	40	38	93.52	90.21	96.95
AUC0-inf, µg•h/mL	40	38	93.79	90.52	97.19

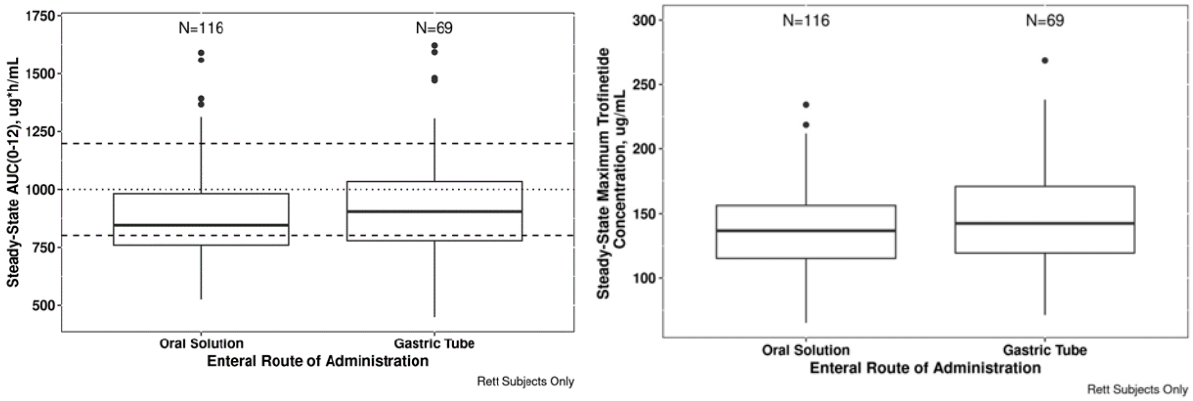
GMR=geometric mean ratio

Source: Study 006 CSR Table 11-5 and 11-4.

Gastrostomy Tube (G-Tube) Administration

The effect of G-tube administration on bioavailability was evaluated using PopPK analysis (Report MS-008) primarily based on the clinical trials in RTT patients (i.e., Studies RETT-001, RETT-002, and 003). In the analysis population, 116 subjects with RTT received oral solution, and 69 subjects were dosed via G-tube. The results indicated that mode of administration (oral or G-tube) is not a significant covariate in the final popPK model. The model- predicted median values of $AUC_{0\text{-}12h,ss}$ and $C_{max,ss}$ were similar between subjects dosed by G-tube compared to subjects dosed by oral solution, confirming that G-tube administration has no effect on trofinetide exposure (Figure 3).

Figure 3 Boxplot of Population Pharmacokinetic Model-Predicted $AUC_{0\text{-}12h,ss}$ and C_{max} Values in Subjects with RTT by Enteral Route of Administration (Report MS-008)



Note: The dashed lines indicate the target exposure range (800 to 1200 µg•h/Ml), and the dotted line represents the median target exposure (1000 µg•h/Ml). The bottom and top of the box represent the 25th and 75th percentiles, respectively; the whiskers represent the 25th/75th percentile+1.5 × IQR; and the line within the box represents the median. The circles represent the values above/below the 25th/75th percentile+1.5 × IQR.

Source: Report MS-008 Figures 24 and 25

Drug-drug Interactions (DDI)

Trofinetide as a substrate of metabolizing enzymes

The DDI potential of trofinetide was investigated by in vitro assays. Trofinetide was found to be stable in human whole blood (Study 10117-ADME) and plasma (Study 10096). Metabolic stability of trofinetide was further investigated in Study XT184115 with human liver microsomes and with a panel of recombinant human cytochrome P450 (rCYP) isozymes. Based on the results, the contribution of metabolism plays minor role in the elimination of trofinetide. These in vitro results also align with the findings in the human mass balance study which indicated the drug was primarily excreted via renal clearance and the contribution of hepatic clearance was minimal. Therefore, no DDI is expected with inducers or inhibitors of major metabolizing enzymes.

Trofinetide as a perpetrator of metabolizing enzymes

Trofinetide was evaluated using human liver microsomes in Study XT185143 to determine the potential for trofinetide as a direct, time- and metabolism-dependent inhibitor of CYP enzymes. Trofinetide was not a direct inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, and CYP3A4/5 based on the in vitro results.

Due to the high concentrations of trofinetide in the gut lumen following oral administration, the $R_{1,gut}$ calculated for CYP3A4/5 inhibition was 24.4 ($R_{1,gut}$≥11), indicating an inhibition signal ($R_{1,gut}$≥11). A PBPK model was used to predict the effect of trofinetide on the PK of midazolam (a sensitive substrate of CYP3A4) following intravenous and oral administrations. The predictions showed that concomitant administration of 12 g BID oral trofinetide with intravenously administered midazolam would not significantly change midazolam's exposure. However, trofinetide would increase the C_{max} and $AUC_{0\text{-}inf}$ of orally administered midazolam by 20% and 33%, respectively, indicating trofinetide is a weak inhibitor of intestinal CYP3A4 enzyme (see Appendix 4.5 PBPK review).

Therefore, safety monitor is recommended when trofinetide is used in combination with orally administered sensitive CYP3A4 substrates for which a small change in substrate plasma concentration may lead to serious toxicities. (b) (4) Loperamide is a substrate of both CYP3A and P-gp, and is a commonly used antidiarrheal, to treat the most frequently reported adverse event (AE) of trofinetide in subjects with RTT. (b) (4) the DDI potential associated with trofinetide on CYP3A4 inhibition might be updated in the future.

The time-/metabolism- dependent inhibition on CYP enzymes was also screened for trofinetide. The results showed that trofinetide only produced a signal of time-/metabolism- dependent inhibition on CYP2B6. However, the Applicant could not calculate the R_2, an index to determine the time-/metabolism-dependent inhibition potential per the FDA in vitro DDI guidance[6]. A PMR will be issued for in vitro study to evaluate the time-dependent inhibition of CYP 2B6 enzyme by trofinetide based on FDA in vitro DDI guidance.

The inhibition potential on the UGT enzymes by trofinetide was investigated in in vitro Study XT195103. The calculated R_1 values for UGT 1A9, 2B7 and 2B15 ranged from 1.04-1.10, above the index limit (R1>1.02) indicating an inhibition potential by trofinetide. However, there are no established substrates for UGT enzymes currently available for clinical DDI evaluations. Hence, clinical study to evaluate the effect of trofinetide on the PK of the substrates of UGT 1A9, 2B7 and 2B15 is not required.

Trofinetide DDI potential associated with transporters

The DDI potential for trofinetide as a substrate of transporters was evaluated in vitro Study XT188150. The test includes transporters: P-gp, BCRP, bile salt export pump (BSEP), OATP1B1, OATP1B3, OAT1, OCT2,

[6] 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

MATE1, and MATE2-K. Based on the results, trofinetide at the clinically relevant concentrations is unlikely a substrate of any of the tested transporters. For inhibition potential by trofinetide on transporters, trofinetide only showed a potential to inhibit OATP1B1 and OATP1B3 based on the calculated R values, 1.33 and 1.34 (R>1.1), respectively. Considering OATP1B1 and OATP1B3 are major transporters associated with hepatic clearance, further clinical DDI evaluation is recommended to evaluate trofinetide as an inhibitor of OATP1B1 and OATP1B3 transporters. For details of in vitro DDI studies, please refer to Appendix 4.3.

Overall, no DDI is expected with inducers or inhibitors of major metabolizing enzymes and transporters. Trofinetide is a weak inhibitor of CYP3A4. Co-administration of trofinetide with orally administered midazolam, a sensitive CYP3A4 substrate, was predicted to increase the AUC of midazolam by approximately 1.33-fold based on PBPK. Time-dependent inhibition on CYP 2B6 was inconclusive based on in vitro data. Trofinetide inhibits UGT enzymes (UGT1A9, 2B7, and 2B15) and OATP1B1 and OATP1B3 transporters in vitro. The DDI potential of trofinetide needs further clinical evaluation on trofinetide's inhibition on OATP1B1 and OATP1B3 transporters and in vitro investigation regarding the time-/metabolism-inhibition. Please refer to section 1.2 Post-Marketing Requirements and Commitments for DDI related PMRs and 2.4 Summary of Labeling for the recommendations in DDI.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

The bioanalytical methods for trofinetide quantification were developed and validated in human blood and urine. The use of whole blood in lieu of plasma for concentration was supported by the very low plasma protein binding (<0.6%) and a lack of distribution of trofinetide into red blood cells indicated by the consistent blood-to-plasma ratios ranging from 0.529 to 0.592 across the observed drug concentration range. The samples were prepared by solid phase extraction and analyzed via HPLC-MS/MS (API 3000). The methods (Inotiv method SAP.1480 for whole blood and Inotiv SAP.2136 for urine) met the acceptance criteria for bioanalytical methods according to the: Bioanalytical Method Validation Guidance for Industry, and were shown to be selective, accurate, and robust. Performance characteristics and validation attributes for the bioanalytical method are presented in Table 6.

Table 6 Bioanalytical method validation results for trofinetide in urine and whole blood

Parameter	Urine		blood
	Low Range Curve	High Range Curve	
Analyte	Trofinetide		
Internal Standard (IS)	NNZ-2566-IS ($^{13}C_5$, ^{15}N-trofinetide)	NNZ-2566-IS	NNZ-2566-IS
Lower Limit of Quantitation (ng/mL)	50ng/mL	50,000ng/mL	100 ng/mL
Average Recovery of Drug (%)	141.8%	71.4%	61.8%
Average Recovery of IS (%)	125.8%	68.1%	64.0%

Standard Curve Concentrations (ng/mL) and linearity R^2	50, 100, 500, 1000, 10000, 30000, 45000 and 50000 ng/mL R-Squared > 0.9949	50, 100, 500, 1000, 10000, 30000, 45000, 50000 µg/mL, R-Squared = >0.997	100, 200, 500, 2,000, 10000, 40,000, 80,000, and 100,000 ng/mL, R-Squared > 0.997
QC Intra-run Precision (%)	0.9% to 3.0%	1.1% to 9.4%	2.4% to 4.2%
QC Intra-run Accuracy (%)	-8.7% to 6.4%	-7.2% to 11.6%	–12.0% to 0.4%
QC Inter-run Precision (%)	2.2% to 7.0%	3.6% to 7.2%	3.3% to 10.1%
QC Inter-run Accuracy (%)	-2.8% to 2.0%	-1.6% to 8.6%	–0.1% to 4.0%
Dilution integrity	Not applicable	Dilution QC: 100 µg/mL (dilution factor: 20) Accuracy: -9.0% Precision: 8.1%	Dilution QC: 200 µg/mL (dilution factor: 5) Accuracy: 0.5% Precision: 2.7%
Short- and Long-Term Stability of trofinetide in Matrix (Days)	24 Hours at room temperature, 21 days at -20°C and -80°C	24 Hours at room temperature, 14 days at -20°C and -80°C	24 Hours at room temperature, 1752 days at -80°C
Extract Stability at room temperature	126 hours at 2-8 °C	229 hours at 2-8 °C	50 hours at 2-8 °C
Freeze-Thaw Stability	4 cycles; freeze at –80°C and/or -20°C and thaw at room temperature		
Selectivity	No interference in 6 lots of blank matrix		
Incurred Sample Reproducibility	Not applicable		100% samples showed difference within ± 20.0% of the mean.

Source: Bioanalytical method validation report (b) (4) 1000-091480-1 and 1000-192136-1

4.2 List of Clinical Studies in the Development Program

Table 7 Summary of Clinical Studies conducted in Development Program for Trofinetide

Study number and title,	**Test product, dosage, regimens, duration of treatment**	**Study population**
Healthy Subject - PK Profile and Initial Tolerability Studies following Oral and IV Administration		
Neu-2566-HV-005 (Study HV-005) A Phase I, Double-Blind, Randomized, Dose Escalation Study to Assess the Safety, Tolerability, and Pharmacokinetics of NNZ-2566 in Healthy Subjects following Oral Administration	Single oral dose of 6 or 30 mg/kg or placebo; oral dose 100 mg/kg BID or placebo BID for one day, multiple doses 100 mg/kg oral BID for 5 days.	Healthy adult subjects; 12 males and 12 females.
ACP-2566-007 (Study 007) A Phase 1, Open-Label, Single-Dose Study to Investigate the Pharmacokinetics (Absorption, Metabolism, and Excretion) of [^{14}C]ACP-2566 Following Oral Administration to Healthy Male subjects	Trofinetide single oral dose of 12 g (as ready-to-use [RTU] solution) containing radiolabeled [^{14}C]-trofinetide (76.05 µCi).	Healthy adult subjects; 8 males.

Neu-2566-HV-001 (Study HV-001) A Phase 1, Single Dose, Double-Blind, Randomized, Dose Escalation Study to Assess the Safety, Tolerability and Pharmacokinetics of NNZ-2566 When Administered as a 10 Minute Infusion	Single-dose, 10-minute IV infusion of trofinetide 0.1, 1.0, 10.0, or 20.0 mg/kg or placebo.	Healthy subjects. 28 males.
Neu-2566-HV-002 (Study HV-002) A Phase I, Single Dose, Double-Blind, Randomized, Dose Escalation Study to Assess the Safety, Tolerability and Pharmacokinetics of NNZ-2566 When Administered as a 12-hour, 24-hour and 72-hour Infusion	Single-dose, IV infusion of trofinetide 1 mg/kg/h over 12 hours or placebo over 12 hours.	Healthy adult subjects; 7 males.
Neu-2566-HV-003 (Study HV-003) A Phase I, Double-Blind, Randomized, Dose Escalation Study to Assess the Safety, Tolerability and Pharmacokinetics of NNZ-2566 When Administered as a Loading Dose (10- minute Infusion) Immediately Followed by a Maintenance Dose (up to 72-hour Infusion)	Single-dose, IV infusion of trofinetide 20.0 mg/kg for 10 minutes, followed by either a 1 mg/kg/h infusion over 12 hours or 3 mg/kg/h over 24 or 48 hours, or 6 mg/kg/h over 72 hours.	Healthy adult subjects; 28 males.
Neu-2566-HV-004 (Study HV-004) A Phase I, Double-Blind, Randomized, Dose Escalation Study to Assess the Safety, Tolerability, and Pharmacokinetics of NNZ-2566 in Healthy Female Subjects, When Administered as a Loading Dose (10-minute Infusion), and as a Loading Dose Followed by a Maintenance Dose (72-Hour Infusion)	Single-dose, IV infusion of trofinetide 6 mg/kg over 10 minutes; trofinetide 20 mg/kg over 10 minutes; or trofinetide 20 mg/kg over 10 minutes, followed by 1, 3, or 6 mg/kg/h infusion over 72 hours.	Healthy adult subjects; 40 females.
Healthy Subject - Bioavailability and Food Effect Study		
ACP-2566-006 (Study 006) A Phase 1, Open-Label, Single-Dose Study to Evaluate the Effects of Food and Evening Dosing on the Pharmacokinetics of Trofinetide Administered Orally in Healthy Adult Subjects	Single oral dose of 12 g as RTU oral solution administered either in the morning either fed (high-fat meal) or fasted (approximately 10 hours) or in the evening and fasted approximately 6 hours).	Healthy adult subjects; 28 males and 13 females.
Healthy Subject - PD and PK/PD Study Reports (TQT Study)		
ACP-2566-008 (Study 008) A Phase 1, Ascending Dose Study to Assess the Effects on QTc Interval, Safety and Tolerability, and Pharmacokinetics of Orally Administered Trofinetide in Healthy Adult Subjects	Trofinetide single oral doses of 12, 18, and 24 g, as RTU oral solution, or placebo. Moxifloxacin single oral dose of 400 mg or placebo.	Healthy adult subjects; 24 males and 16 females.
Patients with RTT - PK and Initial Tolerability Study Report		
Neu-2566-Rett-001 (Study RETT-001) A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group, Dose-Escalation Study of NNZ-2566 in Rett syndrome	Trofinetide oral doses as follows: Day 1 10 mg/kg QD, Day 2 20 mg/kg QD, Days 3-14 35 mg/kg BID; or Day 1 10 mg/kg QD, Day 2 20 mg/kg QD, Days 3 through 26 35 mg/kg BID, Day 27 20 mg/kg QD, Day 28 10 mg/kg QD; or Day 1 17 mg/kg QD, Day 2 35 mg/kg QD, Days 3-26 70 mg/kg BID, Day 27 35 mg/kg QD, Day 28 17 mg/kg QD.	Adolescent and adult subjects with RTT; 56 females aged 16-45 years

Patients with RTT - Controlled Clinical Studies Pertinent to the Clinical Indication		
ACP-2566-003 (Study 003) A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study of Trofinetide for the Treatment of Girls and Women With Rett Syndrome	Trofinetide RTU oral solution or G-tube dosing based on the subject's body weight at Baseline as follows: 12-20 kg: 30 mL (6 g) BID >20 through35 kg: 40 mL (8 g) BID >35 through 50 kg: 50 mL (10 g) BID >50 kg: 60 mL (12 g) BID Placebo	Pediatric, adolescent, and adult subjects with RTT; 187 females 5-20 years of age
Neu-2566-RETT-002 (Study RETT-002) A Randomized, Double-Blind, Placebo-Controlled, Dose-Ranging Study of the Safety and Pharmacokinetics of Oral NNZ-2566 in Pediatric Rett Syndrome	Oral placebo for 14 days followed by titration to trofinetide oral doses of 50, 100, or 200 mg/kg BID or placebo BID for 42 days.	Pediatric and adolescent subjects with RTT; 82 females 5-15 years of age.
Patients with RTT --Open label extension studies to the pivotal study		
ACP-2566-004 (Study 004) ongoing A 40-Week, Open-label Extension Study of Trofinetide for the Treatment of Girls and Women With Rett Syndrome (extension of Study 003)	Trofinetide RTU oral solution dosing based on the subject's weight at Baseline as follows: 12 through20 kg: 30 mL (6 g) BID >20 through 35 kg: 40 mL (8 g) BID >35 through 50 kg: 50 mL (10 g) BID >50 kg: 60 mL (12 g) BID	Pediatric, adolescent, and adult subjects with RTT; 180 females expected.
ACP-2566-005 (Study 005) ongoing A 32-months, Open-label Extension Study of Trofinetide for the Treatment of Girls and Women With Rett Syndrome (extension of Study 004)	trofinetide RTU oral solution dosing based on the subject's weight at Baseline as follows: 12 through20 kg: 30 mL (6 g) BID >20 through 35 kg: 40 mL (8 g) BID >35 through 50 kg: 50 mL (10 g) BID >50 kg: 60 mL (12 g) BID	Pediatric, adolescent, and adult subjects with RTT; 153 females expected.
Younger children with RTT (2-5 yrs)		
ACP-2566-009 (Study 009) ongoing An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age Who Have Rett Syndrome	Trofinetide RTU oral solution as follows: Day 1 (all subjects): 10 mL (2 g) BID Week 2 (all subjects): 20 mL (4 g) BID Week 4 (≥9 to 12 kg): 25 mL (5 g) BID Week 4 (12 to 20 kg): 30 mL (6 g) BID	Subjects 2 to 4 years with RTT; 10 to 15 females
Patients with other diseases		
Neu-2566-FXS-001 (Study FXS-001) A Randomized, Double-Blind, Placebo- Controlled, Parallel-Group, Fixed-Dose Study of NNZ-2566 in Fragile X Syndrome	Oral placebo for 14 days followed by trofinetide oral doses of 35 or 70 mg/kg BID or placebo BID for 28 days.	Adolescent and adult subjects with FXS; 70 males.
Neu-2566-TBI-001/002 (Study TBI-001/002) A Randomized, Double-Blind, Placebo-Controlled, Dose-Escalation Study of NNZ-2566 in Patients With Traumatic Brain Injury (TBI) With and Without Informed Consent	Trofinetide 10-minute 20 mg/kg IV bolus infusion followed by 1, 3, or 6 mg/kg/h IV infusion over 72 hours or placebo.	Adolescent and adult subjects with TBI; 221 males and 30 females.

BID: twice daily; QD, once daily, RTU, ready-to-use.
Source: 2.7.2 Summary of Clinical Pharmacology Studies

4.3 In Vitro DDI Studies

Study 10117-ADME, Study 10096, Study XT184115 for metabolizing stability and phenotyping

In vitro testing for the DDI potential of trofinetide was conducted by incubating phenotyping experiments. Trofinetide was found to be stable in human liver microsomes and plasma with loss of less than half of the parent compound at the end of the 60- (blood) or 30-minute (plasma) incubation at 37°C (Study 10117-ADME and Study 10096). There were no metabolites unique to humans that were not detected in nonclinical toxicity species. In reaction phenotyping experiments (Study XT184115), incubation of trofinetide (0.1 and 1 µM) with recombinant human CYP enzymes (rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6, and rCYP3A4) resulted in ≤27% substrate loss. The highest amount of substrate loss was noted at 0.1 µM trofinetide with rCYP2C8 (27%) and rCYP2D6 (24.5%). Metabolic stability of trofinetide was tested at 0.1 to 10 µM in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) with human liver microsomes and with a panel of recombinant human cytochrome P450 (rCYP) isozymes (Study XT184115). In all instances, turnover was low (<50%), suggesting that metabolism by cytochrome P450 (CYP) enzymes plays little or no role in the elimination of trofinetide. Similar results were obtained in an earlier, preliminary study (Study 10096).

Study XT185143 (inhibition effect on CYP enzymes)

The capacity of trofinetide to inhibit human CYP enzymes, either directly or in a time- and metabolism-dependent manner, was studied in human liver microsomes at concentrations up to 25 mM (50× unbound human therapeutic C_{max}) for all CYPs except up to 15 mM CYP3A4/5 (1/10 estimated local intestinal concentration at the clinical dose level; Study XT185143). Direct inhibition was observed for CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation) with half maximal inhibitory concentration (IC50) values of 10, 18, 4.8, 10, 12, and 13 mM, respectively**.** The inhibition index (R1) values are calculated to be more than 1.02 for most tested enzymes indicating DDI potentials. The static mechanistic models were used to estimate the area under the concentration-time curve ratio (AUCR) of a sensitive index substrate in the presence and absence of trofinetide. The estimated AUCR values were less than the limit of 1.25 indicating minimal DDI risk for abovementioned enzymes, except for CYP2C8 where AUCR was 1.28, only slightly higher the borderline. Considering the AUCR is a conservative approach and trofinetide's elimination is relatively rapid (with t1/2 less than 3 hours), the clinical DDI risk associated with CYP2C8 inhibition is not expected.

Trofinetide functioned as a time/metabolism-dependent inhibitor for only CYP2B6, for which the IC50 was reduced to 5 mM (~9-fold higher than the therapeutic Cmax) by a 30-minute preincubation with the microsomes in the presence of NADPH. However, the time-dependent inhibition by trofinetide on CYP2B6 was inclusion as R2 could not be calculated. Further in vitro investigation on time-dependent inhibition on CYP2B6 will be needed in the PMR setting to characterize the time-dependent inhibition potential by trofinetide on CYP2B6.

Table 8 CYP Inhibition Calculations

Enzyme	Direct inhibition, Zero-min preincubation			Metabolism-dependent inhibition, 30-min preincubation with NADPH					
	IC_{50} (mM)	Maximum inhibition (%)	K_i (mM)	IC_{50} (mM)	Maximum inhibition (%)	K_i (mM)	R_1	R_2	$R_{1,gut}$
CYP1A2	10	81	5.0	--	--	--	1.10	--	--
CYP2B6	18	67	9.0	5.0	100	2.5	1.05	1.19	--
CYP2C8	4.8	100	2.4	--	--	--	1.20	--	--
CYP2C9	> 25.0	46	--	--	--	--	--	--	--
CYP2C19	10	82	5.0	--	--	--	1.10	--	--
CYP2D6	12	79	6.0	--	--	--	1.08	--	--
CYP3A4/5[a]	> 15.0	41	--	--	--	--	--	--	--
CYP3A4/5[b]	13	57	6.5	--	--	--	1.07	--	24.4

Abbreviations: CYP=cytochrome P450; IC_{50}=concentration of drug producing 50% inhibition; K_i=inhibition constant; TDI=time-dependent manner

Notes:

R_1 was calculated using the equation in Figure 1 of the FDA guidance document (FDA 2020). Because K_{inact} was not available for the TDI result, the same equation was used for CYP2B6 TDI.

The gut concentration of trofinetide for the CYP3A4/5 and P-gp and BCRP calculations was set to (12,000 mg)/(315.3 mg/mmol)/(0.250 L) = 152.2 mM.

[a] Midazolam as substrate

[b] Testosterone as substrate

Source: \\CDSESUB1\EVSPROD\nda217026\0028\m1\us\response-to-rfi-regarding-clinical-pharmacology-ddi.pdf Table 2

Table 9 Calculation of AUCR for CYP Enzymes

Enzyme	K_i (mM)	[I]h	[I]g[a]	A_h	A_g	AUCR
CYP1A2	5	0.674142713		0.881190385	1	**1.13**
CYP2B6	9	0.674142713		0.930314992	1	**1.07**
CYP2C8	2.4	0.674142713		0.78070546	1	**1.28**
CYP2C19	5	0.674142713		0.881190385	1	**1.13**
CYP2D6	6	0.674142713		0.898991864	1	**1.11**
CYP3A4/5	6.5	0.674142713	0.679984	0.9060316	0.905294496	**1.22**

Source: Appendix 1

Abbreviations: A_g=effect of reversible inhibitions, gut; A_h=effect of reversible inhibitions, liver; AUCR= area under the concentration-time curve ratio; [I]g=concentration of the interacting drug, gut; [I]h=concentration of the interacting drug, liver; K_i=inhibition constant

[a] [I]g only applies for CYP3A4

Source: \\CDSESUB1\EVSPROD\nda217026\0028\m1\us\response-to-rfi-regarding-clinical-pharmacology-ddi.pdf Table 3

Table 10 Table UGT inhibition calculations

Enzyme	Direct inhibition		R_1 values
	IC_{50} (mM)	K_i (mM)	
UGT1A1	> 25	--	--
UGT1A3	> 25	--	--
UGT1A4	> 25	--	--
UGT1A6	> 25	--	--
UGT1A9	12	6	1.08
UGT2B7	10.0	5	1.10
UGT2B15	23	11.5	1.04
UGT2B17	> 25	--	--

Abbreviations: IC_{50}=concentration of drug producing 50% inhibition; K_i=inhibition constant; UGT=uridine diphosphate glucuronosyltransferase
Note: R_1 was calculated using the equation in Figure 1 of the FDA guidance document (FDA 2020).

Source: \\CDSESUB1\EVSPROD\nda217026\0028\m1\us\response-to-rfi-regarding-clinical-pharmacology-ddi.pdf Table 5

Study XT183140 for trofinetide's induction potential on CYP enzymes

The potential for trofinetide to induce human CYP enzymes was tested at concentrations up to 15 mM in cryopreserved primary human hepatocytes. The hepatocytes were incubated with trofinetide for 3 days and then harvested for analysis of CYP1A2, CYP2B6, and CYP3A4 mRNA as representatives of the groups of enzymes regulated by the aryl hydrocarbon receptor (AhR), the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR). Evidence of induction was observed (i.e., >2-fold increase in mRNA relative to solvent control), at one or both of the highest concentrations tested (i.e., 5 and/or 15 mM), which are 10- and 29-fold higher, respectively, than the Cmax obtained at the clinical dose in Study 003. Based on the results, the induction potential should be ruled out for trofinetide.

Study XT195103 for trofinetide's inhibition potential on UGT enzymes

The capacity of trofinetide to inhibit UGT enzymes was tested at concentrations up to 25 mM. Trofinetide was a direct inhibitor of UGT1A9, UGT2B7, and UGT2B15, with IC50 values of 12, 10, and 23 mM, respectively. For UGT1A1, UGT1A3, UGT1A4, UGT1A6, or UGT2B17, less than 50% inhibition was observed for UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT2B17, respectively. Therefore, the IC50 values for them were reported as > 25 mM and corresponding R1 values should be less than 1.02, indicating minimal inhibition potential for UGT1A1, UGT1A3, UGT1A4, UGT1A6 and UGT2B17. Trofinetide showed inhibition potential on UGT 1A9, 2B7 and 2B15 based on the R1 values above 1.02.

Study XT188150 for trofinetide potential as a substrate or inhibitor of transporters

The capacity of trofinetide to act as a substrate or inhibitor of drug transporters was tested using stably transfected cellular models for organic ion transporter (OAT)1, OAT3, P-glycoprotein (P-gp), BCRP, bile salt export pump (BSEP), organic anion transport polypeptide (OATP)1B1, OATP1B3, organic cation transporter (OCT)2, MATE1, MATE 2 and MATE2-K. Tropfinetide did not appear to be a substrate for any other transporters based on the results except for OATP1B1. (Table 9). However, the inhibition potential on OATP1B1 was only observed at an extremely low concentration (5 µM) of trofinetide and not at the higher concentration of 10 µM. Considering the clinically relevant concentration of trofinetide is about 50 fold higher than µM, the contribution of the transporter in vivo would appear likely to be negligible.

For transporter inhibition, trofinetide was tested at concentrations up to 25 mM and was shown to inhibit P-gp, BCRP, bile salt export pump, OATP1B1, OATP1B3, OAT1, OCT2, MATE1, and MATE2-K with IC50 values of 17.2, 13.5, 8.97, 11.7, 11.5, 10.9, 3.64, 3.68, and 0.951 mM, respectively. For P-gp and BCRP, the Igut /IC50 are less than 10 which indicating low potential for inhibition on intestinal P-gp and BCRP transporters. The potential for trofinetide to inhibit three of the renal transporters (OCT2, MATE1, and MATE2-K) was indicted by Imax,u/IC50 values range between 0.131 and 0.505. Of note, creatinine is a substrate for OCT2, MATE1, MATE2-K, and OAT2, and thus that their inhibition can result in elevation of serum creatinine levels. Such elevations have not been observed in the pivotal clinical study (#003) indicating the in vitro inhibition on these transporters is not clinically relevant.

4.4 Pharmacometric Review

4.4.1 Applicant's Analysis

4.4.1.1 Population PK Analysis

A total of 5595 records in 442 subjects from 13 clinical studies were included in the final population PK (final PopPK) model. Data from Study ACP-2566-009 were pooled with the existing studies, and the model was re-estimated (updated popPK model). The inclusion of Study ACP-2566-009 data brought the total count to 5709 records in 455 subjects (Table I). A nonlinear mixed effects modeling approach with the first-order conditional estimation with interaction (FOCEI) method in NONMEM, version 7.3 (ICON, Maryland) was used for the PopPK analysis.

The body weights of the PopPK modeling population ranged from 9.8 kg to 140 kg (median:61.4 kg) and ages ranged from 2 to 64 years (median: 21 years). The median (range) glomerular filtration rate of the subjects was 123 (59.7 to 285) mL/min/1.73 m^2.

Table I: Summary of studies included in the population PK analysis

Protocol	Title	Subject Population	No. of Subjects	Dose
Neu-2566-HV-001	Phase I, single dose, double-blind, randomized, dose escalation study	Healthy male subjects (19-34 yrs)	20 (Trofinetide) 8 (Placebo)	10 mins IV infusion of 0.1, 1.0, 10, 20 mg/kg
Neu-2566-HV-002	Phase I, single dose, double-blind, randomized, dose escalation study	Healthy male subjects (19-27 yrs)	5 (Trofinetide) 2 (Placebo)	1.0 mg/kg/h IV infusion over 12 hours
Neu-2566-HV-003	Phase I, double-blind, randomized, dose escalation study	Healthy male subjects (18-36 yrs)	29 (Trofinetide) 21 (PK data available)	20 mg/kg IV infusion (10 min) followed by 1 mg/kg/h (12 hrs), 3 mg/kg/h (24 or 48 hrs), 6 mg/kg/h (72 hrs)
Neu-2566-HV-004	Phase I, double-blind, dose escalation study	Healthy female subjects (19-37 yrs)	42 (Trofinetide) 28 (PK data available)	10-min IV infusion (6 or 20 mg/kg) or a 10-min infusion (20 mg/kg) followed by a 72-hr infusion (1, 3, or 7

				mg/kg/h)
Neu-2566-HV-005	Phase I, double-blind, randomized, dose escalation study	Healthy subjects (12 male, 12 female,19-38 ys)	24 (Trofinetide) 18 (PK data available)	Single 6- or 30-mg/kg oral dose, or 100 mg/kg BID for 1 day, followed by a 4-day washout, then 100 mg/kg BID for 5 more days
ACP-2566-006	Phase 1, open-label, single dose study	Healthy Male or female adults (19-45 yrs)	36 (Trofinetide)	Single 12 g oral dose
ACP-2566-007	Phase 1, open-label, single dose study	Healthy males (28-38 yrs)	8 (Radiolabeled [^{14}C]-ACP-2566)	Single 12 g oral dose of [^{14}C]-ACP-2566)
Neu-2566-Rett-001	Phase 2, Randomized, double-blind, placebo-controlled, parallel group, dose-escalation study	Adolescent or adult females with Rett syndrome (15-44 yrs)	36 (Trofinetide) 20 (Placebo) 36 (PK data available)	35 mg/kg or 70 mg/kg after 3 or 5 days of titration
Neu-2566-Rett-002	Phase 2, Randomized, double-blind, placebo-controlled, dose-ranging study	Pediatric and adolescent females with Rett syndrome (5-15 yrs)	58 (Trofinetide) 24 (Placebo) 57 (PK data available)	Oral dose of 50, 100, or 200 mg/kg BID for 42 days
Neu-2566-TBI-001/002	Phase 2 randomized, double-blind, placebo-controlled, dose escalation study	Adult males with traumatic brain injury (TBI) (16-72 yrs)	167 (Trofinetide) 84 (Placebo) 58 (PK data available)	20 mg/kg IV 10 min infusion followed by a continuous IV infusion of 1, 3, or 6 mg/kg/h for 72 hours
Neu-2566-FXS-001	Phase 2 randomized, double-blind, placebo-controlled, parallel group, fixed-dose study	Adolescent or adult males with Fragile X syndrome (12-41 yrs)	47 (Trofinetide) 25 (Placebo) 45 (PK data available)	Placebo BID for 14 days, followed by 35 or 70 mg/kg oral dose BID for 28 days
ACP-2566-003	Phase 3 randomized, double-blind, placebo-controlled, parallel-group study	Female subjects with Rett syndrome (5-20 yrs)	93 (Trofinetide) 94 (Placebo) 92 (PK data available)	Body weight-banded doses (6, 8, 10, or 12 grams BID) of trofinetide or placebo for 12 weeks
ACP-2566-009	Phae 2/3 open-label study	Female subjects with Rett syndrome (2-4 yrs)	14 (Trofinetide) 13 (PK data available)	Begin with 2 g BID treatment, followed by dose escalation based on weight band

Source: Adapted from Applicant's PopPK report ACP-2566-MS-010, Table 1 and 2, Page 20 and 21 and 5.2 Tabular Listing of All Clinical Studies

Listings of the baseline demographics and laboratory variables for these subjects are given in Table II.

Table II: Summary Statistics of Subject Demographic Characteristics and Laboratory Values, by Study, for the Trofinetide Population PK Analysis Population

Variable		Study Neu-2566-HV-					Study ACP-2566-			Study Neu-2566-Rett-		Study ACP-2566-		Study Neu-2566-FXS-	Study Neu-2566-TBI-	Overall (n = 455)
		001 (n = 20)	002 (n = 5)	003 (n = 21)	004 (n = 29)	005 (n = 12)	006 (n = 41)	007 (n = 8)	008 (n = 20)	001 (n = 35)	002 (n = 58)	003 (n = 92)	009 (n = 13)	001 (n = 44)	001 (n = 57)	
Age	Mean	22.1	24	24	23.4	24.2	32.9	31.9	33.9	24.1	9.88	11	3	25.2	35.6	21.8
	(SD)	(2.83)	(3.08)	(5.12)	(3.05)	(4.67)	(7.05)	(3.44)	(5.21)	(6.17)	(3.51)	(4.71)	(0.816)	(8.74)	(14.9)	(12.2)
	Median	21	24	22	23	23	32	31.5	33	21.8	9.41	10	3	24.7	30	21
	Min, Max	19, 28	19, 27	18.8, 36.	519, 30	19, 38	19, 45	28, 38	27, 44	15.9, 40.	85.21, 15.	95, 20	2, 4	13, 40.9	17, 64	2, 64
ALT (U/L)	Mean	21.2	23.6	22.2	14.3	14.2	23.3	28.9	17.8	21	17.9	20.7	16.8	25	60.3	25.4
	(SD)	(8.26)	(11.9)	(13.4)	(5.91)	(4.56)	(15.6)	(12.9)	(12)	(12.4)	(7.08)	(9.65)	(4.72)	(28.2)	(75.9)	(32.4)
	Median	20.5	17	17	13	14	19	23.5	13.5	18	16	19	15	17	40	18
	Min, Max	8, 49	13, 42	7, 56	5, 28	8, 22	6, 77	16, 48	8, 56	7, 53	6, 38	7, 69	13, 29	6, 165	9, 548	5, 548
AST (U/L)	Mean	26	20.2	21.6	19.1	17.5	21.5	23.5	18.9	20.2	24.7	23.3	29.8	17.7	90.6	30.6
	(SD)	(15.7)	(2.77)	(4.99)	(5.74)	(3.45)	(6.85)	(6.02)	(9.28)	(9.53)	(7.43)	(6.57)	(5.68)	(10.4)	(149)	(57.7)
	Median	23	19	20	17	18	20	23	16.5	18	23	22	31	15.5	57	22
	Min, Max	17, 90	17, 24	15, 31	13, 33	12, 23	11, 44	16, 34	12, 55	10, 46	13, 64	12, 50	19, 37	10, 78	17, 1130	10, 1130
Body Mass Index (kg/m^2)	Mean	22.8	22.6	23.4	23.1	24.6	25.4	26.7	27.8	22	16.7	17.9	14.6	27	26.6	22.2
	(SD)	(2.6)	(2.72)	(2.88)	(2.65)	(2.04)	(2.86)	(2.27)	(3.78)	(7.11)	(3.96)	(4.37)	(1.68)	(5.86)	(4.63)	(5.93)
	Median	22.4	24	23.4	22.3	25	25.8	27	27.3	19.1	15.8	17	13.9	26.8	26.3	22.1
	Min,	18.3,	19.3,	18.4,	20,	20.7,	20.5,	23,	20.9,	14.6,	11.2,	10.6,	11.9,	15.6,	18.7,	10.6,
	Max	28.4	25.4	30.4	29.4	28.1	29.9	29.6	38.3	46.1	31	34.8	17.2	43.3	40.7	46.1
Glomerular Filtration Rate	Mean	101	111	114	119	123	108	105	107	137	129	162	150	116	97.3	125
	(SD)	(15.7)	(23)	(15.2)	(7.52)	(5.76)	(15.2)	(7.43)	(17.2)	(14)	(27.5)	(36.9)	(26.9)	(19.5)	(23.1)	(32.5)
	Median	102	120	118	121	123	109	101	112	138	130	154	138	116	94.1	123
	Min,	59.7,	81.5,	85.9,	103,	110,	76.4,	97.1,	75.9,	113,	74.5,	98.6,	116,	64.6,	60.7,	59.7,
	Max	125	135	134	130	131	140	116	138	176	213	285	186	169	143	285
Bilirubin, Total (mg/dL)	Mean	0.722	0.807	0.805	0.498	0.512	0.595	0.688	0.556	0.331	0.29	0.298	0.238	0.677	0.604	0.484
	(SD)	(0.431)	(0.42)	(0.615)	(0.256)	(0.253)	(0.265)	(0.196)	(0.223)	(0.111)	(0.212)	(0.141)	(0.087)	(0.431)	(0.395)	(0.345)
	Median	0.614	0.877	0.643	0.468	0.526	0.5	0.65	0.525	0.3	0.2	0.26	0.2	0.6	0.5	0.4
	Min,	0.351,	0.292,	0.292,	0.175,	0.234,	0.2,	0.5,	0.22,	0.2,	0.1,	0.11,	0.2,	0.2,	0.17,	0.1,
	Max	2.22	1.29	3.16	1.4	1.17	1.2	1.1	0.99	0.6	1	0.88	0.5	3	2.7	3.16
Body Weight (kg)	Mean	73.8	70.1	75.6	63.4	70.9	74.1	81.1	80.6	41	26.9	30.5	13.4	81.6	83.2	56.5
	(SD)	(10.3)	(8.92)	(8.63)	(9.66)	(9.21)	(10.4)	(6.73)	(11.5)	(12.2)	(11.3)	(12.7)	(2.27)	(21.2)	(14.9)	(27.1)
	Median	74.5	69.5	75	61.6	71.2	73.7	78.2	82.8	37.7	22.5	29.4	13.6	78.4	82	61.4
	Min,	59,	62.3,	62,	52.1,	54.6,	56.1,	72.6,	61.2,	23.3,	15.1,	13.3,	9.8,	37.4,	56,	9.8,
	Max	95	84.2	95	89	83.7	98.5	90.8	98	79	62.1	78.2	18.1	140	129	140
Disease State, N (%)	Healthy subject	20 (100)	5 (100)	21 (100)	29 (100)	12 (100)	41 (100)	8 (100)	20 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	156 (34.3)
	Rett syndrome	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	35 (100)	58 (100)	92 (100)	13 (100)	0 (0)	0 (0)	198 (43.5)
	Fragile X syndrome	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	44 (100)	0 (0)	44 (9.67)
	Traumatic brain injury	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	57 (100)	57 (12.5)
Route of Administration, N (%)	IV	20 (100)	5 (100)	21 (100)	29 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	57 (100)	132 (29)
	Oral	0 (0)	0 (0)	0 (0)	0 (0)	12 (100)	41 (100)	8 (100)	20 (100)	22 (62.9)	40 (69)	54 (58.7)	9 (69.2)	44 (100)	0 (0)	250 (54.9)
	Gastric tube	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	13 (37.1)	18 (31)	38 (41.3)	4 (30.8)	0 (0)	0 (0)	73 (16)
Fed/Fast Status, N (%)	Fed	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	22 (53.7)	8 (100)	0 (0)	0 (0)	0 (0)	92 (100)	0 (0)	0 (0)	0 (0)	122 (26.8)
	Fasted	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	19 (46.3)	0 (0)	20 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	39 (8.57)
	Missing	20 (100)	5 (100)	21 (100)	29 (100)	12 (100)	0 (0)	0 (0)	0 (0)	35 (100)	58 (100)	0 (0)	13 (100)	44 (100)	57 (100)	294 (64.6)
Sex, N (%)	Male	20 (100)	5 (100)	21 (100)	0 (0)	6 (50)	28 (68.3)	8 (100)	12 (60)	0 (0)	0 (0)	0 (0)	0 (0)	44 (100)	52 (91.2)	196 (43.1)
	Female	0 (0)	0 (0)	0 (0)	29 (100)	6 (50)	13 (31.7)	0 (0)	8 (40)	35 (100)	58 (100)	92 (100)	13 (100)	0 (0)	5 (8.77)	259 (56.9)

Source: Applicant's PopPK report, Page 42, Table 4 and Applicant's PopPK report ACP-2566-MS-010, Page 48-49, Table 4

Population PK Model

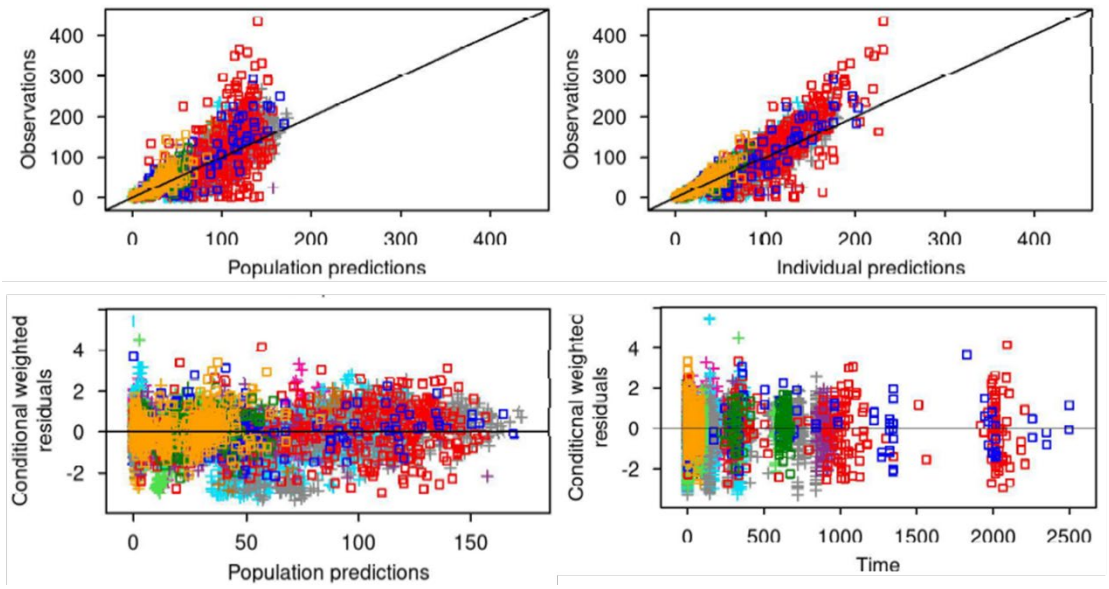
To fully characterize the PK of trofinetide, guide dose selection, and confirm the appropriateness of the proposed dosing regimens, the applicant developed a preliminary PopPK model initially. The applicant added completed studies on an ongoing basis and refined at different stages through the project development.

The trofinetide PK was described by 2-compartment PK model with first-order absorption and linear elimination, and 2 separate exponential error models for healthy subjects and subjects with RTT. Effects of fed status, diarrhea occurrence, and supratherapeutic doses (18- and 24-g doses) on oral bioavailability (F1) and first-order absorption rate constant (ka) on fed status were also included in the final model.

Following the covariate analysis, effects from body weight, GFR, and both Rett and TBI disease status on clearance (CL) were included, as well as age and FXS disease status effects on the central volume of distribution (Vc), and both Rett and TBI disease status effects on the peripheral volume of distribution (Vp). Interindividual variability was estimated for first-order absorption rate constant (ka), CL, Vc, Vp, and intercompartmental clearance using exponential error models. The final PK model also included a proportional shift in CL in subjects with TBI and a proportional shift in Vc for both subjects with RTT and subjects with FXS.

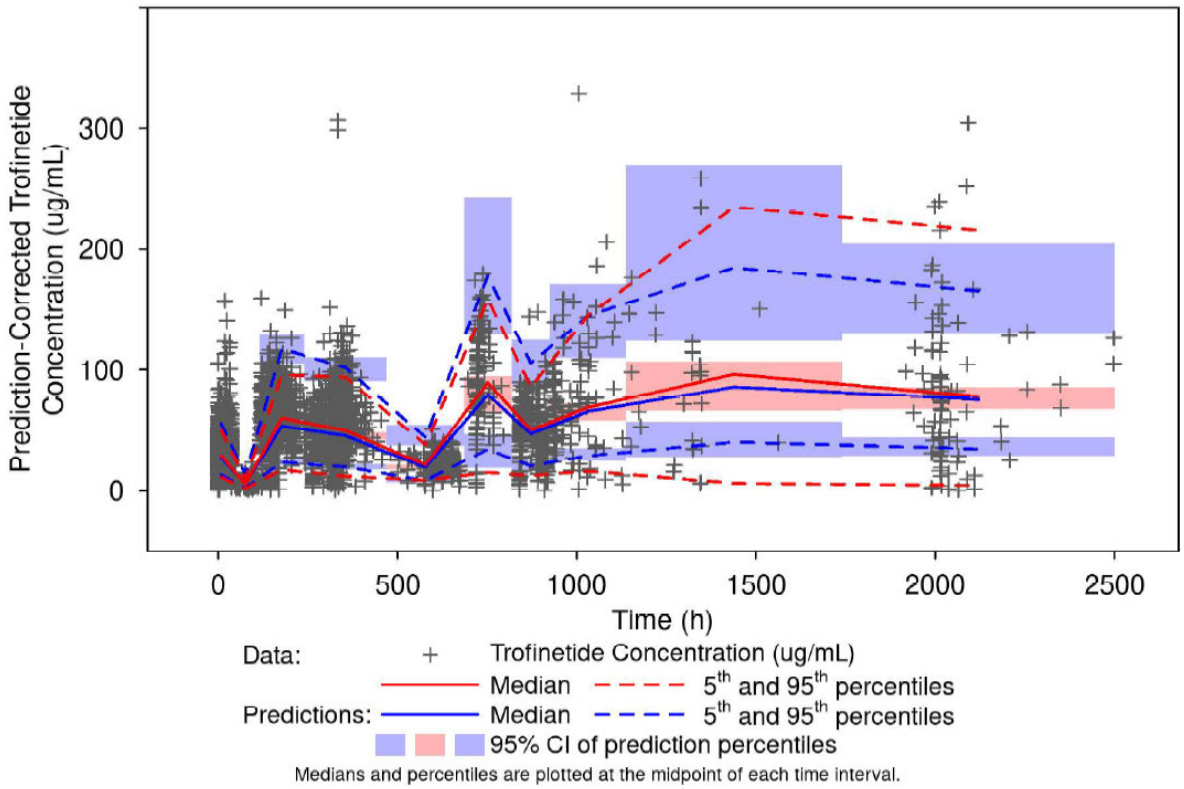
The updated popPK model for trofinetide was assessed with diagnostics plots including goodness-of-fit (Figure I) and pcVPC (Figure II).

Figure I: Goodness-of-Fit Plots for Updated PopPK Model



Source: Adapted from Applicant's PopPK report ACP-2566-MS-010, Figure 3, Page 63

Figure II: Updated PopPK Model of Trofinetide: Prediction-corrected Visual Predictive Check



Abbreviations: CI, confidence interval

Source: Applicant's PopPK report ACP-2566-MS-010, Page 64, Figure 4

Parameter estimates from the updated population PK model are presented in Table III.

For a typical subject with body weight of 58 kg, age of 22.4 years old and eGFR of 124 mL/min/1.73 m^2, the estimated CL was 11.7 L/hr, Vc was 25 L, Vp was 35.4 L, Q was 1.42 L/hr, Ka was 0.394 hr^{-1} and F1 was 0.832. A dose of 18 g reduced F1 by 13.2% and a dose of 24 g reduced F1 by 28.4%. Fed status resulted in a 13.3% decrease in F1 and a 9.69% decrease in ka. Diarrhea occurrence led to a 15.7% decrease in F1.

Interindividual variability on CL, Vc, Vp, Q, and F1 were 13.6%, 30.8%, 30.2%, 65.3%, and 20.2%, respectively. The proportional residual variance estimates were moderate with 28.1%, and 37.4% for healthy and subjects with RTT, TBI, or FXS, respectively. The shrinkage standard deviations for the random effects were 37.5%, 31.9%, 9.8%, 41.0%, and 54.8% for CL, Vc, Q, Vp and F1, respectively. This suggests that the updated PopPK model can adequately characterize IIV of Q. However, shrinkage estimates were moderate for CL, Vc and large for Vp and F1, which suggests that these parameters with large shrinkage are less reliable and should be interpreted with caution.

Table III: Updated PopPK Model: Trofinetide Parameter and Covariate

Parameter		Final Parameter Estimate		Magnitude of Variability	
		Population Mean	%RSE	Final Estimate	%RSE
CL	Central clearance (L/h)	11.7	1.99	13.6 %CV	17.4
	Exponent of (WTKG/58) for CL	0.486	7.40		
	Proportional shift in CL for RTT = 1	-0.146	29.4		
	Proportional shift in CL for TBI = 1	0.229	17.8		
	Exponent of (GFR/124) for CL	0.272	19.9		
V_c	Central volume (L)	25.0	3.78	30.8 %CV	12.5
	Exponent of (AGE/22.4) for V_c	0.556	7.98		
	Proportional shift in V_c for FXS = 1	1.16	20.0		
Q	Distribution clearance (L/h)	1.42	5.97	65.3 %CV	17.7
V_p	Peripheral volume (L)	35.4	5.69	30.2 %CV	14.3
	Proportional shift in V_p for RTT = 1	0.805	23.4		
	Proportional shift in V_p for TBI = 1	-0.753	4.43		
k_a	First-order absorption rate constant (1/h)	0.394	4.00	NE	NA
	Shift in k_a for FED	-0.0969	23.6		
F1	Oral bioavailability	0.832	3.59	20.2 %CV	16.0
	Shift in F1 for FED	-0.133	7.28		
	Shift in F1 for 18-g dose	-0.132	20.0		
	Shift in F1 for 24-g dose	-0.284	5.65		
	Shift in F1 for diarrhea	-0.157	19.5		
Residual Variability Healthy Subjects		0.0788	1.53	28.1 %CV	NA
Residual Variability Subjects With RTT, TBI, or FXS		0.140	3.19	37.4 %CV	NA
Minimum Value of the Objective Function = 25986.125					

Abbreviations: %CV, coefficient of variation expressed as a percent; FXS, Fragile X syndrome; GFR, glomerular filtration rate; IIV, interindividual variability; NA, not applicable; NE, not estimated; RTT, Rett syndrome; %RSE, relative standard error expressed as a percent; TBI, traumatic brain injury; WTKG, body weight in kilograms.
Shrinkage estimates: 37.5% for IIV in CL, 31.9% for IIV in VC, 9.8% for IIV in Q, 41.0% for IIV in VP, and 54.8% for IIV in F1.

Source: Applicant's PopPK report ACP-2566-MS-010, Page 50, Table 5

Covariate Analysis

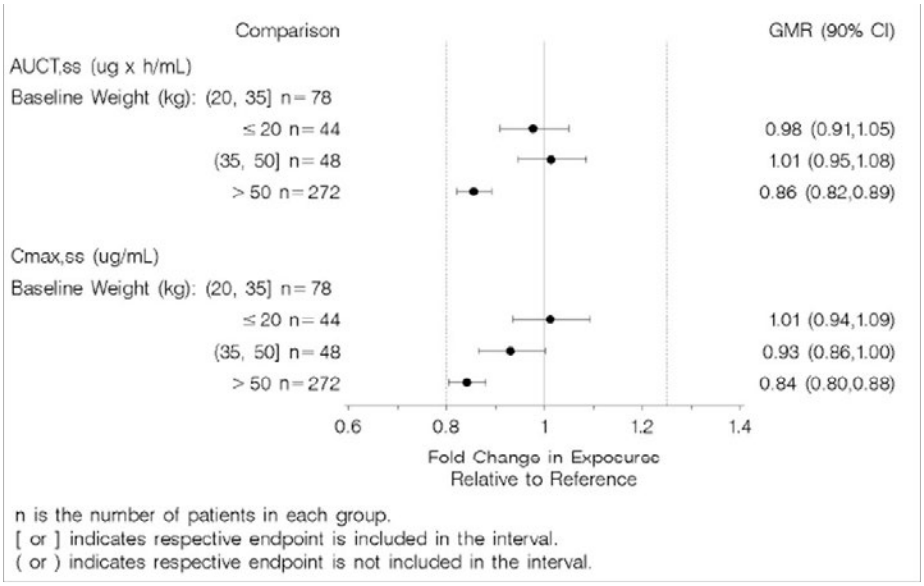
The applicant conducted covariate analysis used a standard forward selection backward elimination method to identify statistically significant (α= 0.001) predictors of PK variability that also explained a sufficient proportion of interindividual variability (IIV) for the respective PK parameter upon which they were tested (that is, reducing IIV by 5%). The continuous covariates evaluated included age, body weight, body mass index (BMI), estimated glomerular filtration rate, total bilirubin, alanine aminotransferase, and aspartate aminotransferase. The only categorical covariates evaluated were enteral route of administration (oral solution versus gastric tube) and a combination of sex and disease state, to account for the inclusion of specific sexes (males only or females only) in the majority of the studies.

Testing of exploratory covariates (intrinsic/extrinsic factors) revealed the following:

- Body weight and eGFR was identified as statistically significant covariates affecting trofinetide CL.
- Age was identified as a statistically significant covariate affecting trofinetide Vc.
- Both RTT and TBI disease status were identified as having a statistically significant effect on CL and Vp and FXS disease status had an effect on Vc.
- Food effect, diarrhea occurrence, and supratherapeutic doses (18 g and 24 g doses) on F and fed status on ka were identified as having a statistically significant effect.

The effect of specific covariates on the $AUC_{0\text{-}12,ss}$ and $C_{max,ss}$ of relative to the reference population is presented in Figure III-Figure VII.

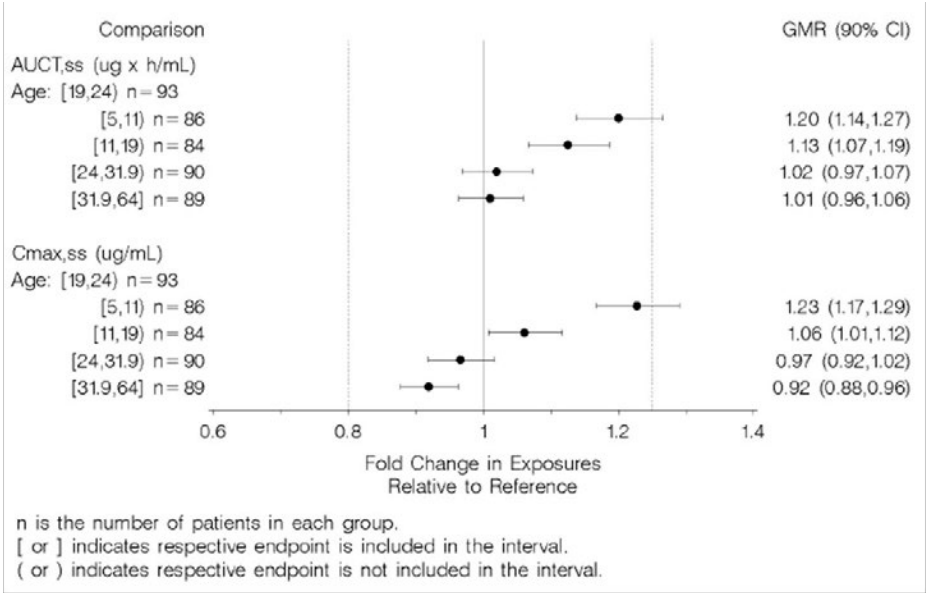
Figure III: Forest Plot of Geometric Mean Ratios (90% Confidence Intervals) of Body Weight Effects on Trofinetide $AUC_{0\text{-}12,ss}$ and $C_{max,ss}$ Following the Body Weight-Based Banded Dosing



Abbreviations: $AUC_{0\text{-}12,ss}$=$AUC_{T,ss}$=area under the blood concentration-time curve from 0 to 12 hours at steady state; CI=confidence interval; $C_{max,ss}$=maximum (peak) observed drug concentration at steady state; GMR=geometric mean ratio.

Source: Applicant's PopPK report ACP-2566-MS-008, Page 119, Figure 21D

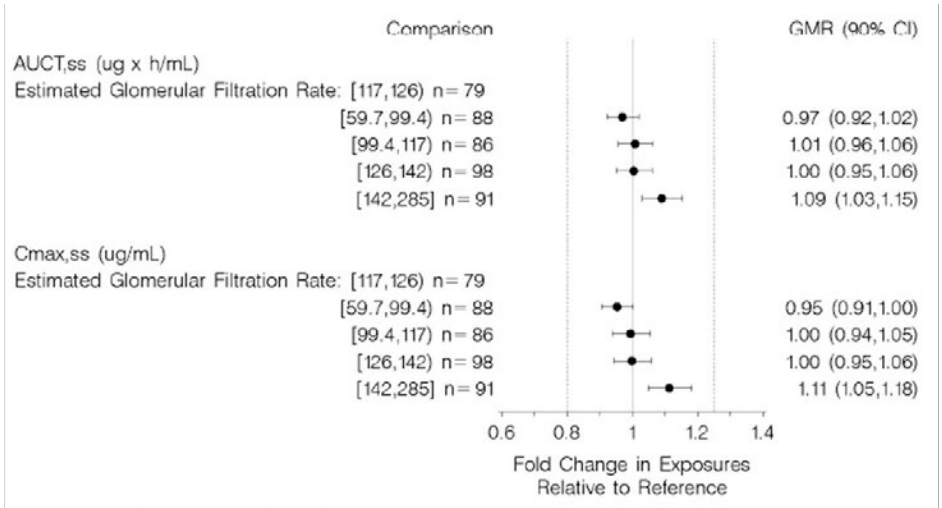
Figure IV: Forest Plot of Geometric Mean Ratios (90% Confidence Intervals) of Age Effects on Trofinetide $AUC_{0\text{-}12,ss}$ and $C_{max,ss}$ Following the Body Weight-Based Banded Dosing



Abbreviations: $AUC_{0\text{-}12,ss}$=$AUC_{T,ss}$=area under the blood concentration-time curve from 0 to 12 hours at steady state; CI=confidence interval; $C_{max,ss}$=maximum (peak) observed drug concentration at steady state; GMR=geometric mean ratio.

Source: Applicant's PopPK report ACP-2566-MS-008, Page 119, Figure 21C

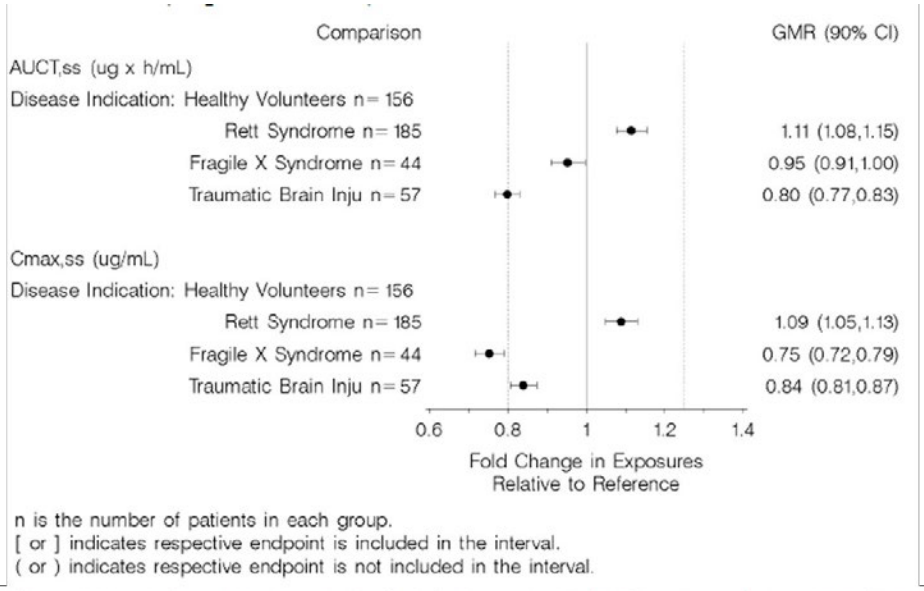
Figure V: Forest Plot of Geometric Mean Ratios (90% Confidence Intervals) of eGFR Effects on Trofinetide $AUC_{0-12,ss}$ and $C_{max,ss}$ Following the Body Weight-Based Banded Dosing



Abbreviations: $AUC_{0-12,ss}$=$AUC_{T,ss}$=area under the blood concentration-time curve from 0 to 12 hours at steady state; CI=confidence interval; $C_{max,ss}$=maximum (peak) observed drug concentration at steady state; eGFR=estimated glomerular filtration rate; GMR=geometric mean ratio.

Source: Applicant's PopPK report ACP-2566-MS-008, Page 118, Figure 21A

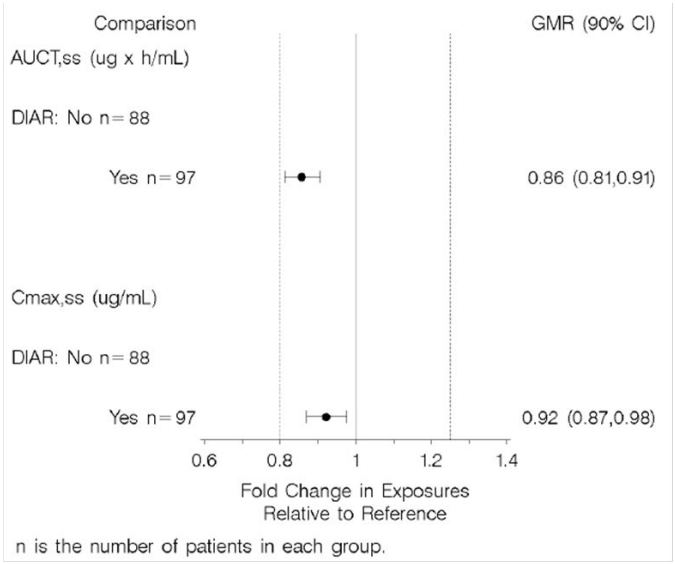
Figure VI: Forest Plot of Geometric Mean Ratios (90% Confidence Intervals) of Disease Indication Effects on Trofinetide $AUC_{0-12,ss}$ and $C_{max,ss}$ Following the Body Weight-Based Banded Dosing



Abbreviations: $AUC_{0-12,ss}$/$AUC_{T,ss}$=area under the blood concentration-time curve from 0 to 12 hours at steady state; CI=confidence interval; $C_{max,ss}$=maximum (peak) observed drug concentration at steady state; GMR=geometric mean ratio; Inju=injury.

Source: Applicant's PopPK report ACP-2566-MS-008, Page 118, Figure 21B

Figure VII: Forest Plot of Geometric Mean Ratios (90% Confidence Intervals) of Diarrhea Effects on Trofinetide $AUC_{0-12,ss}$ and $C_{max,ss}$ Following the Body Weight-Based Banded Dosing



Abbreviations: $AUC_{0-12,ss}$=$AUC_{T,ss}$=area under the blood concentration-time curve from 0 to 12 hours at steady state; CI=confidence interval; $C_{max,ss}$=maximum (peak) observed drug concentration at steady state; DIAR=diarrhea; GMR=geometric mean ratio.

Source: Applicant's PopPK report ACP-2566-MS-008, Page 121, Figure 23

Reviewer's Comments

Overall, the applicant's refined population PK model is adequate to describe trofinetide PK profiles following oral administration.

4.4.1.2 Steady-State Exposure Prediction

To support the proposed weight-banded dosing regimen, the updated population PK model, using data from 14 studies, was used to generate individual measures of steady-state trofinetide exposures ($AUC_{0-12,ss}$, minimum observed drug concentration at steady state[$C_{min,ss}$], time of the maximum observed drug concentration at steady state [$T_{max,ss}$], and maximum observed drug concentration at steady state [$C_{max,ss}$]) for the subjects in Studies ACP-2566-003 and ACP-2566-009. The following proposed body weight-banded dosing scenarios: 5 g BID for subjects 9 to < 12 g, 6 g BID for subjects 12 to < 20 kg, 8 g BID for subjects 20 to < 35 kg, 10 g BID for subjects 35 to < 50 kg, and 12 g BID for subjects 50 kg were simulated by integration of the predicted concentration-time profile for each subject based on the final PopPK model and individual empiric Bayesian PK parameter estimates.

Summary statistics were computed for the steady-state exposures, stratified by dose, and are shown in Table IV, along with the predicted steady-state exposures for Study ACP-2566-003 subjects from the final model.

Given only 200 mg/kg BID dose manifested a positive clinical outcome from phase 2 study (RETT-002) result, the applicant aimed at an exposure range of 90% quantile of AUC from the subjects who received 200 mg/kg BID trofinetide, i.e., 790-967 µg*h/mL. The applicant further rounded this exposure range to a

median $AUC_{0\text{-}12,ss}$=1000 µg•h/mL with a range expanding to ±20% around the median (800 to 1200 µg•h/mL).

A distribution plot and a boxplot comparing the $AUC_{0\text{-}12,ss}$ values for each body weight group to the exposure range ($AUC_{0\text{-}12,ss}$ = 800 - 1200 g*h/mL) are shown in Figure VIII and Figure IX, respectively. The plots show that the distribution of $AUC_{0\text{-}12,ss}$ values overlapped with the exposure range of 800-1200 g*h/mL, and that the median peak $AUC_{0\text{-}12,ss}$ values were largely contained within that exposure range for all body weight ranges following proposed dosing from Studies ACP-2566-003 and ACP-2566-009.

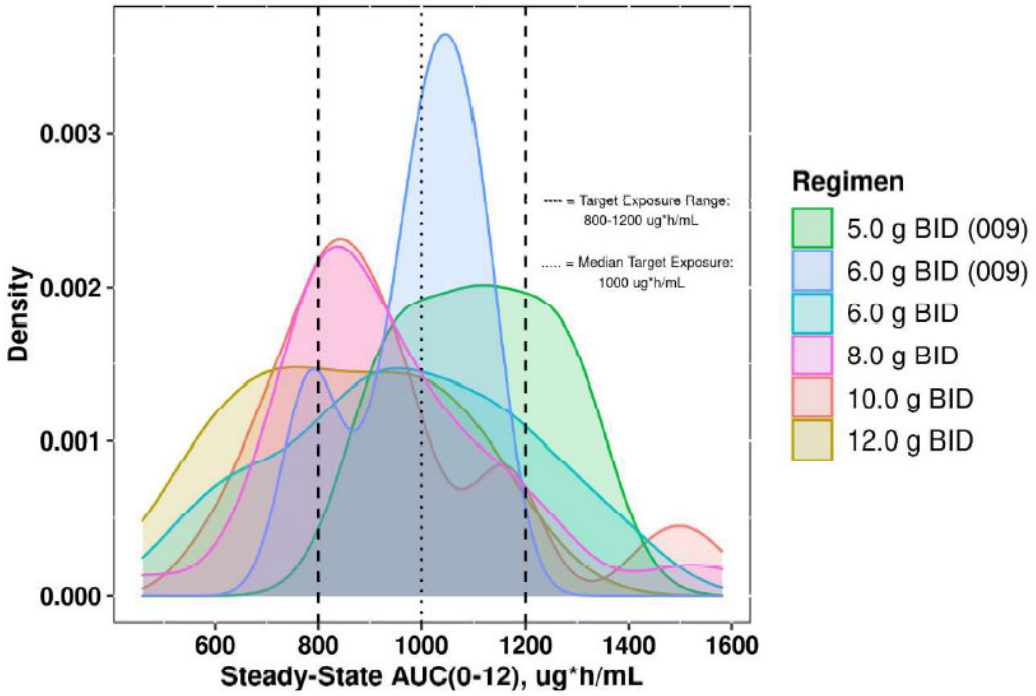
Table IV: Summary Statistics of Population Pharmacokinetic Model-Predicted Steady-State Exposures, Stratified by Dose, for Subjects in Studies ACP-2566-003 and ACP-2566-009

Exposure	Statistic	Study ACP-2566-009 Subjects (Re-Estimated Model)			Study ACP-2566-003 Subjects (Final Model)				
		5 g BID (n = 3)	6 g BID (n = 10)	ACP-2566-009 Overall (n = 13)	6 g BID (n = 23)	8 g BID (n = 41)	10 g BID (n = 21)	12 g BID (n = 7)	ACP-2566-003 Overall (n = 92)
$C_{min,ss}$ [µg/mL]	Mean	20.254	19.412	19.606	19.673	17.174	20.176	14.609	18.424
	(SD)	(5.967)	(6.717)	(6.318)	(6.282)	(6.277)	(12.397)	(4.613)	(8.090)
	Median	18.376	18.284	18.376	18.879	16.149	17.388	13.839	17.391
	Min, Max	15.45, 26.93	8.06, 27.87	8.06, 27.87	9.50, 34.92	8.28, 43.31	7.56, 66.26	8.89, 21.28	7.56, 66.26
	5th, 95th	15.45, 26.93	8.06, 27.87	8.06, 27.87	11.33, 29.35	10.25, 25.56	8.70, 34.46	8.89, 21.28	8.89, 29.35
$C_{max,ss}$ [µg/mL]	Mean	203.130	174.955	181.457	161.077	156.836	146.281	143.906	155.518
	(SD)	(31.908)	(16.223)	(22.798)	(38.939)	(34.521)	(29.386)	(33.641)	(34.318)
	Median	215.760	173.375	173.940	171.320	154.940	139.490	150.660	153.095
	Min,	166.84,	144.13,	144.13,	90.47,	71.23,	103.20,	95.53,	71.23,
	Max	226.79	195.69	226.79	234.20	268.61	238.18	188.98	268.61
	5th,	166.84,	144.13,	144.13,	107.93,	112.66,	111.80,	95.53,	107.93,
	95th	226.79	195.69	226.79	218.58	210.30	183.28	188.98	211.85
$AUC_{0\text{-}12,ss}$ [µg × h/mL]	Mean (SD)	1108.277 (166.587)	987.867 (121.438)	1015.654 (135.918)	966.544 (241.915)	926.502 (221.162)	933.340 (247.676)	852.974 (209.972)	938.909 (230.026)
	Median	1109.2	1013.6	1015.500	968.820	889.020	850.720	839.570	899.040
	Min,	941.23,	784.13,	784.13,	539.92,	446.77,	584.02,	560.11,	446.77,
	Max	1274.4	1122.1	1274.40	1391.60	1593.40	1558.20	1148.40	1593.40
	5th,	941.23,	784.13,	784.13,	586.47,	669.23,	688.18,	560.11,	586.47,
	95th	1274.4	1122.1	1274.40	1367.40	1312.70	1471.70	1148.40	1391.60
$T_{max,ss}$ (h)	Mean (SD)	1.714 (0.214)	1.775 (0.226)	1.761 (0.216)	1.974 (0.195)	1.976 (0.237)	2.170 (0.275)	2.026 (0.162)	2.024 (0.243)
	Median	1.755	1.732	1.755	1.937	1.986	2.150	2.047	1.996
	Min, Max	1.48, 1.91	1.46, 2.11	1.46, 2.11	1.68, 2.54	1.27, 2.60	1.74, 2.97	1.87, 2.29	1.27, 2.97
	5th, 95th	1.48, 1.91	1.46, 2.11	1.46, 2.11	1.71, 2.28	1.60, 2.32	1.82, 2.62	1.87, 2.29	1.71, 2.44

Abbreviations: $AUC_{0\text{-}12,ss}$, area under the concentration-time curve from time 0 to 12 hours at steady state; BID, twice daily; $C_{max,ss}$, maximum observed drug concentration at steady state; $C_{min,ss}$, minimum observed drug concentration at steady state; Max, maximum; Min, minimum; n, number of subjects; SD, standard deviation; $T_{max,ss}$, time of the maximum observed drug concentration at steady state.

Source: Applicant's PopPK report ACP-2566-MS-010, Page 52, Table 7

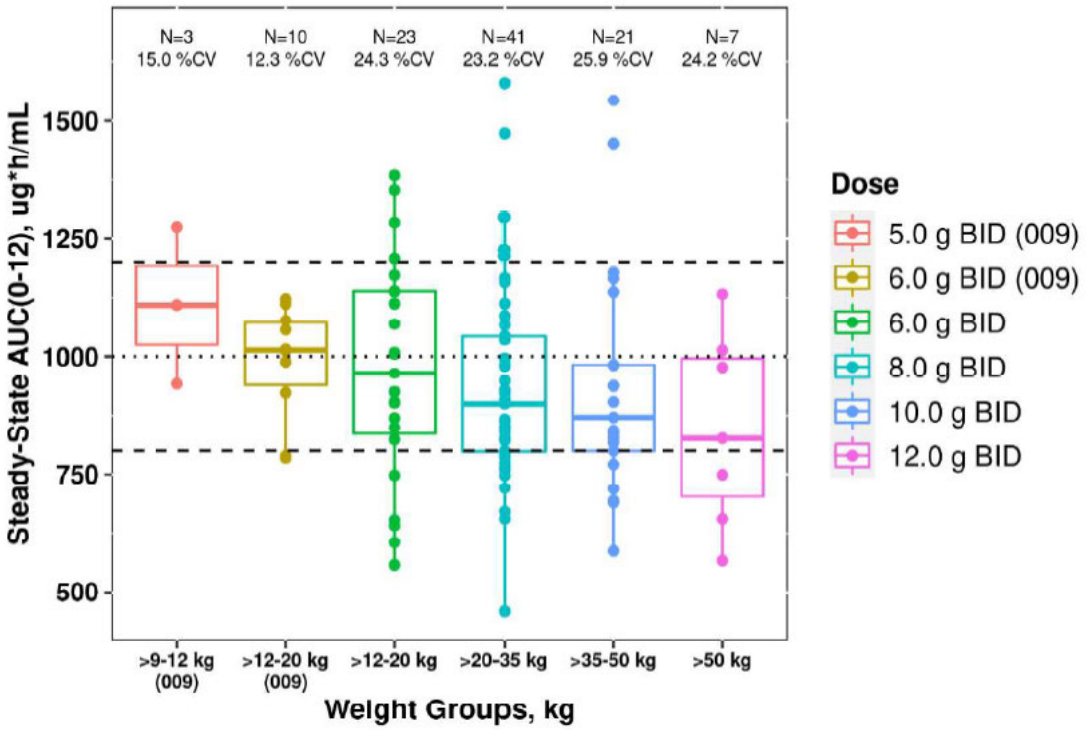
Figure VIII. Distribution of Population Pharmacokinetic Model-Predicted $AUC_{0\text{-}12,ss}$ Values in the Subjects From Studies ACP-2566-003 and ACP-2566-009, by Body Weight-Banded Dosing Regimen



Abbreviations: $AUC_{0\text{-}12,ss}$/AUC(0-12), area under the concentration-time curve from time 0 to 12 hours at steady state; BID, twice daily.

Source: Applicant's PopPK report ACP-2566-MS-010, Page 67, Figure 7

Figure IX. Boxplot of Population Pharmacokinetic Model-Predicted $AUC_{0\text{-}12,ss}$ Values in the Subjects From Studies ACP-2566-003 and ACP-2566-009, by Body Weight-Banded Dosing Regimen



Abbreviations: $AUC_{0\text{-}12,ss}$/AUC(0-12), area under the concentration-time curve from time 0 to 12 hours at steady state; BID, once daily; %CV, coefficient of variation expressed as a percent; IQR, interquartile range; N, number of subjects.

Note: The dashed lines represent the target exposure range ($AUC_{0\text{-}12,ss}$ = 800-1200 µg × h/mL). The dotted line represents the median target exposure ($AUC_{0\text{-}12,ss}$ = 1000 µg × h/mL).

Note: The bottom and top of each box represent the 25th and 75th percentiles, respectively; the whiskers represent the 25th/75th percentile + 1.5 × IQR; the line within each box represents the median. The circles represent the values above/below the 25th/75th percentile + 1.5 × IQR.

Source: Applicant's PopPK report ACP-2566-MS-010, Page 68, Figure 8

Reviewer's Comments

The Applicant's PopPK analysis results confirmed that the distribution of $AUC_{0\text{-}12,ss}$ values were largely contained within the exposure range of 800-1200 µg•h/mL, and that the median peak $AUC_{0\text{-}12,ss}$ fell within the same exposure range for all body weight bands following proposed dosing regimen. The reviewer was able to confirm the applicant's findings on comparison of the distribution of $AUC_{0\text{-}12,ss}$ values for each body weight group with the exposure range. Please see more details in Reviewer's Analysis.

4.4.1.3 Exposure-Response Analysis

To characterize the relationships between trofinetide exposure and coprimary (RSBQ and CGI-I scores) or secondary efficacy endpoints (CSBS-DP-IT Social Composite and RTT-COMC scores), the Applicant developed E-R models using data from subjects with RTT with available trofinetide exposure estimates and placebo subjects from Studies in subjects with Rett syndrome.

The overall procedures followed for the development of the E-R models for RSBQ total scores, CGI-I, CSBS-DP-IT Social Composite, and RTT-COMC scores were as follows:

- generation of individual estimates of exposure based on the updated PopPK model
- exploratory data analysis
- base structural model development incorporating drug exposure effects
- evaluation of covariate effects
- final model refinement
- model evaluation

Stationary covariates evaluated in the E-R analyses of efficacy and safety measures were age, baseline body weight, and baseline BMI.

Efficacy and safety data were obtained from Studies Neu-2566-Rett-001, Neu-2566-Rett-002, and ACP-2566-003. All participants were females 5 years of age with Rett syndrome (RTT).

Participants in Study Neu-2566-Rett-001 were administered 35 or 70 mg/kg twice daily (BID) for up to 28 days. Participants in Study Neu-2566-Rett-002 were administered 50, 100, or 200 mg/kg BID for up to 42 days. Patients in Study ACP-2566-003 were randomized to either placebo or trofinetide for up to 12 weeks of treatment; trofinetide was administered as weight-banded dosing regimens of 6 g BID for 12 to < 20 kg, 8 g BID for 20 to < 35 kg, 10 g BID for 35 to < 50 kg, or 12 g BID for 50 kg.

The dataset for use in the E-R efficacy modeling of RSBQ total scores included patients from Studies Neu-2566-Rett-002 (per protocol population) and ACP-2566-003 (full analysis set) receiving either placebo or trofinetide with available trofinetide exposure measures.

The dataset for use in the E-R efficacy modeling of CGI-I scores included patients from Studies Neu-2566-Rett-001 (per protocol population), Neu-2566-Rett-002 (per protocol population), and ACP-2566-003 (full analysis set) receiving either placebo or trofinetide with available trofinetide exposure measures.

The dataset for use in the E-R efficacy modeling of CSBS-DP-IT social composite and RTT-COMC scores included patients from Study ACP-2566-003 (full analysis set) receiving either placebo or trofinetide with available trofinetide exposure measures.

The dataset for use in the E-R safety modeling of TEAEs included patients from the safety analysis population from Studies Neu-2566-Rett-001, Neu-2566-Rett-002, and ACP-2566-003 receiving either placebo or trofinetide with available trofinetide exposure measures.

The final population pharmacokinetic (PK) model for trofinetide was used to generate empiric Bayesian PK parameter estimates for individual subjects in the analysis dataset. These estimates were used to generate predicted trofinetide concentration-time profiles over 12 hours for individual subjects. The predicted concentration-time profiles were then used to compute appropriate measures of trofinetide exposure via integration (area under the concentration-time curve [AUC], AUC from time 0 to 12 hours [AUC_{0-12}], maximum observed drug concentration [C_{max}], and average observed drug concentration [C_{avg}]) for each individual. The measures of trofinetide exposure evaluated for the efficacy analysis included average daily consecutive between-visit exposure estimates of C_{avg}, C_{max}, and AUC_{0-12}.

For safety analysis of TEAEs, exposure estimates evaluated included the average of daily C_{max}, AUC_{0-12}, and C_{avg} from first to last dose date for all patients. Trofinetide exposure measures were set to zero for placebo patients.

Efficacy endpoints included RSBQ total scores, CGI-I scores, CSBS-DP-IT social composite scores, and RTT-COMC scores. RSBQ scores were collected at baseline and Days 14, 28, 42, 54, and 66 from Study Neu-2566-Rett-002 and at Screening, baseline, and Weeks 2, 6, and 12 from Study ACP-2566-003. The RSBQ data used in E-R analyses was RSBQ total scores. CGI-I scores were collected on Days 5, 14, 17, and 28 for Cohort 0 and on Days 5, 14, 26, and 40 for Cohorts 1 and 2 in Study Neu-2566-Rett-001. In Study Neu-2566-Rett-002, CGI-I scores were collected on Days 21, 28, 42, 54, and 66. In Study ACP-2566-003, CGI-I scores were collected on Weeks 2, 6, and 12. In Study ACP-2566-003, CSBS-DP-IT social composite scores were collected at baseline and Weeks 2, 6, and 12. In Study ACP-2566-003, RTT-COMC scores were collected at baseline and Weeks 2, 6, and 12.

Adverse events (AEs) of interest, such as decreased appetite, diarrhea, irritability, seizures, vomiting, and weight loss/weight decreased, were recorded from the time informed consent was obtained through the duration of the study. The endpoints used for E-R efficacy modeling included RSBQ, CGI-I, CSBS-DP-IT social composite, and RTT-COMC scores. The RSBQ scores were not measured in Study Neu-2566-Rett-001. The observed RSBQ, CGI-I, CSBS-DP-IT social composite, and RTT-COMC scores (without transformation) were used for modeling.

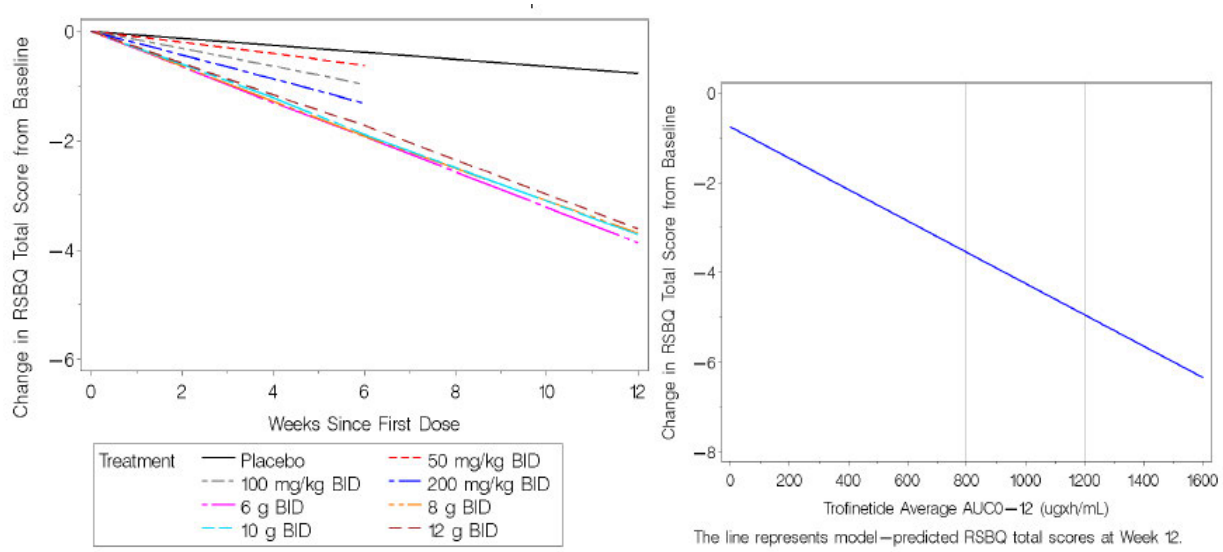
Exposure-Efficacy Relationships

Rett Syndrome Behaviour Questionnaire (RSBQ) Total Score

An E-R model describing the effects of trofinetide exposures on RSBQ total scores was developed using data from 264 subjects (1022 records) from Studies RETT-002 and 003. A linear function described the relationship between the trofinetide average AUC_{0-12} and the slope for time whereby higher exposure was predictive of a reduction (improvement) in RSBQ total scores. Baseline body weight was a significant predictor of the slope, with heavier subjects having a greater response in RSBQ total scores. Assuming the mean weight and mean trofinetide average AUC_{0-12} for each weight-based banded dosing regimen, the model-predicted reductions (improvement) in RSBQ total score at Week 12 were 2.1, 4.0, 6.8, and 10.2, respectively, compared to a reduction in RSBQ score of 1.2 for placebo at Week 12. No other tested

covariates (age, BMI, or baseline RSBQ scores) were found to be a statistically significant predictor of the variability in RSBQ scores. Assuming the mean weight and mean trofinetide average AUC_{0-12} for each weight-based banded dosing regimen, the model-predicted reductions (improvement) in RSBQ total score at Week 12 were 2.1, 4.0, 6.8, and 10.2, respectively, compared to a reduction in RSBQ score of 1.2 for placebo at Week 12. No other tested covariates (age, BMI, or baseline RSBQ scores) were found to be a statistically significant predictor of the variability in RSBQ scores.

Figure X. Model-Predicted Change in RSBQ Total Scores from Baseline Versus Weeks (Left) and Versus Trofinetide AUC_{0-12} (Right)



Source: Applicant's E-R analysis report ACP-2566-MS-009, Page 203 and 204, Figure 21 and 22

Clinical Global Impression - Improvement (CGI-I) Scores

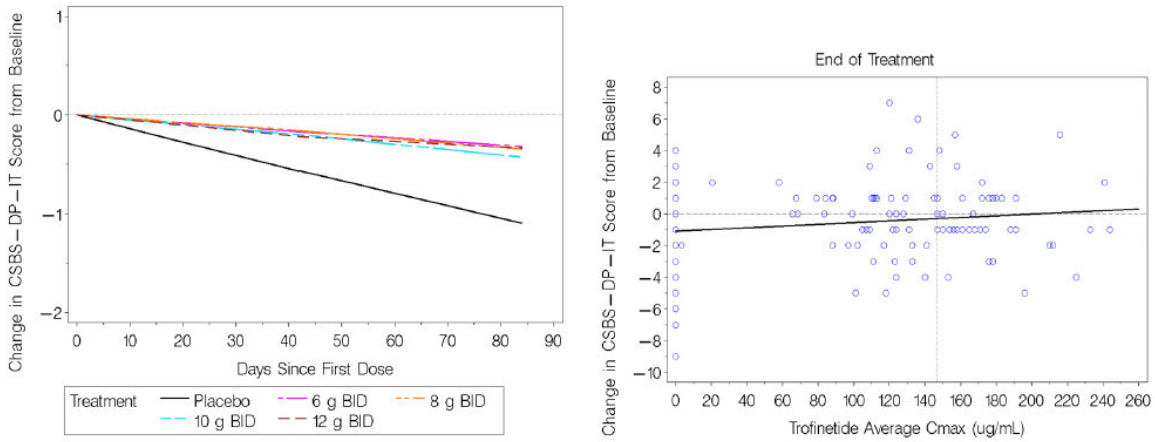
E-R analyses were performed to describe the effect of trofinetide exposure on the efficacy endpoint CGI-I scores collected from 316 subjects (989 records) for up to 12 weeks from Studies RETT-001, RETT-002, and 003. Exploratory analysis demonstrated that mean CGI-I response appeared to be slightly lower over time in the trofinetide treatment group compared to placebo, however, there was no clear relationship between trofinetide exposure and CGI-I scores, and none of the exposure measures resulted in an acceptable model fit. There was no clear E-R relationship.

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

An E-R model was developed to describe the effect of trofinetide exposure on the efficacy endpoint CSBS-DP-IT Social Composite Scores collected from 182 subjects (679 records) for up to 12 weeks from Study 003. Exploratory analysis demonstrated an increase (improvement) in CSBS-DP-IT Social Composite Scores with an increase in trofinetide exposure. An exponential model as a function of time best described the CSBS-DP-IT Social Composite Scores data following placebo treatment. When the effect of trofinetide exposure was tested, a statistically significant E-R relationship was found for CSBS-DP-IT Social Composite

Scores and all trofinetide exposure measures. Trofinetide average C_{max} was the most significant exposure measure as a linear function and was chosen for inclusion in the base E-R model. As trofinetide average Cmax increased, there was an increase (improvement) in CSBS-DP-IT Social Composite Scores. No covariates met the criteria for inclusion in the final CSBS-DP-IT Social Composite Score model. Assuming the median trofinetide average C_{max} value of 147 µg/mL, the reduction in model-predicted CSBS-DP-IT Social Composite Scores at Week 12 was 0.33, smaller than the reduction of 1.09 for placebo, indicating treatment with trofinetide improved CSBS-DP-IT Social Composite Scores response.

Figure XI. Model-Predicted Change in CSBS-DP-IT Social Composite Scores from Baseline Versus Days (Left) and Versus Trofinetide Cmax (Right)



Abbreviations: C_{max}=maximum (peak) observed drug concentration; BID=twice daily; CSBS-DP-IT=Communication and Symbolic Behavior Scales-Developmental Profile Infant-toddler Checklist

Notes: The dashed horizontal line in the both panels represents a reference line at no change in CSBS-DP-IT Social Composite Scores. The solid line in the right-hand panel represents the model-predicted CSBS-DP-IT Social Composite Scores at Week 12; the dashed vertical line in the right-hand panel represents the median C_{max} of 147 µg/mL; the open circles in the right-hand panel represent the observed data.

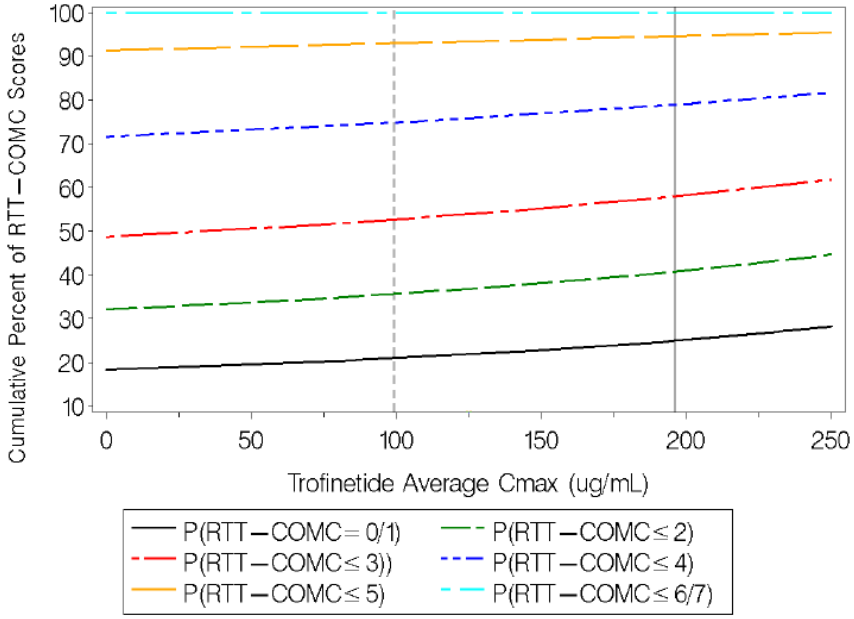
Source: Applicant's E-R analysis report ACP-2566-MS-009, Page 229 and 230, Figure 43 and 44

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

An E-R model was developed to describe the effect of trofinetide exposure on the efficacy endpoint RTT-COMC scores collected from 181 subjects (672 records) for up to 12 weeks from Study 003. Exploratory analysis demonstrated a reduction (improvement) in RTT-COMC scores with an increase in trofinetide exposure. Due to small sample size with RTT-COMC scores of 0 (N=2) and 7 (N=3), these data records were combined with scores of 1 and 6, respectively, for modeling analyses. A proportional odds model was fitted to the placebo-only data using linear and exponential functions of time in days and found no placebo response over time. Trofinetide exposure measures (average daily AUC0-12, Cmax, and Cavg) were tested as linear, power, and exponential functions. All exposure measures and functional forms resulted in statistically significant changes in the objective function ($p<0.05$). Average trofinetide Cmax was the most significant exposure measure and was chosen for inclusion in the base E-R model. As trofinetide average Cmax increased, there was a higher probability of lower RTT-COMC scores, indicating an improvement in response. No covariates met the criteria for inclusion in the final RTT-COMC score model. The model-predicted cumulative probability of RTT-COMC score ≤3 was 0.55, assuming the median trofinetide

average Cmax of 147 µg/mL compared to 0.49 for placebo as shown in Figure XII. proportion of subjects in all categories of RTT-COMC scores across the range of trofinetide average Cmax.

Figure XII. Model-Predicted Cumulative Percent of RTT-COMC Scores Versus Trofinetide Cmax



The vertical lines represent the 25th to 75th percentiles of Cmax for the target dose.

Abbreviations: C_{max}=maximum (peak) observed drug concentration; BID=twice daily; P=cumulative percent; RTT-COMC=Rett Syndrome Clinician Rating of Ability to Communicate Choices.

Source: Applicant's E-R analysis report ACP-2566-MS-009, Page 242, Figure 53

Safety Adverse Events

E-R analyses describing the effect of trofinetide average daily exposure on the probability of AEs of decreased appetite, diarrhea, irritability, seizures, vomiting, and weight decreased were performed using data from 323 subjects from Studies RETT-001, RETT-002, and 003.

No E-R modeling was performed for occurrence of decreased appetite, irritability due to low incidence of these TEAEs. None of the exposure measures (AUC_{0-12}, C_{avg}, and C_{max}) evaluated were found to be a statistically significant predictor (α=0.05) of the probability of seizures. Therefore, the incidence of seizure AEs was not considered related to trofinetide exposure.

Overall, 39.01% of subjects had at least one occurrence of diarrhea during the treatment period, with 15.94% in placebo subjects and 56.22% in trofinetide subjects. The majority of diarrhea AEs were mild (67.5%) and moderate (31%) with only 1.6% severe. Exploratory analysis demonstrated a trend for a higher occurrence of diarrhea with higher trofinetide exposure for all exposure measures. All exposure measures (AUC_{0-12}, Cmax, and Cavg) and functional forms were significant predictors of the probability of diarrhea and the linear function of trofinetide average AUC_{0-12} was selected for inclusion in the base E-R model for the probability of diarrhea. As trofinetide average AUC_{0-12} increased, the model-predicted

probability of diarrhea increased. No covariates met the criteria for inclusion in the final diarrhea model. Using the trofinetide AUC_{0-12} range of 800 and 1200 µg•h/mL, the model-predicted probability of diarrhea was 0.71 and 0.89, respectively.

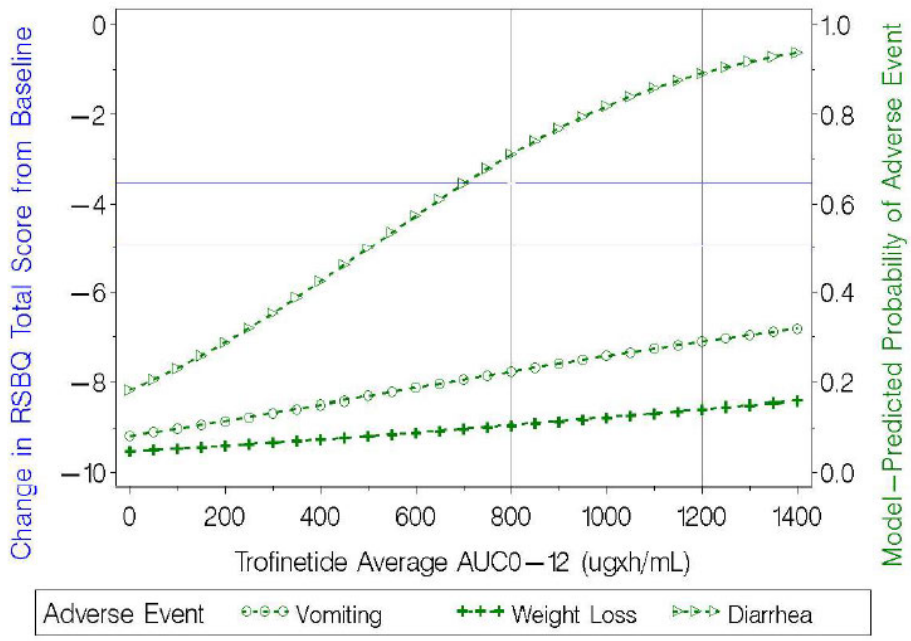
Overall, 13.93% of subjects had at least one occurrence of vomiting during the treatment period, with 8.7% in placebo subjects and 17.84% in trofinetide subjects. The majority of vomiting TEAEs (84.4%) were mild, 13.3% were moderate, and 2.2% were severe. Exploratory analysis demonstrated a trend for a higher occurrence of vomiting at higher exposure for all exposure measures. All exposure measures (AUC_{0-12}, Cavg, and Cmax) and functional forms were significant predictors of the probability of vomiting and trofinetide average Cmax as an exponential function was selected for inclusion in the base E-R model. Assuming the model-predicted median trofinetide average Cmax of 147 µg/mL and the median age of 11 years, the model-predicted probability of vomiting was 0.23 compared to 0.09 in subjects administered placebo.

Overall, 7.12% of subjects had at least one occurrence of weight decreased during the treatment period, with 4.35% in placebo subjects and 9.19% in trofinetide subjects. Exploratory analysis demonstrated a relatively flat relationship between observed AE of weight decreased and trofinetide exposures, except at the highest quartile of exposure. Trofinetide average Cmax as a linear function was selected for inclusion in the base E-R model. As trofinetide average Cmax increased, the model-predicted probability of weight decrease increased. No covariates met the criteria for inclusion in the final weight decreased model. Assuming the model-predicted median trofinetide average Cmax of 147 µg/mL, the model-predicted probability of an AE of weight decreased was 0.11 compared to 0.05 following placebo treatment.

Comparison of Exposure-Response and Exposure-Safety Relationships

The comparison plot for efficacy and the probability of TEAEs of diarrhea, vomiting, and weight decreased is provided in Figure XIII.

Figure XIII. Relationships for RSBQ Total Scores and the Probability of TEAEs of Diarrhea, Vomiting, and Weight Decreased Versus Trofinetide AUC_{0-12}



The horizontal lines represent the model−predicted RSBQ total scores at Week 12 for the target exposure range. The dashed lines represent the probability of each adverse event. The vertical lines represent the target exposure range from 800 to 1200 ugxh/mL.

Abbreviations: AUC_{0-12}, area under the concentration-time curve from time 0 to 12 hours; RSBQ, Rett Syndrome Behaviour Questionnaire; TEAE, treatment-emergent adverse event.
Note: Treatment emergent adverse events of weight loss and weight decreased are used interchangeably throughout the report.

Source: Applicant's E-R analysis report ACP-2566-MS-009, Page 271, Figure 82

Reviewer's Comments

The applicant chose trofinetide Cmax, which was the most significant exposure measure for inclusion in the base E-R model for co-secondary efficacy endpoints (CSBS-DP-IT and RTT-COMC), Mechanistic basis for using this metric in the E-R analysis is unclear.

Reviewer's Analysis

Methods

Data Sets

Data set used is listed in **Table V**.

Table V: Analysis Data Sets

Datasets

Study	Name	Link to EDR
Phase 1 Studies: HV-001, HV-002, HV-003, HV-004, HV-005, 006, 007, and 008 Phase 2 Studies: RETT-001, RETT-002, FXS-001, and TBI-001/002 Phase 3 Studies: Study 003, and Study 009	poolpk4.xpt adpc.xpt adsl.xpt adae.xpt adcm.xpt	\\CDSESUB1\EVSPROD\nda217026\0001\m5\datasets\acp-2566-ms-010\analysis\legacy\datasets\acp-2566-ms-010-define.zip \\CDSESUB1\EVSPROD\nda217026\0001\m5\datasets\acp-2566-003\analysis\adam\datasets\

Software

PopPK model fitting was performed in NONMEM 7.4.3 and Pirana 2.9.9. Primary analysis and plotting were performed in R 4.1.2.

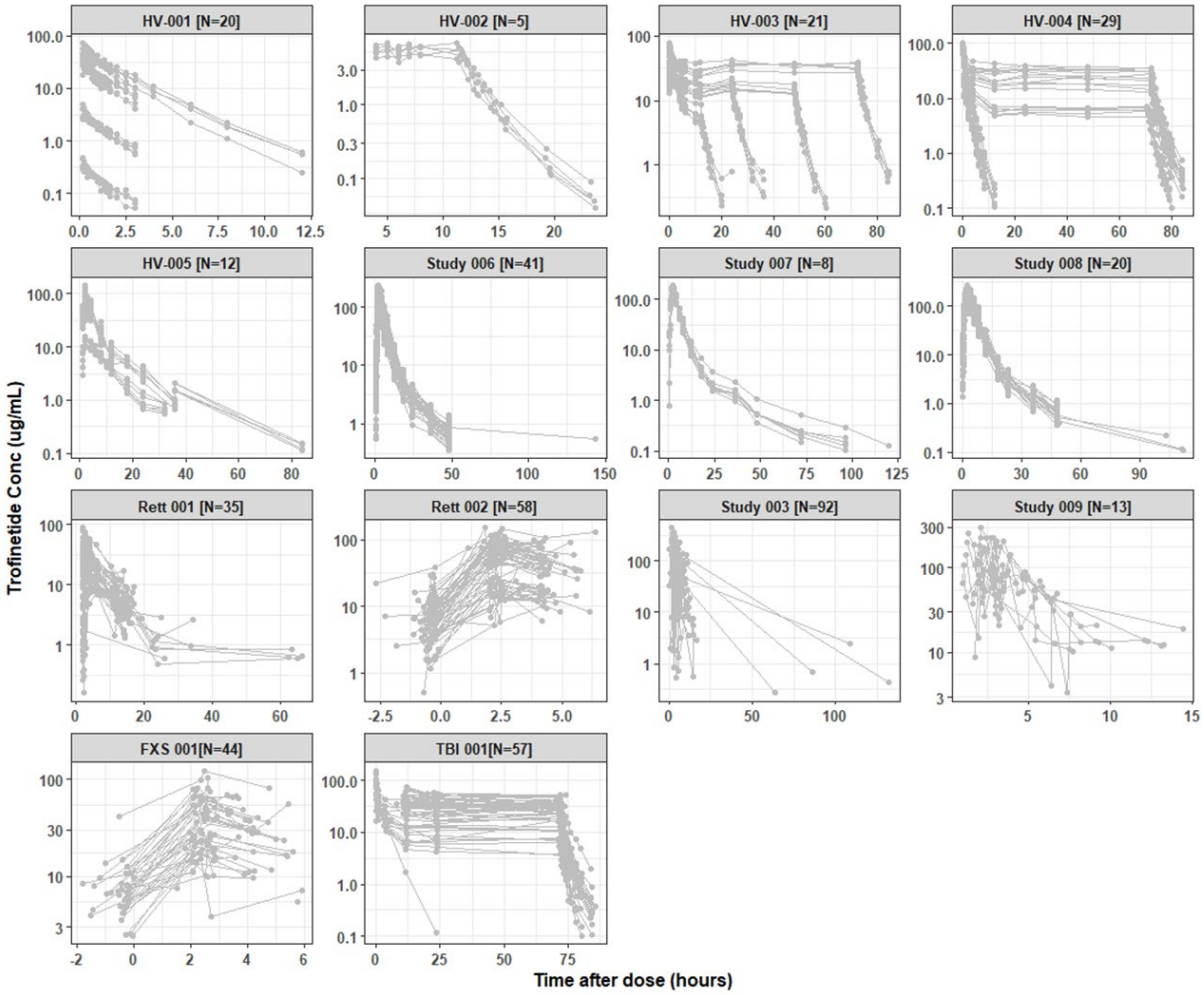
Results

The reviewer was able to reproduce the applicant's PopPK results with NONMEM (version:7.4.3). The applicant's PopPK analysis is adequate for characterizing the PK profiles for trofinetide. In addition, the reviewer conducted an independent analysis to evaluate the adequacy of the applicant's proposed dosing recommendations.

Overview of Observed Data

Figure 14 presents PK profiles of in adults following administration by study.

Figure XIV: Observed Trofinetide Concentration Versus Time after Dose by Study

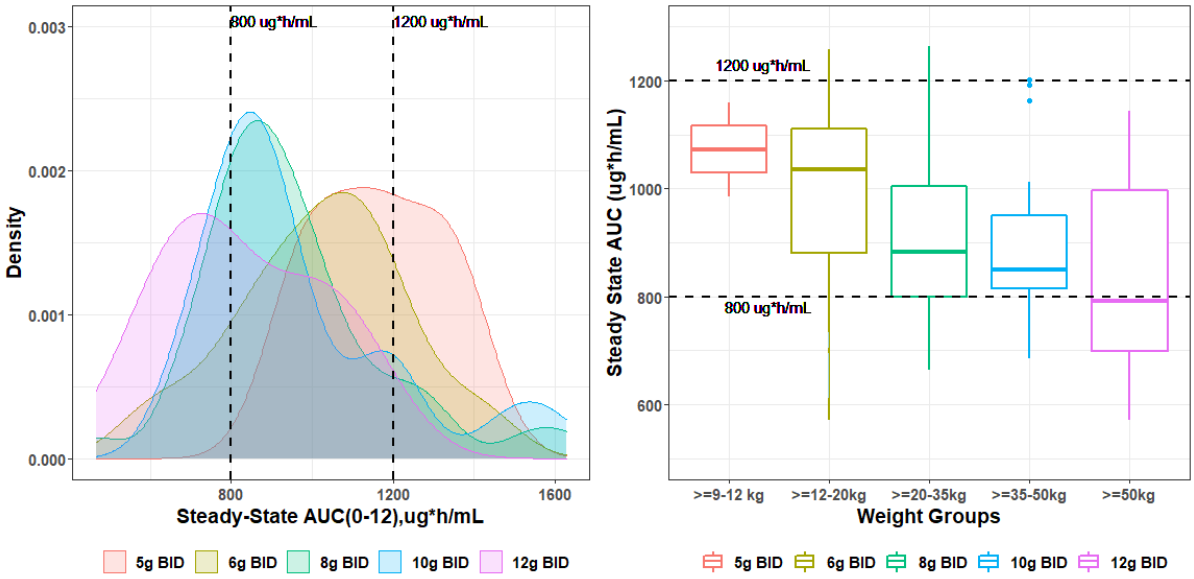


Source: Reviewer's Analysis

Evaluation of Proposed Dosing Regimen

The Applicant's updated PopPK model was used to support weight-banded dosing recommendations adults and pediatric patients 2 years of age and older with Rett syndrome. Reviewer conducted PK simulations using demographic data of all the subjects (N=464) in trofinetide clinical development program to generate steady-state exposures ($AUC_{0\text{-}12,ss}$ minimum observed drug concentration at steady state [$C_{min,ss}$], and maximum observed drug concentration at steady state [$C_{max,ss}$]) following proposed body weight-banded dosing regimens. Figure 15 and 16 show the comparison the distribution of $AUC_{0\text{-}12,ss}$ values for each body weight group with the exposure range ($AUC_{0\text{-}12}$,ss = 800 - 1200 µg*h/mL), which indicates that distribution of $AUC_{0\text{-}12,ss}$ values were largely contained within the exposure range (800-1200 µg•h/mL), and the median peak $AUC_{0\text{-}12}$,ss fell within the same exposure range for all body weight bands following proposed dosing regimen.

Figure XV: Distribution (Left) and Boxplot (Right) of Population Pharmacokinetic Model-Predicted $AUC_{0\text{-}12,ss}$ Values in the Subjects from Study 003 and Study 009, by Body Weight-Banded Dosing Regimen



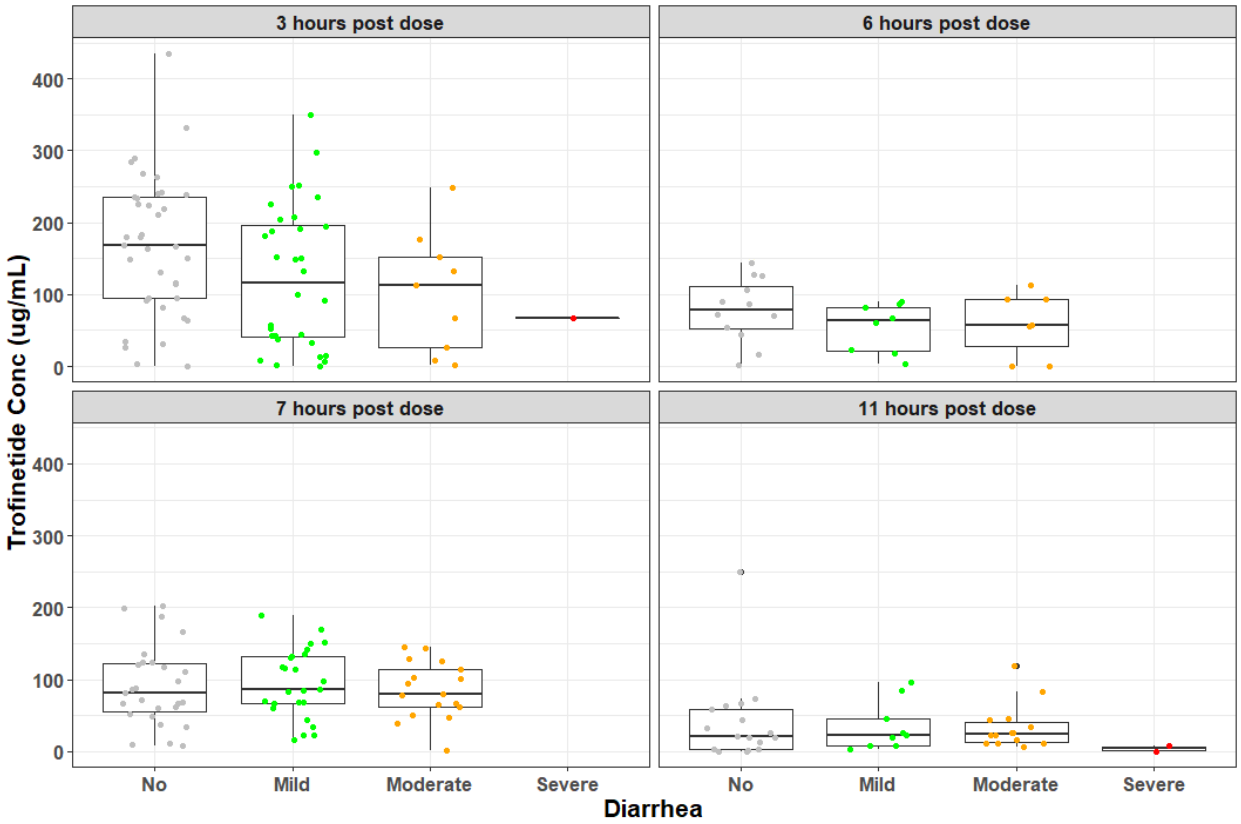
Source: Reviewer's Analysis

Evaluation of Diarrhea Effect on Trofinetide PK

Diarrhea was the most reported TEAE in the clinical studies of trofinetide in RTT. While TEAEs of diarrhea were frequently observed, overall, 49.7% of subjects in Study 003 had at least one occurrence of diarrhea during the treatment period, with 20.7% in placebo subjects and 80.6% in trofinetide subjects. Most diarrhea AEs were mild (61.2%) and moderate (37.2%) with 1.7% severe.

Figure XVI presents a comparison of trofinetide concentration at different time points after-dose time by diarrhea severity of the subjects from study ACP-2566-003. Three hours post dose, compared to the patients who didn't experience diarrhea at that time point, the median trofinetide plasma concentrations in patients who experienced mild, moderate and severe diarrhea were lower, ~31%, ~33% and ,~60% respectively. It is worthy to note that there was only one pk data from the patient who experienced severe diarrhea three hours post dose. It is also observed that trofinetide plasma concentration observed in patients who experienced mild and moderate diarrhea vs. the patients without diarrhea at the other time points (6, 7 and 11 hours after dose) were similar, with a difference in median trofinetide concentration less than 25%.

Figure XVI: Comparison of Steady State Trofinetide Concentration at Different Time Points After-Dose in Study ACP-2566-003, by Diarrhea Severity of the Subjects

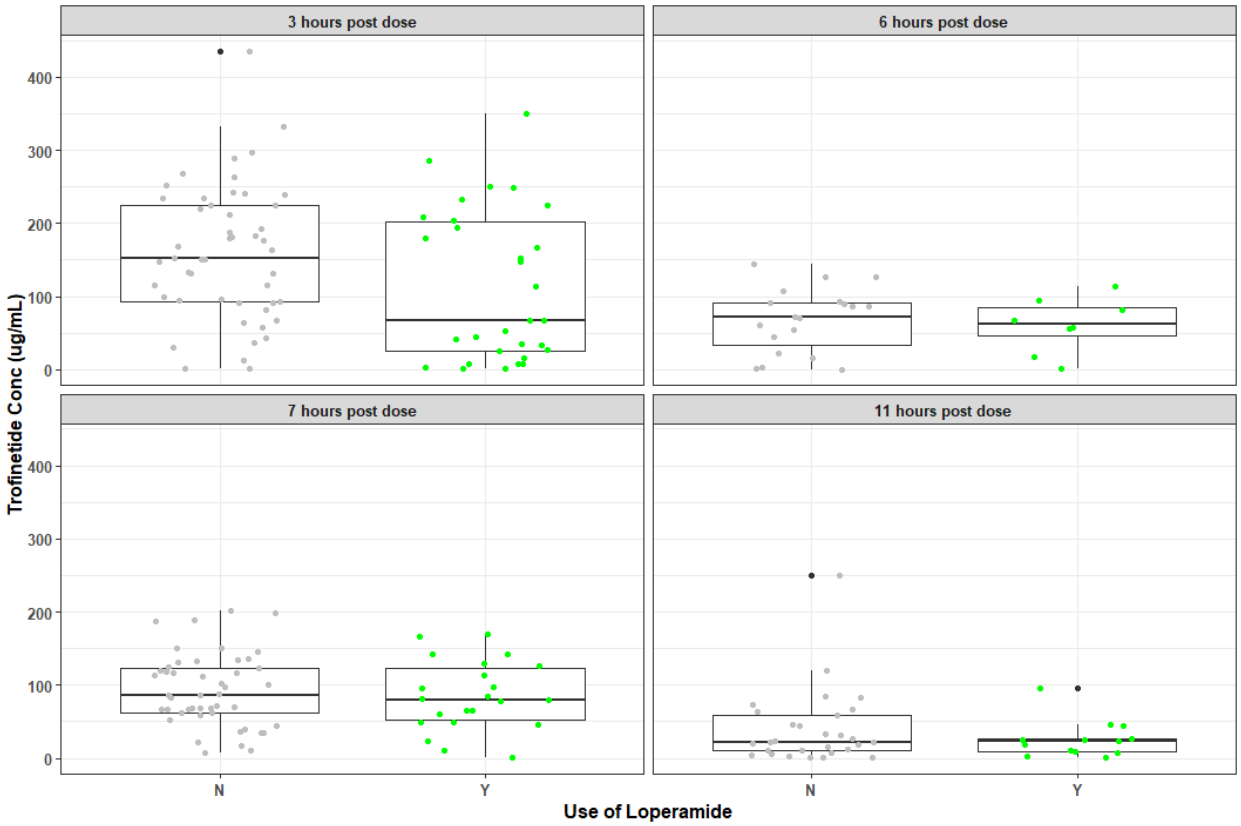


Note: Patients with dose adjustment were excluded from the analysis.

Source: Reviewer's Analysis

Figure XVII presents a comparison of trofinetide concentration at different time points after-dose time by use of loperamide from study ACP-2566-003. Three hours post dose, compared to the patients who didn't take loperamide at that time point, the median trofinetide plasma concentrations in patients was ~56% lower. It is unclear if the reduction in plasma concentration three hour after dose was caused by loperamide since it might be confounded with diarrhea status and other baseline demographic variables. It is also observed that trofinetide plasma concentration observed in patients who took the Loperamide vs. the counterpart who didn't take the loperamide at the other time points (6, 7 and 11 hours after dose) were comparable, with a difference in median trofinetide concentration less than 15%.

Figure XVII: Comparison of Trofinetide Steady State Concentration at Different Time Points After-Dose in Study ACP-2566-003, by Concomitant Use of Loperamide

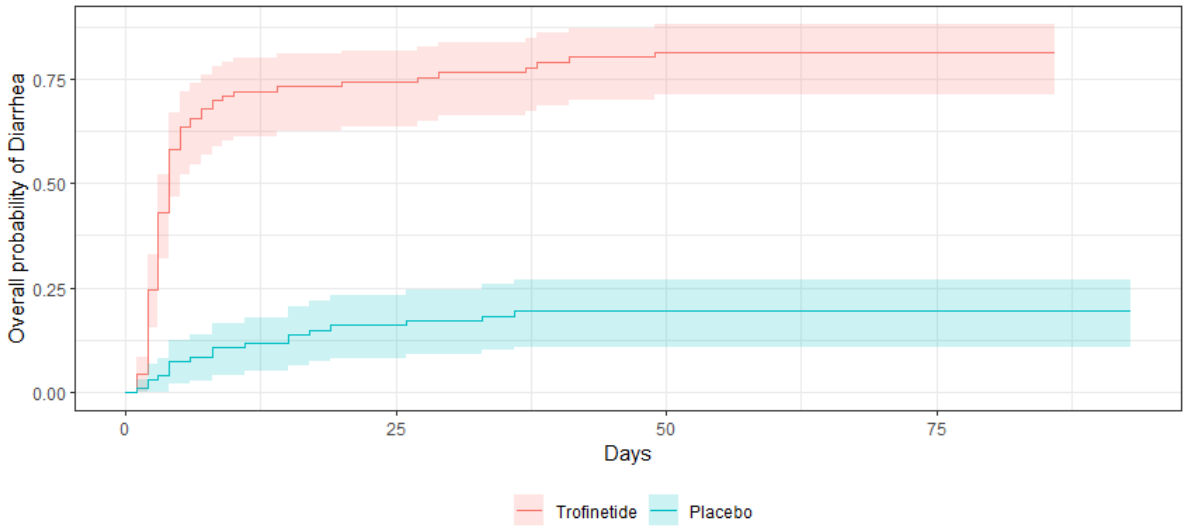


Note: Patients with dose adjustment were excluded from the analysis.

Source: Reviewer's Analysis

Simulations were conducted using the updated popPK model to evaluate the impact of diarrhea on typical patient representing each weight-banded group. Since majority of subjects in trofinetide treatment group had diarrhea within 1 week of starting the treatment (Figure 18), to simplify the simulation scenarios, subjects were assumed to experience diarrhea immediately after oral administration of trofinetide. The simulated trofinetide PK profile for typical patients with median age, median body weight and median eGFR with or without diarrhea were plotted for each body-weight group (Figure XVII). Simulations suggest that about 15.7 % reduction in steady-state Cmax and AUC_{0-12} would be observed for all the typical subjects (age: 2-25 yrs, body weight:10-75kg, and eGFR: 113-163 mL/min/1.73 m^2). Overall, diarrhea is not expected to impact trofinetide PK.

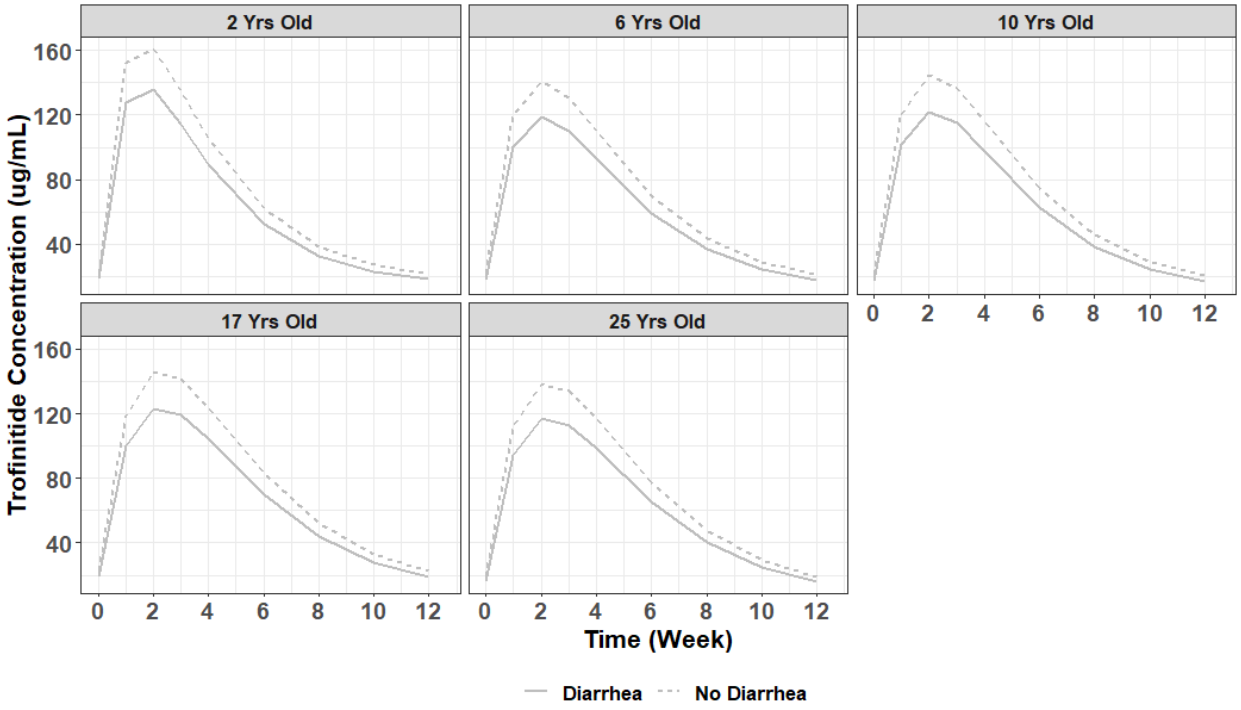
Figure XVIII: Time to Event Analysis for Occurrence of Diarrhea in Study ACP-2566-003, by Trofinetide and Placebo Arm



Trofinetide				
At Risk	93	23	15	15
Events	0	69	75	75
Placebo				
At Risk	94	76	69	69
Events	0	15	18	18

Source: Reviewer's Analysis

Figure XIX: Simulated Steady-State Concentration-Time Profiles of Trofinetide following Oral Administration for Typical Subjects Experienced or Not Experienced Diarrhea



Typical subject was simulated for each weight-banded group:

Subject (b) (6) A 2-year-old subject with body weight of 10 kg, and eGFR of 163 mL/min/1.73 m^2

Subject A 6-year-old subject with body weight of 17 kg, and eGFR of 137 mL/min/1.73 m^2

Subject A 10-year-old subject with body weight of 28 kg, and eGFR of 143 mL/min/1.73 m^2

Subject A 17-year-old subject with body weight of 40 kg, and eGFR of 138 mL/min/1.73 m^2

Subject A 25-year-old subject with body weight of 75 kg, and eGFR of 113 mL/min/1.73 m^2

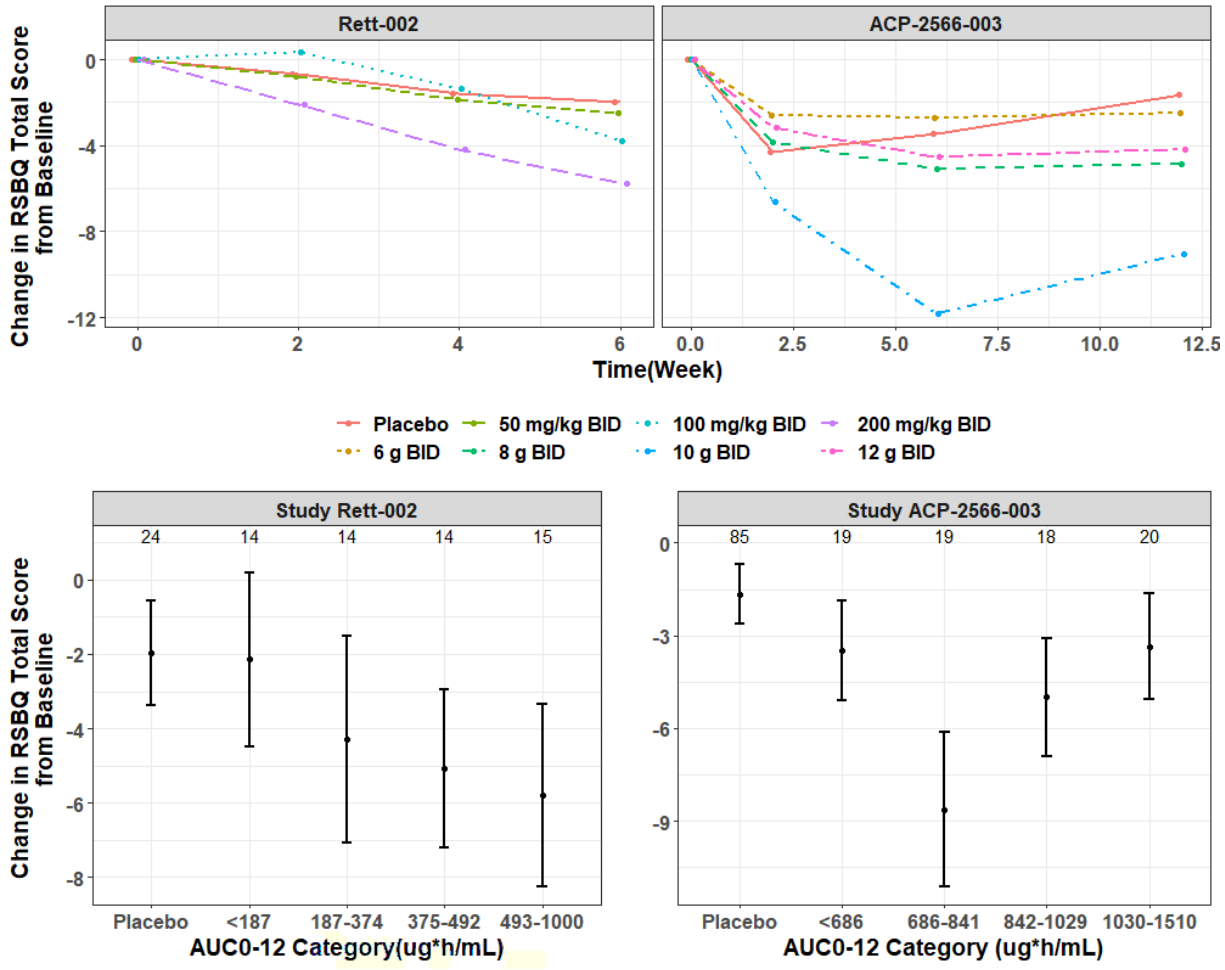
Source: Reviewer's Analysis

A PK/PD analysis of the drug exposure and efficacy data suggests a correlation between trofinetide exposure (AUC_{0-12}) and change in RSBQ total score or CSBS-DP-IT score as shown in Figure XIX and Figure XX. In general, the results from reviewer's analysis are in agreement with the applicant's findings.

Figure XXI shows the comparison for efficacy and the probability of TEAEs of diarrhea, vomiting, and weight decreased. A trofinetide dose/exposure-related effect was observed (Figure XXII), whereby higher trofinetide exposure was predictive of an increase in the probability of occurrence of diarrhea, vomiting, and weight decreased. For diarrhea, at the trofinetide AUC_{0-12} range of 800 to 1200 µg*h/mL, the model-predicted probability of diarrhea was 0.71 and 0.89, respectively. If the trofinetide dose was reduced to half, with AUC_{0-12} reducing to the range of 400 to 600 µg*h/mL, the model-predicted probability of diarrhea was 0.42 and 0.57, respectively. Meanwhile, the model predicted reduction of RSBQ total scores from baseline range from -3.5 to -4.9 at the trofinetide AUC_{0-12} ranged of 800 to 1200 µg*h/mL, while the reduction of RSBQ total scores from baseline was estimated to be from -2.2 to -2.9 at the end of the treatment (Figure XXI). Note that, this assumes that efficacy and PK follow similar time course. However,

we are unable to verify this assumption. The dose modification in efficacy study was done according to applicant's protocol (acp-2566-003 Protocol and Protocol Amendments). If the subject cannot tolerate administration of the full assigned dose (for example, if the subject experiences diarrhea) any time before the Week 6 visit, the Investigator may instruct the caregiver to reduce study drug to a dose as low as half the assigned dose. In addition, up to four doses (in total, consecutive or non-consecutive) may be held within the first 6 weeks. The dose reduction would help with management of diarrhea. The E-R model suggests that there would be a decrease of about 1.3 unit in RSBQ total score change. But the clinical trials data suggests in subjects with diarrhea, there was no significant loss in efficacy compared to placebo. Therefore, the labeling recommendation for 50% reduction in dose to manage the diarrhea is acceptable.

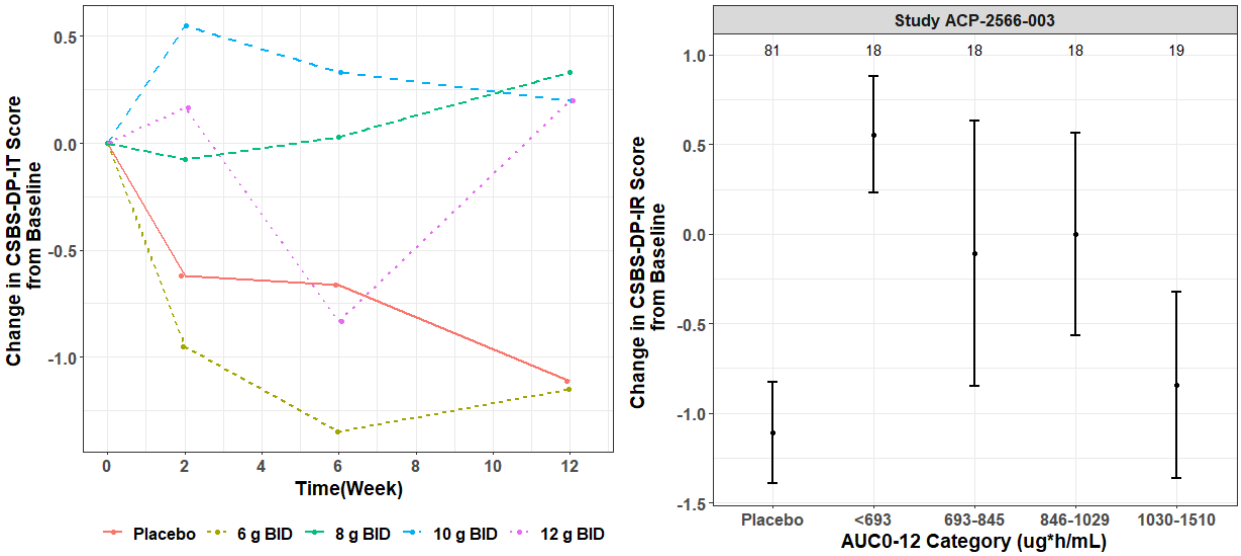
Figure XX. Change in RSBQ Total Scores from Baseline Versus Weeks (Top Panels) and Versus Trofinetide AUC_{0-12} Quantiles (Bottom Panels) from Phase 2 Study Rett-002 and Phase 3 Study ACP-2566-003



Note: The line and error bar in the bottom panels represent mean+/-sd of change in RSBQ total score from baseline at week 12.

Source: Reviewer's Analysis

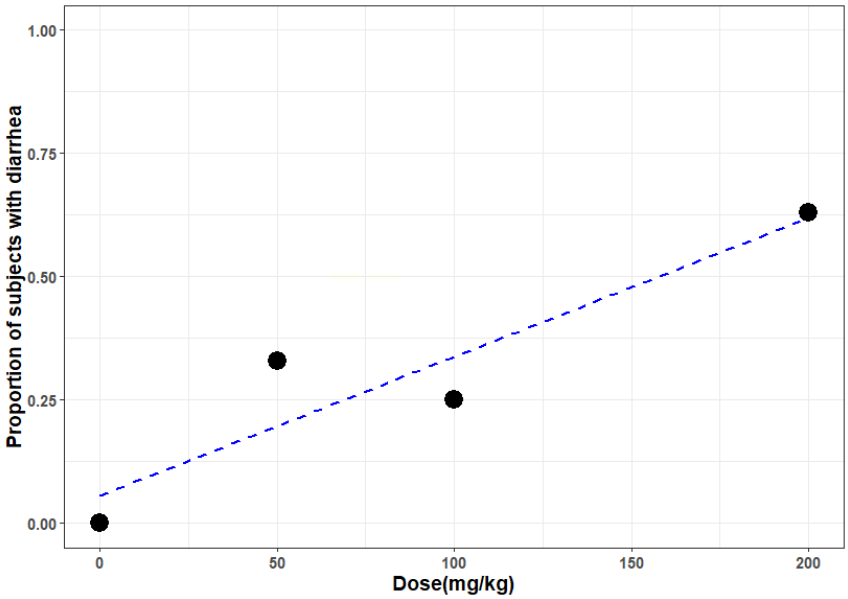
Figure XXI. Change in CSBS-DP-IT Score from Baseline Versus Weeks (Left) and Versus Trofinetide Cmax (Right)



The line and error bar in the right panel represent mean+/-sd of change in CSBS-DP-IT score from baseline at week 12.

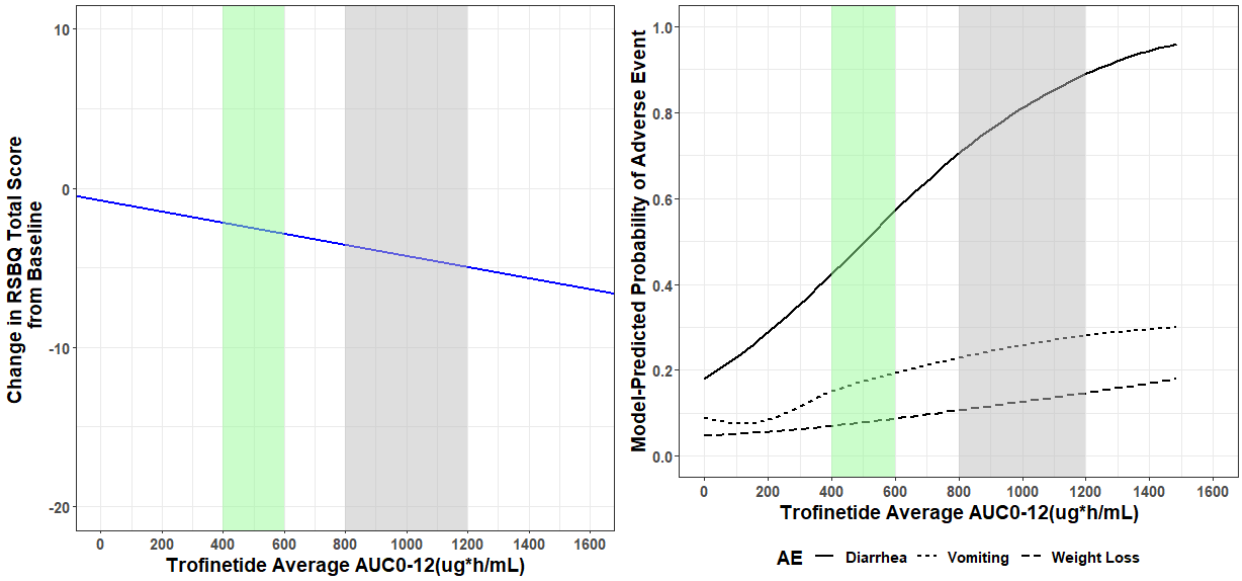
Source: Reviewer's Analysis

Figure XXII. Relationship of Dose and the Proportion of Subjects with Diarrhea from Phase 2 Study Rett-002



Source: Reviewer's Analysis

Figure XXIII. Relationships for RSBQ Total Scores (Left) and the Probability of TEAEs of Diarrhea, Vomiting, and Weight Decreased Versus Trofinetide AUC_{0-12} (Right)



Note: The grey shade represents the exposure range of 800-1200 ug*h/mL and the green shade represents the exposure range of 400-600 ug*h/mL after the trofinetide dose was reduced to half.

Source: Reviewer's Analysis

Listing of Analyses Codes and Output Files

File Name	Description	Location
PK_analysis_Trofinetide.R	PK and PopPK analysis file	\Review\2022\NDA 217026 Trofinetide\PPK analysis\Reviewer\dataset\study9\pk
ER_analysis_Trofinetide.R	E-R Analysis	\Review\2022\NDA 217026 Trofinetide\PPK analysis\Reviewer\dataset\study9\er

4.5 PBPK Analyses

Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's physiologically based pharmacokinetic (PBPK) analyses to:

- evaluate the drug-drug interaction (DDI) potentials of trofinetide as an inhibitor of CYP3A
- predict the PK of trofinetide in subjects with renal impairment

The Division of Pharmacometrics has reviewed the PBPK analyses reports (acp-2566-ms-002, -003 and -005), the response to FDA's information request submitted on November 15th, 2022 (sequence 0021), and

the modeling supporting files, and concluded that the PBPK analyses are adequate to evaluate the effects of trofinetide on the PK of midazolam and trofinetide was predicted to have a weak inhibitory effect on the sensitive CYP3A substrate midazolam.

At the time of the conclusion of this review, the applicant hasn't submitted the requested data to demonstrate the performance of the renal impairment population models described in the report #acp-2566-ms-002. (b) (4)

Background

Trofinetide is a synthetic analog of the tripeptide glycine-proline-glutamate (GPE), a product of the naturally occurring cleavage of insulin-like growth factor 1 (IGF-1) protein, is currently being developed for treatment of Rett syndrome in adults and pediatric patients 2 years of age and older. Trofinetide is administered twice daily orally or via gastrostomy tube as a ready-to-use oral solution. The recommended dose is based on body weight using banded weight ranges. The solution can be taken with or without food.

Trofinetide exposure increased in an approximately dose-proportional manner in healthy subjects following IV infusion, and in pediatric and adult female patients with Rett syndrome (5- to 45-year-old) following multiple oral doses of trofinetide (35-200 mg/kg BID). Minimal accumulation in exposure was observed following multiple doses of trofinetide. After a standardized FDA high-fat meal, the C_{max} and AUC of trofinetide decreased 21% and 6% compared to the fasted state following a single 12-g trofinetide.

Trofinetide has high solubility (>500 mg/mL in water, 2.3.S.1.3) and low plasma protein binding (~5%). In the human ADME study, 95% of the administered dose was recovered, with approximately 15% and 80% of the administered dose recovered in the feces (7.8% parent) and the urine (73.3% parent), respectively. These data suggest that trofinetide was well-absorbed and confirmed the results from the in vitro metabolism studies that trofinetide was minimally metabolized. Trofinetide is determined *in vitro* to be a competitive inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, and CYP3A4/5 with IC 50 values of 10, 18, 4.8, 10, 12, and >15 mM, respectively, and a time-dependent inhibitor of CYP2B6. Trofinetide is not a substrate of major transporters, but it inhibited probe substrate transport mediated by BCRP, OATP1B1, OATP1B3, OAT1, OCT2, MATE1 and MATE-2K, with IC50 values of 17.2, 13.5, 8.97, 11.7, 11.5, 10.9, 3.64, 3.68, and 0.951 mM, respectively. The applicant has not completed any clinical DDI studies to evaluate these *in vitro* findings. Refer to the Clinical Pharmacology review section for detail information on trofinetide regarding its ADME properties, *in vitro* and clinical studies.

Methods

The PBPKPlus module of GastroPlus (version 9.8) were used to build the PBPK model and run the simulations. ADMET Predictor® (Version 9.5) was applied to obtain in silico predicted estimates of key physicochemical and biopharmaceutical properties from the chemical structure of trofinetide. The PBPK model for trofinetide was developed using clinical studies with full PK profiles. The model was initially

developed from studies in which trofinetide was administrated intravenously (IV) (Neu-2566-HV-001, -002 and -003), then the oral absorption component of the model was captured using data from studies in which trofinetide was administrated orally (Neu-2566-HV-005 and Neu-2566-RETT-001). The final model was further updated with the data from the food effect study (ACP-2566-006), QTc study (ACP-2566-008) and human ADME study (ACP-2566-007) which were conducted using the clinically relevant doses of trofinetide. The trofinetide PBPK model consists of an ACAT model for the intestinal dissolution and absorption, a permeability-limited model for all systemic tissue distribution and a model for elimination via renal excretion which was define by glomerular filtration rate times the unbound fraction in plasma. The final model input parameters were summarized in ***Table i***. To simulate drug interaction with midazolam, the midazolam model in the GastroPlus database was used without any modification.

Table i Final input parameters in the trofinetide model

Property	Value	Reference
LogD (at pH = 4.0)	-2.93	Acadia Pharmaceuticals, Inc.[a]
Aqueous Solubility (pH = 3.96)	800 mg/mL	Acadia Pharmaceuticals, Inc.[b,c]
pKa Base Acid Acid	 8.61 4.42 2.97	Acadia Pharmaceuticals, Inc.[a]
Effective Permeability	Previous value: 0.39 (cm/s × 10^{4}) Updated Value: 0.60 (cm/s × 10^{4}) for 12-gram dose	Model fit parameters
Fraction Unbound in Plasma (Human)	94.5% to 96.0%	Acadia Pharmaceuticals, Inc.[d]
Blood/Plasma Concentration Ratio (Rat)	0.525	Acadia Pharmaceuticals, Inc.[d]
Absorption Scale Factor Coefficient: C3 and C4	 5, 0.4	Model fit parameters
Specific PStc	1.215 × 10^{-3} (mL/s/mL)	Model fit parameter

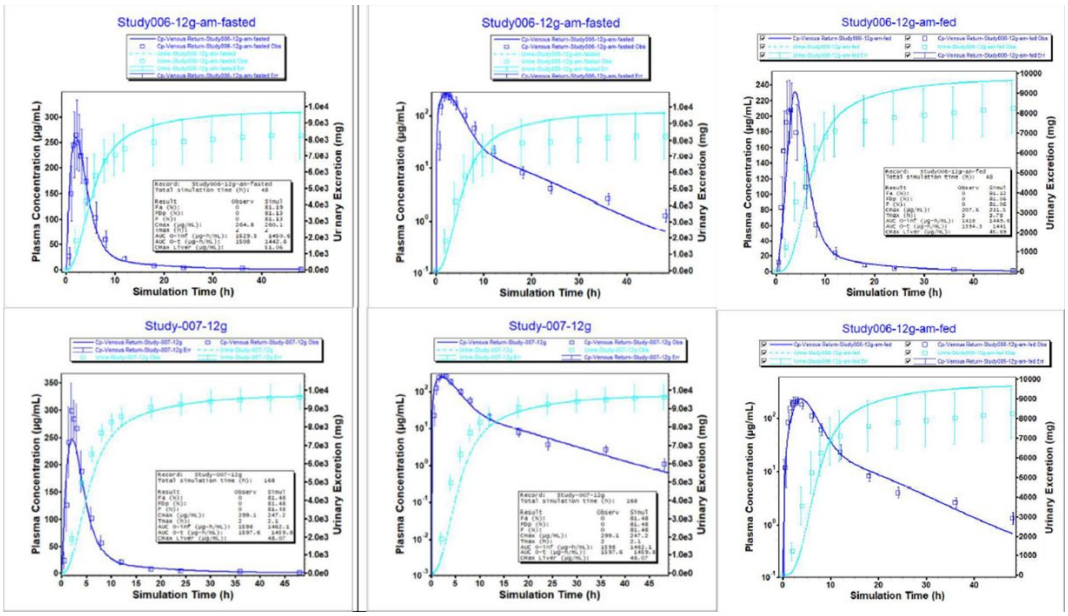
Abbreviations: C3/C4, absorption scale factor coefficients; LogD, octanol/water distribution coefficient; pKa, negative logarithm of the ionization constant of an acid; PStc, permeability-surface area product.

Results

1. Can the PBPK model adequately describe the PK profiles of trofinetide?

Yes. The trofinetide PBPK model could reasonably well describe trofinetide concentration- or amount-time profiles in plasma or urine, respectively, following administration of single 12-g dose of trofinetide (**Figure i)**

***Figure i*. Simulated and observed PK profiles in plasma and urine following oral administration of single dose of trofinetide in healthy subjects under the fasted and fed states**



Abbreviations: AUC0-inf, area under the plasma concentration-time curve from time zero to infinity; AUC0-t, area under the plasma concentration-time curve from time zero to the last measurable time point; Cmax, maximum plasma concentration; Cp, plasma concentration; Err, error bars; F, bioavailability; Fa, fraction of dose absorbed; FDp, fraction of dose reaching the portal vein; Obs, observed; Observ, observed; Simul, simulation; Tmax, time of maximum concentration.

Source: Figure 2 in PBPK report acp-2566-ms-002 Addendum

2. Can PBPK analyses predict the effect of trofinetide on the PK of sensitive CYP3A substrate midazolam?

Yes, the PBPK analyses could predict the effect of trofinetide on the PK of midazolam and the results are shown in ***Table ii***.

Table ii Predicted effects of single or multiple oral doses of 12 g trofinetide on a single dose of intravenous or oral midazolam

Trofinetide dosing regimen	Midazolam dosing regimen	AUC ratio	C_{max} ratio
12g SD	2mg IV	1.00	1.00
12g SD	15mg PO	1.30	1.18
12g BID for 6 days	15mg PO	1.33	1.20

Oral midazolam was administered concurrently with oral trofinetide on Day 1 following a single dose (SD) of trofinetide or on Day 6 following twice daily doses (BID) of trofinetide. Intravenous midazolam was administered 2 hours after oral trofinetide.

Reviewer's comment:

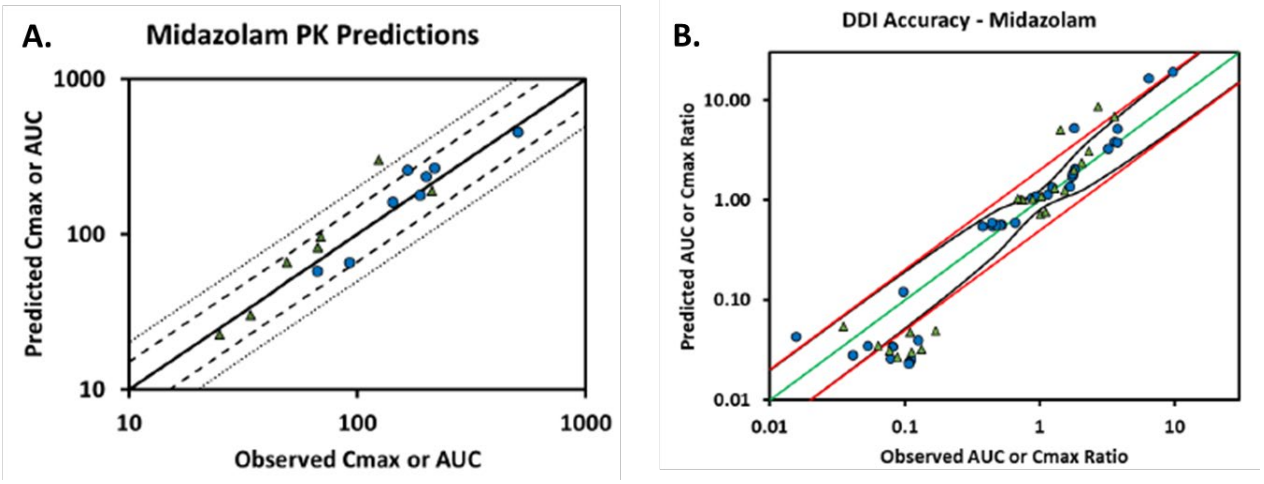
There are three factors determining the accuracy of midazolam DDI prediction: trofinetide plasma concentration, CYP3A inhibition parameter (Ki), and midazolam PBPK model.

- *There is uncertainty about whether the trofinetide concentration at the intestine was well predicted. The permeability of trofinetide could not be determined because the concentration transported in the Caco-2 study was below the limit of detection of the analytical method*

(Study 16763). The absorption parameters in the model were adjusted to capture the terminal portion of the oral PK profiles of the 100 mg/kg solution dose in Study Neu-2566-HV-005 which was verified with PK data from Study ACP-2566-007. In addition, the effective permeability was optimized to appropriately account for the observed absorption after oral administration of the 12g dose (ACP-2566-007). As shown in **Figure i***, the trofinetide PBPK model could reasonably describe the time courses of trofinetide plasma concentrations and the amount of urinary excretion. Because trofinetide is predominantly eliminated via urinary excretion, the time course of urinary excretion may also reflect the time course of the amount absorbed. Therefore, it may be assumed that the absorption of trofinetide at the intestine was reasonably simulated.*

- *In vitro CYP inhibition study showed that trofinetide inhibited the sensitive CYP3A substrate midazolam with an IC50 value of > 15 mM. The Ki value was assumed to be half of the IC50 value which is reasonable as the substrate concentration used in this experiment was determined by midazolam K_m value (XT185143). The IC50 value of trofinetide may be less likely to be affected by nonspecific binding, the major cause for inaccuracy of in vitro inhibition parameters, because trofinetide is highly soluble (>500 mg/mL) and has low plasma protein binding (5%). Moreover, a low microsomal protein concentration (0.05 mg/mL) was used in the inhibition study.*
- *For midazolam PBPK model, the reviewer issued an Information Request (11/03/2022) requesting the applicant to provide the data that could demonstrate the ability of the midazolam model to predict the observed midazolam PK following intravenous and oral administration of midazolam and predict the clinical drug interactions with various known weak to strong CYP3A inhibitors available in the public domain. The results are summarized in* ***Figure ii****.* The midazolam PBPK model could reasonably well capture the midazolam plasma concentration-time profiles in the absence or presence of a perpetrator (data not shown) and predict midazolam PK parameters (**Figure iiA**). The midazolam model could predict the DDIs of ranitidine, diltiazem and fluconazole following oral administration with IV and oral midazolam, and the predicted AUC increases were all within the bioequivalence bounds of the observed values (**Figure iiB**). It should be noted that interactions between voriconazole and midazolam following oral administration of both drugs were overpredicted (**Figure iiB**). Given that the PBPK analyses can described its interaction with both IV and oral midazolam following IV administration of voriconazole, the voriconazole concentrations in the intestine might be overestimated using the default voriconazole PBPK model.

Figure ii Performance of the midazolam PBPK model to predict the midazolam PK and DDI with CYP3A inhibitors



A. Blue (circles) and green (triangles) represent $AUC_{0\text{-}inf}$ and C_{max}, respectively. Solid, dashed, and dotted lines show identity line, 1.5-fold, and 2-fold prediction errors, respectively.

B. Blue (circles) and green (triangles) represent $AUC_{0\text{-}t}$ and C_{max}, respectively. Red lines represent 2-fold prediction error, and black lines represent fold prediction error per Guest's criteria.

Source: Midazolam PBPK-DDI model report submitted in response to IR submitted on November 15th, 2022 (sequence 0021).

Conclusions

The PBPK analyses are adequate to evaluate the effect of trofinetide on the PK of midazolam. Trofinetide was predicted to have a weak inhibitory effect on sensitive CYP3A substrates such as midazolam.

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