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ORIGINAL RESEARCH ARTICLE

Physiologically-Based Pharmacokinetic Modeling of Trofinetide in Moderate Renal Impairment for Phase 1 Clinical Study Dose Selection with Model Validation

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Abstract

Background and Objectives Trofinetide, the first approved treatment for Rett syndrome (RTT), is primarily excreted unchanged in the urine; therefore, it is important to assess the extent to which the exposure is affected in patients with renal impairment. Pharmacokinetic modeling overcomes the challenge of dose finding in phase 1 studies that include special populations where there is the potential for increased exposure to study drug. The objectives of this phase 1 study were to evaluate trofinetide pharmacokinetics, safety, and tolerability in a population with moderate renal impairment and normal renal function. The observed pharmacokinetic profiles were used to validate the dosing adjustments in moderate renal impairment that were previously predicted using a physiologically-based pharmacokinetic (PBPK) model.

Methods The PBPK model was first used to predict dose adjustments that are necessary to achieve similar exposure in the four stages of renal impairment (mild, moderate, severe, end stage renal disease) as in healthy controls. The predicted dose adjustment from 12 to 6 g for the moderate renal impairment category was then applied to the phase 1 clinical study. Subsequent validation of the PBPK model was achieved by comparing the model-predicted and clinically observed exposures in subjects with moderate renal impairment. In a phase 1, open-label study, trofinetide exposure was assessed in healthy ($n = 10$) and moderate renal impairment ($n = 10$) participants receiving single oral doses of 12 g or 6 g, respectively. Observed exposures [area under the blood concentration–time curve from time 0 to infinity (AUC_{inf}) and maximum concentration (C_{max})] were compared with predicted exposures from simulations in virtual healthy and moderate renal impairment populations ($n = 100$) to validate a PBPK model of renal impairment that had previously predicted doses across renal impairment categories.

Results Dose-normalized geometric mean ratios for C_{max} were comparable [1.02 (90% CI 0.69–1.50)] while AUC_{inf} was approximately two-fold higher [1.81 (90% CI 1.31–2.50)] in moderate renal impairment participants compared with healthy controls. These observed values closely aligned with predicted distributions. Treatment-emergent adverse events were reported in two (20.0%) participants with moderate renal impairment and four healthy participants (40.0%).

Conclusion PBPK modeling of trofinetide in a virtual population with moderate renal impairment predicted a 50% dose reduction compared to individuals with normal renal function. Comparison of observed pharmacokinetic results from a phase 1 study in subjects with moderate renal impairment and matched healthy participants to the model-predicted exposures validated this dose reduction. No new safety concerns for trofinetide emerged.

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Graphical abstract

A Phase 1 Clinical Study and Physiologically Based Pharmacokinetic Modeling to Evaluate the Impact of Renal Impairment on the Pharmacokinetics of Trofinetide

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BACKGROUND

Renal impairment has not been reported as a comorbid condition in Rett syndrome, a rare neurodevelopmental disorder

Trofinetide, **the first approved treatment for Rett syndrome**, is primarily excreted in the urine, so it is important to assess the extent to which renal impairment affects exposure

PBPK modeling suggested a 50% dose reduction in a virtual population with moderate renal impairment to achieve the same exposure following the recommended trofinetide dose in a healthy population

METHODS

Phase 1 study

Trofinetide **6 g** single oral dose
Moderate renal impairment
(*N* = 10)

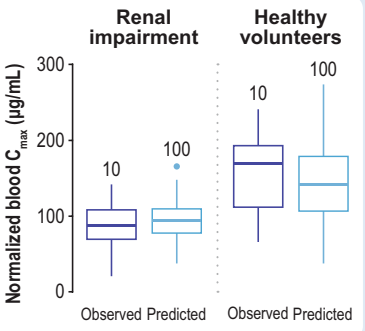
Trofinetide **12 g** single oral dose
Matching healthy controls
(*N* = 10)

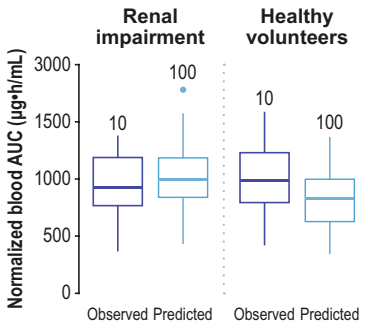
Validation of the PBPK model

Observed AUC and C_{max} = **Predicted** AUC and C_{max}

Observed AUC and C_{max} ≠ **Predicted** AUC and C_{max}

RESULTS





CONCLUSIONS

- Observed and predicted trofinetide exposures confirm that a **50% dose reduction** in individuals with **moderate renal impairment** achieves the same target exposure as the recommended dose in individuals with normal renal function
- Agreement between the observed and predicted exposures validates the PBPK model of renal impairment

Abbreviations: *AUC* area under the blood concentration-time curve from time 0 to infinity, C_{max} maximum drug concentration, *PBPK* physiologically based pharmacokinetic

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Plain Language Summary

Trofinetide is the first approved treatment for Rett syndrome, a rare genetic condition that affects brain development. People with Rett syndrome do not usually experience problems with their kidneys but, because trofinetide is mostly removed from the body in the urine, it is important to check if the dose needs to be reduced when the kidneys do not work properly. Researchers first used computer modeling to predict how much the recommended dose of trofinetide in people with normal kidney function would have to be reduced in virtual populations with mild, moderate, or severe kidney disease, or kidney failure. To check if the computer model was correct, the blood levels of trofinetide were compared between 10 people with moderate kidney disease given a 50% reduced dose as predicted by the model and 10 healthy people given the recommended dose. The levels of trofinetide measured in people with moderate kidney disease were similar to the levels in healthy people and were similar to the predicted levels in the model, so together these results supported the 50% dose reduction in people with moderate kidney disease. The similarity between the results in the study and the model confirms that the model is good at predicting how much the dose needs to change in people with kidney disease.

Key Points

Renal impairment has not been reported as a comorbid condition in Rett syndrome, but since trofinetide is primarily excreted unchanged in the urine (percentage of dose excreted ~70%), it is important to assess the extent to which the exposure is affected when renal impairment is present.

Observed exposures from a phase 1 study and predicted exposures from physiologically based pharmacokinetic (PBPK) modeling supported a 50% dose reduction in individuals with moderate renal impairment to achieve the same exposure as individuals with normal renal function given the recommended dose.

Agreement between the observed and predicted exposures validates the recommended dose adjustments in the moderate renal impairment population from the PBPK model.

1 Introduction

Rett syndrome (RTT) is a rare, debilitating, neurodevelopmental disorder, that primarily affects females [1], but also a smaller number of males [2]. There was no available treatment for RTT until the FDA-approval of trofinetide (DAYBUE™; Acadia Pharmaceuticals) in March 2023, which is indicated in adult and pediatric patients 2 years of age and older. Trofinetide is an oral solution dosed according to body weight: twice daily doses of 5 g (≥ 9 to < 12 kg), 6 g (≥ 12 to < 20 kg), 8 g (≥ 20 to < 35 kg), 10 g (≥ 35 to < 50 kg), or 12 g (> 50 kg).

RTT is characterized by multisystem comorbidities and the need for polypharmacy to manage symptoms that include seizures and gastrointestinal problems such as constipation [3, 4]. Comorbidities that could affect trofinetide exposure include hepatic or renal impairment; however, both are rarely reported in RTT. Furthermore, a physiologically-based pharmacokinetic (PBPK) modeling study excluded any clinically relevant impact of hepatic impairment on trofinetide exposure [5], which is consistent with the fact that less than 5% of trofinetide undergoes metabolic elimination [6]. The issue of polypharmacy could also contribute to increased exposure due to drug–drug interactions; however, PBPK modeling indicated that trofinetide did not affect CYP3A4-mediated drug metabolism in the liver and was a weak inhibitor of CYP3A4 activity in the intestines, so there is limited potential for drug interactions with trofinetide [7]. Trofinetide shows linear pharmacokinetics across the dose range tested in pediatric patients with RTT (twice daily 50 mg/kg, 100 mg/kg, or 200 mg/kg) [8], which is consistent with population pharmacokinetic modeling in healthy adults following single and repeated ascending doses (oral doses ranged from 6.0 to 100 mg/kg) [9]. Although renal impairment is not reported as a comorbidity in RTT [3], trofinetide is primarily excreted unchanged in urine [6]. Therefore, given the predominance of renal elimination, it is important to confirm whether renal impairment could potentially impact the clearance of trofinetide in individuals with RTT, and lead to increased exposure that would necessitate dose modification.

PBPK modeling can help guide dose finding in phase 1 studies that include populations where there is the potential for excessive exposure to study drug. PBPK modeling can provide simulations of various clinical scenarios to aid in designing clinical trials, or to evaluate the need for a clinical trial, and can be used to predict clearance in renally impaired subjects, particularly if the drug is exclusively renally eliminated and is not a substrate of a kidney transporter. Given that trofinetide meets both of these criteria [6] (CEREP Report Study No. 16763: P-Glycoprotein Transporter Interaction Studies; unpublished data), a previously validated PBPK model [7] was refined to predict exposure in virtual subjects based on the classification of renal function according to the estimated glomerular filtration rate [eGFR = 60–89, 30–59, 15–29, < 15 mL/min/1.73 m^2 not on dialysis (requiring dialysis),

respectively, for mild, moderate, and severe renal impairment, and end stage renal disease (ESRD)], and to predict dose adjustments that are necessary to achieve similar total exposure in the four stages of renal impairment. Predicted dose adjustments were then applied to a phase 1 clinical study in populations with moderate renal impairment and normal renal function, and the observed exposure data were compared with the model-predicted exposures for validation. The selection of the moderate renal impairment category provides a clinically relevant condition under which to verify the PBPK-predicted influence of renal impairment on trofinetide exposure and clearance. Ethical and clinical challenges precluded the investigation of more severe categories of renal impairment in the phase 1 study.

The objectives were to evaluate the pharmacokinetics and the safety and tolerability of a single oral dose of trofinetide in study participants with moderate renal impairment relative to healthy participants, and to verify the PBPK model for renal impairment by comparing observed versus predicted pharmacokinetic and exposure data.

2 Methods

2.1 PBPK modeling to guide dose selection in the phase 1 study

A previously validated PBPK model that has been used to predict potential drug–drug interactions and the impact of hepatic impairment on trofinetide pharmacokinetics [5, 7], was used to predict dose adjustments according to the four stages of renal impairment.

The model incorporated trofinetide physicochemical and biopharmaceutical properties and information on absorption, dissolution, and elimination that was determined experimentally or optimized during model development (Table 1). Renal elimination of trofinetide was defined in the PBPK model by GFR multiplied by the fraction of unbound drug in the plasma (0.9525). It was assumed that trofinetide is absorbed from the gut via passive diffusion, is eliminated in urine by passive renal filtration, and has a blood to plasma concentration ratio (R_{bp}) value of 0.525 for healthy adults (since confirmed in a pharmacokinetic radiolabeled study in which blood-to-plasma total radioactivity ratios ranged from 0.529 to 0.592) [6]. The renal impairment physiologies in the PBPK model were defined within GastroPlus® software based on the classification of renal function according

Table 1 Trofinetide physicochemical and biopharmaceutical properties for trofinetide model development

Property	Value	Reference
LogD (at pH = 4.0)	− 2.93	Acadia Pharmaceuticals[a]
Aqueous solubility (pH = 3.96)	800 mg/mL[a]	Acadia Pharmaceuticals[b,c]
pKa		Acadia Pharmaceuticals[a]
Base	8.61	
Acid	4.42	
Acid	2.97	
Effective permeability	0.60 (cm/s × 10^4)	Model fit parameter
Fraction unbound in plasma (human)	94.5–96.0%	Acadia Pharmaceuticals[d]
R_{bp} (rat)	0.525	Acadia Pharmaceuticals[d]
ASF C3 and C4	5.0 and 0.4	Model fit parameters
Specific PStc	1.215 × 10^{-3} (mL/s/mL)	Model fit parameter

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ASF C3 and C4 absorption scale factor coefficients 3 and 4, *LogD* octanol/water distribution coefficient, *pKa* negative logarithm of the ionization constant of an acid, *PStc* permeability-surface area product, R_{bp} blood to plasma concentration ratio

[a]Data on file (Trofinetide: physicochemical characterization. Final report, May 2020)

[b]The defined aqueous solubility used in the presented modeling was set to 800 mg/mL, based on initial assessments. However, the updated aqueous solubility reported in the indicated reference is approximately 575 mg/mL. Sensitivity analyses have demonstrated that this difference in aqueous solubility does not impact the predictions

[c]Data on file (Dissolution rates of crystalline and amorphous trofinetide in water and organic solvents. Final report, June 2019)

[d]Data on file (Investigator's brochure for trofinetide. Edition 15.0, 18 March 2021)

to the previously described eGFR values [10]. Systemic physiological changes collected from multiple literature sources were included in the renal impairment physiologies [11].

Simulated doses were adjusted in 1-g increments for each renal impairment physiology to most closely match the predicted blood area under the concentration–time curve from time 0 to infinity (AUC_{inf}) for the healthy subject administered a single 12-g trofinetide dose (blood AUC_{inf} of 856 µg·h/mL). The model predicted that renal impairment would result in a clinically meaningful increase in the AUC_{inf} for trofinetide with lesser impact on the maximum trofinetide concentration (C_{max}), and the extent of exposure increase was dependent upon the degree of renal impairment. The dose adjustment from the PBPK model for moderate renal impairment predicted to result in an AUC_{inf} value (854 µg·h/mL) that most closely matched the AUC_{inf} for the healthy subject was 6 g (ACP-2566-MS-003 study; unpublished data) and this trofinetide dose was applied to the phase 1 study.

2.2 Phase 1 Study Design

This Phase 1, open-label, single-dose study was conducted at four sites in the US and consisted of a screening period (day − 28 to day − 1), treatment period (days 1–6), and a safety follow-up period (30 days after last dose), with an overall study duration of approximately 9 weeks (Fig. 1). The study was performed in accordance with Health Insurance Portability and Accountability Act regulations, Food and Drug Administration Good Clinical Practice (GCP) Regulations, and International Council for Harmonisation GCP Guidelines, and clinical safety data management. Written informed consent was obtained from each participant prior to any screening procedures.

2.3 Study Population

Eligible study participants were adult males or females 18–75 years of age with eGFR ≥ 90 mL/min/1.73 m^2 (healthy participants) or with eGFR 30–59 mL/min/1.73 m^2 (moderate renal impairment) on screening that was confirmed with additional testing at least 72 h later, who had hemoglobin levels > 9.5 g/dL (moderate renal impairment) or > 11.5 g/dL (healthy participants) at screening, and liver function tests ≤ 2.5 × upper limit of normal (moderate renal impairment only). Participants with moderate renal impairment were excluded if they had a current functioning kidney transplant, or any disease or condition, other than renal impairment, that might affect drug absorption, metabolism, or excretion. Healthy and moderate renal impairment participants were excluded if they had a history or current evidence of a serious and/or unstable cardiovascular, respiratory, endocrine, gastrointestinal, renal (healthy participants only), hepatic, hematologic, immunologic, genitourinary, psychiatric or neurologic abnormality or disease or other medical disorder that would interfere with the ability to evaluate participants; body mass index (BMI) > 38 kg/m^2 or < 18 kg/m^2 or body weight > 100 kg or < 50 kg; and uncontrolled hypertension [systolic blood pressure (SBP) > 160 mmHg and diastolic blood pressure (DBP) > 95 mmHg in moderate renal impairment participants or SBP ≥ 140 mmHg and DBP ≥ 90 mmHg in healthy participants].

2.4 Intervention

Healthy control participants received a single oral 12-g trofinetide dose. Participants with moderate renal impairment were to receive a single oral 6--g trofinetide dose; however, two participants with moderate renal impairment received an 8-g dose of trofinetide due to dosing error. Trofinetide was provided as a 200-mg/mL solution. On day 1, participants received a single oral dose of trofinetide with up to 250 mL water in the morning after an overnight fast of approximately

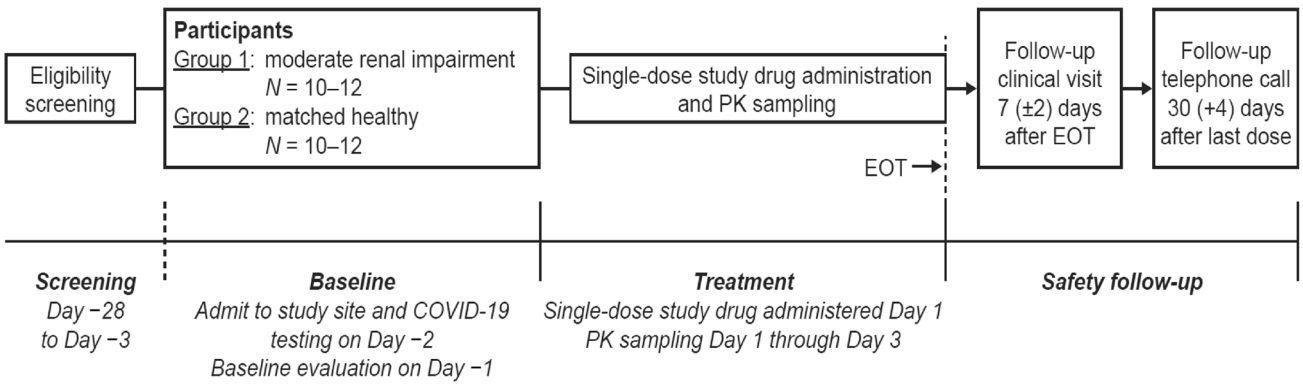

Fig. 1 Schematic of study design. *EOT* end of treatment, *PK* pharmacokinetic

10 h and continued to fast 4 h after dosing. Water was restricted for 1-h postdose and then was allowed ad libitum.

2.5 Pharmacokinetic Assessments

Pharmacokinetic parameters were assessed in whole blood and urine samples. Trofinetide concentrations were determined in lithium-heparinized whole blood and in urine using a validated liquid chromatography with tandem mass spectrometry assay. Trofinetide was validated in blood for a range of 0.10–100 µg/mL and in urine for a range of 0.05–50 mg/mL.

Whole blood concentrations of trofinetide were measured from a blood sample obtained on day 1, 1 h before dosing, and at the following timepoints after dosing: 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, and 120 h. Blood samples continued to be collected at the same timepoints throughout the treatment period (days 2–6). The following single-dose pharmacokinetic exposure parameters were determined in whole blood: C_{max}, time to C_{max} (T_{max}), area under the blood concentration–time curve from time 0 to the time of the last detectable drug concentration ($AUC_{0\text{-}t}$), and AUC_{inf}.

Urine was collected with the volume recorded as voided after dosing and lasting through day 6. For the first 12 h after dosing on day 1, urine was collected over 2-h intervals. During the 12- to 48-h period after dosing, urine was collected over 6-h intervals. During each subsequent 24-h period, urine was collected up to day 6. Pooled urine samples were assayed for trofinetide concentration. Pharmacokinetic parameters assessed in urine included renal clearance of drug (CL_r), and the amount and percentage of dose excreted renally as unchanged drug (Ae_u and $\%fe_u$).

2.6 Safety Assessments

Safety and tolerability assessments included the frequency of treatment-emergent adverse events (TEAEs), physical examinations, vital signs, 12-lead electrocardiogram (ECG), and clinical laboratory tests.

2.7 Statistical Analysis

The sample size for this study was based upon clinical considerations. No statistical methods were used to determine the sample size. However, a retrospective sample size reassessment showed the sample size was powered with at least 80% to target a 95% confidence interval within 60% and 140% of the geometric mean estimate of clearance and volume of distribution in blood for trofinetide in the moderate renal impairment and healthy groups. Up to 24 participants (10–12 per group) were to be enrolled in the study, and a minimum of two participants were required for each eGFR subset within the ranges based on modification of diet in renal disease equation for moderate renal impairment (30–39, 40–49, and 50–59 mL/min/1.73 m^2) [12, 13]. Healthy control participants were enrolled to individually match with enrolled participants with moderate renal impairment based on age (± 10 years in age, but between 18 and 75 years of age), race, gender, and BMI (± 20% in BMI, but still within BMI limits) characteristics. Mean exposure values (AUC and C_{max}) were summarized in the participants who received the 6-g dose ($n = 8$; and matched healthy controls) while statistical comparisons were based on data from all participants with moderate renal impairment ($n = 10$; and matched healthy controls) using exposure values normalized to a 6-g dose. Correlation of dose-normalized AUC and C_{max} with creatinine clearance (CL_{cr}) that was estimated using the Cockcroft–Gault equation [14], was evaluated using Pearson's coefficient to measure the strength and direction of the relationship between two variables (coefficient values range from − 1 to + 1).

The safety analysis set consisted of all participants who received trofinetide. The pharmacokinetic analysis set included all participants in the safety analysis set who had sufficient blood drug concentration data to estimate at least one pharmacokinetic parameter. All inferential statistical testing was two-sided, and log-transformation of values was performed where applicable. For dose-dependent pharmacokinetic parameters (AUC and C_{max}), group comparisons of the dose-normalized, log-transformed values were performed using analysis of variance. The 90% confidence interval for the least squares geometric mean ratio comparing the moderate renal impairment participants versus their matched healthy controls was constructed. All statistical analyses were implemented using SAS® version 9.4 or higher.

3 PBPK Model Validation

Upon completion of the phase 1 study, pharmacokinetic data from the subjects with moderate renal impairment and the matched healthy subjects were available to allow validation of the predicted exposures from the PBPK model based on simulated trofinetide dosing scenarios using validated healthy adult physiologies and physiologies representing moderate renal impairment.

3.1 Analysis Dataset

Trofinetide blood concentration data, body weight, height, race, sex, eGFR, and other physiologic data were obtained from the phase 1 study and incorporated into datasets for use in the GastroPlus software (version 9.8.2) [15] that was used for PBPK simulations. Microsoft® Excel® and R software

(version 4.1.3) [16] were used to compile and post process the GastroPlus simulated output. Other analyses and generation of graphs were performed using a family of Tidyverse packages in R.

To validate the PBPK model-based trofinetide exposure predictions, the observed trofinetide exposures (individual concentrations, C_{max}, and AUC_{inf}) from participants evaluated in the phase 1 study were compared with population predicted exposures for age-, bodyweight-, sex-, and eGFR-matched virtual populations that consisted of 100 virtual subjects with moderate renal impairment and 100 virtual healthy controls. The eGFR values from the study participants were calculated using the modification of diet in renal disease equation: eGFR (mL/min/1.73 m^2) = 175 × (creatinine, mg/dL)$^{-1.154}$ × (age, years.)$^{-0.203}$ × 1.212 (if black) × 0.742 (if female) [12, 13] and were assumed to be equivalent to the actual GFRs and were used in the virtual population physiologies. GFR values in the PBPK physiologies were not calculated but were assigned based on the renal impairment classifications. Separate exposure comparisons were prepared for the healthy and the moderate renal impairment populations.

3.2 Renal Impairment Validation Process

Model-based population simulations were performed to predict trofinetide pharmacokinetic exposures. The population simulation module in GastroPlus runs a series of simulations, for a selected number of virtual subjects, created by random sampling of physiological input parameters to generate a predicted population outcome. The PBPK physiologies, including organ weights, volumes, and blood flows, were generated by the Population Estimates for Age-Related Physiology (PEAR Physiology™) module, a part of the PBPKPlus module in GastroPlus. The default variability (%CV) within GastroPlus was used for all parameters to simulate the potential interindividual variability expected in trofinetide pharmacokinetics.

Using the defined physiologic characteristic distribution for the healthy or the moderate renal impairment populations from the phase 1 study, and the PBPK model settings developed for trofinetide, a virtual population (or stochastic) simulation was run using the single trofinetide dose of 12 g (healthy control) or 6 g (moderate renal impairment). The population-simulation module is used for evaluating the combined effects of variations in population physiology and formulation variables that are not precise values but for which distributions of values can be estimated. All assayed trofinetide concentrations were reported in terms of blood concentrations and were converted to plasma concentrations for modeling purposes using the R_{bp} of 0.525. Individual trofinetide plasma C_{max} and AUC_{inf} values were calculated and provided in the stochastic simulation output from GastroPlus. The individual hematocrit for each virtual subject was used to convert C_{max} and AUC_{inf} values from plasma to blood. Predicted exposure measures including the trofinetide blood concentration versus time profiles, C_{max}, and AUC_{inf} were determined. Changes in the trofinetide R_{bp} due to reduced hematocrit values for the moderate renal impairment physiology were taken into account in the predictions of blood concentrations and pharmacokinetic parameters using Eq. 1:

$$Rbp_{adj} = \frac{Hct_{adj}}{Hct_{adult}} \times \left(Rbp_{adult} - \left(1 - Hct_{adult}\right)\right) + \left(1 - Hct_{adj}\right)$$

where Rbp_{adult} is the blood to plasma concentration ratio in healthy adults (0.525 for trofinetide), Hct_{adult} is the hematocrit (expressed as a fraction) in healthy adults (0.45), Hct_{adj} is the hematocrit (expressed as a fraction) in the assessed population blood, and Rbp_{adj} is the adjusted blood to plasma concentration ratio for the assessed population.

Validation of the model consisted of graphical displays comparing the predicted virtual population output to the observed results. The C_{max} and AUC_{inf} distributions for the moderate renal impairment participants were based on values normalized to a 6-g trofinetide dose to allow a proper comparison to the stochastic simulation output of a 6-g dose.

3.3 Predicted Pediatric Dosing: Deterministic Simulations

To provide additional support for dose adjustment recommendations in pediatric subjects with moderate renal impairment, additional PBPK deterministic simulations in pediatric physiologies were completed. The ages ranged from 2 to 13 years with weights ranging from approximately 9 to 52 kg. Initial 2-year physiologies were created based on the 10th and 50th body weight and height percentiles for pediatric patients with RTT (9.3 and 11.6 kg) (ACP-2566-MS-006 Study; unpublished data).

Virtual female pediatric physiologies encompassing the pediatric body weight range were created in GastroPlus with either normal renal function or with mild or moderate renal impairment and assumed similar systemic physiological changes as identified for renal impairment in adults including changes in GFR, hematocrit, plasma protein levels, hepatic CYP450 enzymes, and gastric emptying rates [11, 17]. Deterministic simulations for virtual pediatric subjects represented by the mild renal impairment physiologies employed the recommended dose for healthy individuals based on the pediatric dosing guidelines. For the moderate renal impairment physiologies, the simulation output was

presented for single oral doses that were 50% of the recommended dose for healthy individuals with normal renal function.

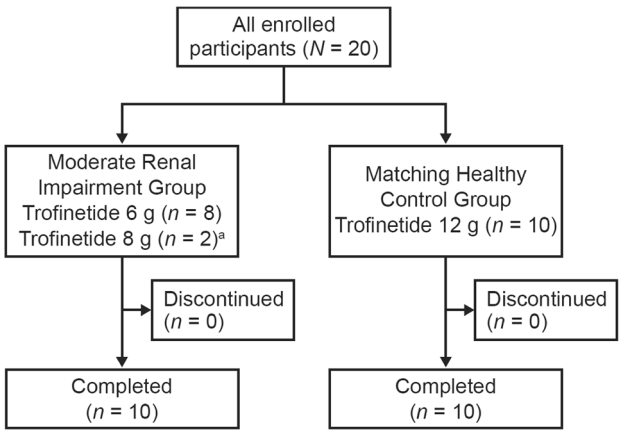

Fig. 2 Study participant disposition. [a]Two participants with moderate renal impairment received a single oral trofinetide dose of 8 g (dosing error)

4 Results

4.1 Demographics and Baseline Characteristics

Twenty participants were enrolled into the phase 1 study, which was initiated on August 12, 2021, and completed on December 1, 2022. Ten participants had moderate renal impairment and received a single trofinetide dose of 6 g ($n = 8$) or 8 g ($n = 2$). The 10 healthy participants received a single trofinetide dose of 12 g. All 20 participants completed the study and were included in the safety analysis set and pharmacokinetic analysis set populations (Fig. 2).

Overall, 12 (60.0%) participants were White and eight (40.0%) were Black or African American. Mean (SD) age at screening was 62.1 (9.6) years and ranged from 42 to 74 years. Thirty percent of participants were male ($n = 6$). Participants with moderate renal impairment covered the entire eGFR range of 30–59 mL/min/1.73 m^2 (Table 2).

Table 2 Summary of demographics and baseline characteristics (safety analysis set)

Parameter statistic	Moderate renal impairment ($n = 10$)	Matching healthy control ($n = 10$)	Total ($N = 20$)
Sex, *n* (%)			
Female	7 (70.0)	7 (70.0)	14 (70.0)
Male	3 (30.0)	3 (30.0)	
Race, *n* (%)			
Black or African American	4 (40.0)	4 (40.0)	8 (40.0)
White	6 (60.0)	6 (60.0)	12 (60.0)
Age, years			
Mean (SD)	63.5 (10.2)	60.7 (9.3)	62.1 (9.6)
Median (min, max)	66.0 (46.0, 74.0)	59.0 (42.0, 73.0)	63.5 (42.0, 74.0)
Height (cm) at baseline			
Mean (SD)	162.7 (7.5)	155.5 (13.3)	159.1 (11.1)
Median (min, max)	163.6 (154.0, 174.0)	153.0 (136.0, 177.0)	160.1 (136.0, 177.0)
Weight (kg) at baseline			
Mean (SD)	82.9 (10.8)	70.6 (10.9)	76.7 (12.3)
Median (min, max)	85.85 (67.7, 98.0)	68.20 (58.8, 90.5)	73.75 (58.8, 98.0)
BMI, kg/m^2 at baseline			
Mean (SD)	31.4 (4.3)	29.2 (1.8)	30.3 (3.4)
Median (min, max)	32.3 (24.2, 36.6)	29.3 (25.7, 31.7)	30.1 (24.2, 36.6)
eGFR (mL/min/1.73 m^2) at screening			
Mean (SD)	48.0 (10.0)	109.9 (17.4)	79.0 (34.6)
Median (min, max)	51.0 (31.0, 60.0)	108.7 (89.0, 134.0)	74.5 (31.0, 134.0)
eGFR (mL/min/1.73 m^2) at baseline			
Mean (SD)	45.6 (6.9)	105.0 (20.6)	75.3 (34.0)
Median (min, max)	45.5 (34.0, 58.0)	93.1 (90.0, 148.0)	74.0 (34.0, 148.0)

BMI body mass index, *eGFR* estimated glomerular filtration rate, *SD* standard deviation, *max* maximum, *min* minimum

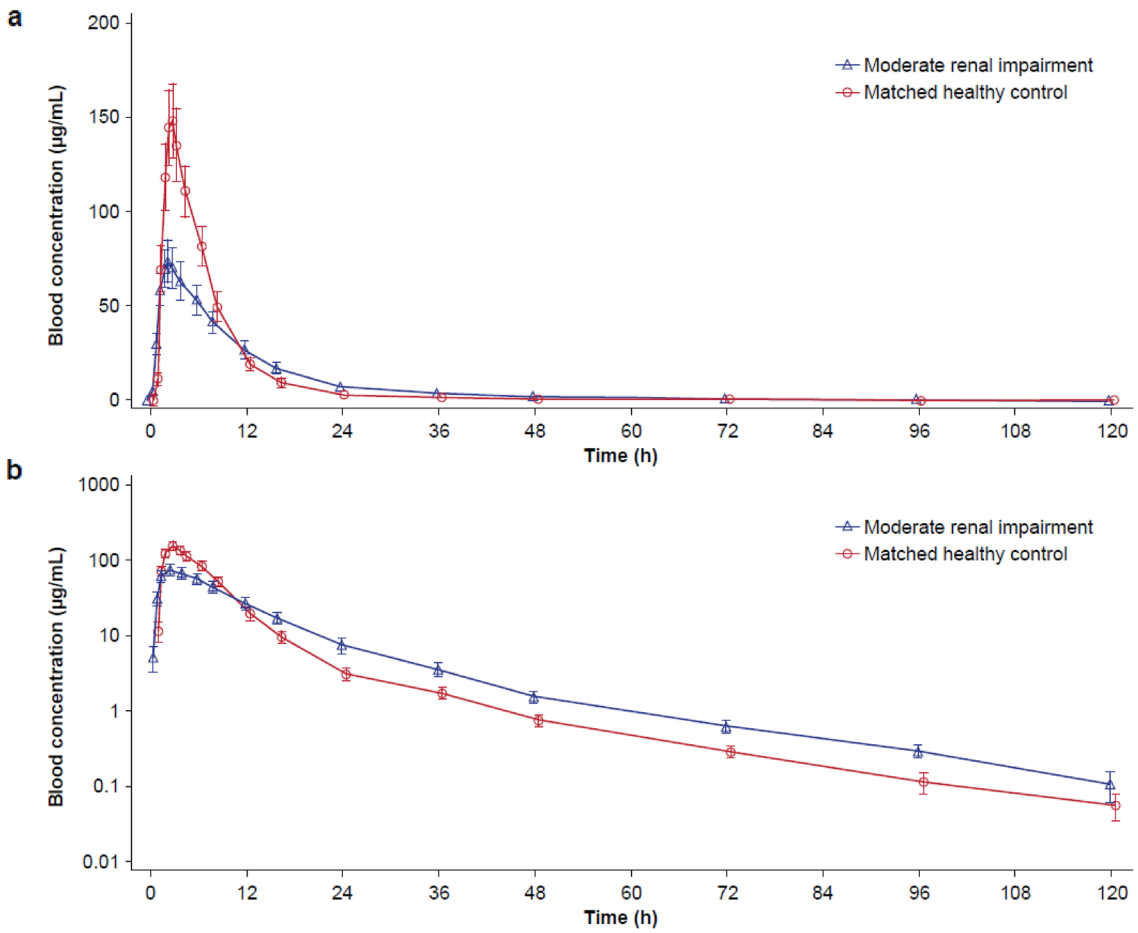

Fig. 3 Mean (±SE) whole blood concentration–time profiles of trofinetide in the moderate renal impairment (6 g) and matched healthy control (12 g) populations in linear **a** and semi-log **b** scales (pharmacokinetic analysis set). Two renally impaired participants received erroneous doses (8 g instead of 6 g) and, along with their matching controls, were excluded from the analysis

4.2 Trofinetide Pharmacokinetics in Blood

Following single trofinetide doses of 6 g in the participants with moderate renal impairment ($n = 8$) and 12 g in the matched healthy controls ($n = 8$), the pharmacokinetic characteristics were in general qualitatively similar (Fig. 3). The C_{max} value following the administration of 6-g trofinetide in participants with moderate renal impairment was approximately 50% the value observed in participants with normal renal function administered 12-g trofinetide. In contrast, overall systemic exposure (assessed by AUC_{inf}) was only lower by approximately 19% in participants with moderate renal impairment. There was a slight shift in T_{max} (2.5 vs. 2.8 h) between participants with moderate renal impairment and their matched controls (Table 3). Dose-normalized geometric mean ratios for C_{max} were comparable while AUC was significantly higher (approximately two-fold) in participants with moderate renal impairment compared with matched healthy controls (Table 4).

4.3 Trofinetide Pharmacokinetics in Urine

The amount and percent of the drug excreted unchanged in urine (Ae_u and $\%fe_u$) and renal clearance (CL_r) was comparable in participants with moderate renal impairment and their healthy matched controls (Table 3).

4.4 Correlation of Pharmacokinetic Parameters and Estimated CL_{cr}

Statistically significant negative correlations based on Pearson's coefficients were demonstrated for dose-normalized AUC and CL_{cr} in the moderate renal impairment group but there was a notably lesser degree of correlation for dose-normalized C_{max} and CL_{cr}, which did not reach statistical significance (Table 5).

Table 3 Mean (SD) pharmacokinetic parameters in blood for trofinetide in participants with moderate renal impairment (6 g) and matching healthy control (12 g) (pharmacokinetic analysis set)

PK parameters, units Mean (SD), unless otherwise noted	Moderate renal impairment $n = 8$	Matched healthy control $n = 8$
PK in blood		
C_{max}, µg/mL	76.4 (32.8)	157.0 (50.7)
$AUC_{0\text{-}t}$, µg·h/mL	866 (353)	1029 (374)
AUC_{inf}, µg·h/mL	872 (355)	1033 (374)
%AUC_{ext}	0.814 (0.414)	0.400 (0.125)
T_{max}[a], h	2.5 (1.5, 4.0)	2.8 (2.0, 6.0)
$t_{½}$, h	21.9 (4.29)	20.8 (6.72)
CL/F, L/h	8.2 (4.1)	13.6 (6.7)
PK in urine		
Total Ae_u, g	4.07 (0.95)	6.44 (2.71)
Total %fe_u	67.9 (15.8)	53.7 (22.6)
CL_r, L/h	5.17 (1.74)	6.47 (2.10)

Two renally impaired participants were misdosed and received 8 g of trofinetide instead of 6 g, and were excluded from this analysis along with their matching controls

Ae_u amount of drug excreted into urine, *$\%AUC_{ext}$* percentage of area under the curve extrapolated from time to last detectable drug concentration to infinity, *AUC_{inf}* area under the concentration–time curve from time 0 to infinity, *$AUC_{0\text{-}t}$* area under the concentration–time curve from time 0 to time of last detectable drug concentration, *CL/F* apparent systemic clearance, *CL_r* renal clearance of drug, *C_{max}* maximum (peak) observed drug concentration, *$\%fe_u$* percentage of cumulative amount of drug excreted in urine, *PK* pharmacokinetic, *SD* standard deviation, *$t_{½}$* apparent terminal elimination half-life, *T_{max}* time to maximum drug concentration

[a]Median (minimum, maximum)

4.5 Safety

Overall, seven TEAEs were reported in six participants (30.0%): two (20.0%) with moderate renal impairment who received 6 g ($n = 1$) or 8 g ($n = 1$) trofinetide and four healthy participants (40.0%) who received 12 g trofinetide. The majority (71.4%) of TEAEs were mild in severity. There were no deaths or serious TEAEs, and no participants with TEAEs leading to discontinuation from the study (Table 6). There was one severe TEAE of increased blood creatine phosphokinase and a moderate TEAE of ear infection, reported by the same participant who received 6 g trofinetide in the moderate renal impairment group. Both events were considered unrelated to trofinetide and resolved. Of the two participants in the moderate renal impairment group that received the 8 g dose, one participant did not report any TEAEs, and the other reported a mild headache on day 1 which was recurrent and considered related to treatment and resolved on day 14. There were no clinically meaningful changes in individual laboratory, vital signs, physical findings, or ECG results.

4.6 Renal Impairment Validation: Virtual Population Simulations

Virtual subjects were generated in GastroPlus based on physical characteristics of the healthy and the moderate renal impairment populations from the phase 1 study (Table 7). Age, body surface area, height, and sex were well matched between the two groups. The GFR values for all subjects met the criteria for healthy subjects and moderate renal impairment. A reasonable overlap of the predicted distribution from the stochastic simulations and the trofinetide blood concentrations from the phase 1 study was observed for both groups with some of the observed trofinetide concentrations from the moderate renal impairment cohort falling below the 5th percentile of the stochastic simulation (Fig. 4).

4.7 Renal Impairment Validation: Comparison of Trofinetide Exposures

For the moderate renal impairment group, the 6-g C_{max} and AUC_{inf} values from the phase 1 study closely aligned with the C_{max} and AUC_{inf} distributions predicted from the 100 virtual healthy and 100 moderate renal impairment subjects in the PBPK stochastic simulations (Fig. 5). For the matched

Table 4 Statistical comparison of pharmacokinetic parameters for trofinetide in participants with moderate renal impairment (6 g or 8 g) and matching healthy controls (12 g) (pharmacokinetic analysis set)

Parameter (unit)	Moderate renal impairment (*n*)	Matched healthy control (*n*)	GMR (moderate/healthy)	Lower 90% CI	Upper 90% CI
C_{max}/dose (µg/mL/g)	10	10	1.02	0.69	1.50
$AUC_{0\text{-}t}$/dose (µg·h/mL/g)	10	10	1.80	1.31	2.49
$AUC_{0\text{-}\infty}$/dose (µg·h/mL/g)	10	10	1.81	1.31	2.50

For dose-normalized PK parameters, group comparisons of the dose-normalized, log-transformed values were performed using ANOVA

ANOVA analysis of variance, *$AUC_{0\text{-}t}/dose$* area under the blood concentration–time curve from time 0 to time of last detectable drug concentration normalized to dose, *$AUC_{0\text{-}\infty}/dose$* area under the blood concentration–time curve from time 0 to infinity normalized to dose, *CI* confidence interval, *$C_{max}/dose$* maximum (peak) observed drug concentration normalized to dose, *GMR* geometric mean ratio, *PK* pharmacokinetic

Table 5 Correlation between dose normalized C_{max}, AUC_{0-t}, and AUC_{inf} of trofinetide and estimated CL_{cr} in the participants with moderate renal impairment (6 g or 8 g) and matching healthy controls (12 g) (pharmacokinetic analysis set)

Parameter	Moderate renal impairment n^a=10		Matched healthy control n^a=10		Overall N^a=20	
	Pearson's coefficient	2-sided *p* value	Pearson's coefficient	2-sided *p* value	Pearson's coefficient	2-sided *p* value
C_{max}/dose (µg/mL/g)	− 0.577	0.0808	− 0.450	0.1919	− 0.347	0.1919
AUC_{0-t}/dose (µg·h/mL/g)	− 0.759	0.0109	− 0.200	0.5790	− 0.716	0.0004
AUC_{inf}/dose (µg·h/mL/g)	− 0.758	0.0110	− 0.202	0.5748	− 0.718	0.0004

AUC_{0-t}/dose area under the concentration–time curve from time 0 to time of last detectable drug concentration normalized to dose, *AUC_{inf}/dose* area under the concentration–time curve from time 0 to infinity normalized to dose, *CI* confidence interval, *CL_{cr}* creatinine clearance, *C_{max}/dose* maximum observed blood drug concentration normalized to dose

[a]Two participants received 8 g of trofinetide instead of 6 g

Table 6 Overall summary of treatment-emergent adverse events (safety analysis set)

Parameter	Moderate renal impairment (*n* = 10) *n* (%)	Matching healthy control (*n* = 10) *n* (%)	Total (*N*= 20) *n* (%)
Any TEAE	2 (20.0)	4 (40.0)	6 (30.0)
Any treatment related TEAE	1 (10.0)	3 (30.0)	4 (20.0)
Any serious TEAE	–	–	–
Any TEAE leading to withdrawal	–	–	–
Any TEAE leading to death	–	–	–
MedDRA preferred term			
Thrombocytopenia	–	1 (10.0)	1 (5.0)
Diarrhea	–	1 (10.0)	1 (5.0)
Ear infection	1 (10.0)	–	1 (5.0)
Blood creatine phosphokinase increased	1 (10.0)	–	1 (5.0)
Headache	1 (10.0)	–	1 (5.0)
Chromaturia	–	1 (10.0)	1 (5.0)
Hematuria	–	1 (10.0)	1 (5.0)
Severity of TEAE			
Mild	1 (10.0)	4 (40.0)	5 (25.0)
Moderate	–	–	–
Severe	1 (10.0)	–	1 (5.0)

Adverse events were coded using the MedDRA version 25.0.

MedDRA Medical Dictionary for Regulatory Activities, *TEAE* treatment-emergent adverse event

healthy control group, the observed median C_{max} and AUC_{inf} values from the phase 1 study were slightly higher than the stochastic simulation values.

4.8 Predicted Pediatric Dosing: Deterministic Simulations

Virtual pediatric physiology characteristics in matched healthy and mild and moderate renal impairment subjects aged from 2 to 13 years were generated in GastroPlus (Table 8). Compared with the virtual healthy pediatric physiologies administered the recommended weight-based dosing (range 5–12 g), the predicted trofinetide blood AUC_{inf} was approximately 19.5% higher for the mild renal impairment physiologies receiving the same dose. The predicted trofinetide blood AUC_{inf} was similar for the moderate renal impairment physiologies that were simulated using doses that are 50% lower than the recommended dose for healthy subjects at the defined body weight (range 2.5–6 g).

5 Discussion

The overall systemic exposure for trofinetide based on the observed clinical results from the phase 1 study and the predicted results from the PBPK model simulations support a

Table 7 Summary of virtual subject populations used in population simulations of trofinetide oral administration in subjects with moderate renal impairment (n=100) and healthy subjects (n=100)

Virtual population (n=100)	Characteristic	Mean (SD)	%CV	Median (Min, Max)	Geometric mean	Geometric %CV
Moderate renal impairment (6 g trofinetide)	Age (years)	59.9 (8.1)	13.6	59 (46, 74)	59.4	13.8
	Body surface area (m^2)	1.85 (0.13)	7.1	1.82 (1.60, 2.21)	1.84	7.0
	Height (cm)	161 (8.9)	5.5	160 (143, 186)	161	5.5
	GFR (mL/min)	46.6 (6.8)	14.5	45.1 (31.9, 59.6)	46.2	14.7
	Hematocrit	0.436 (0.042)	9.6	0.436 (0.352, 0.541)	0.434	9.6
Healthy (12 g trofinetide)	Age (years)	55.2 (8.3)	15.0	54.5 (42, 70)	54.6	15.1
	Body surface area (m^2)	1.79 (0.16)	9.0	1.78 (1.50, 2.06)	1.78	9.1
	Height (cm)	161 (9.0)	5.6	161 (142, 180)	161	5.6
	GFR (mL/min)	115 (18.9)	16.5	111 (90.1, 167)	113	15.8
	Hematocrit	0.458 (0.042)	9.1	0.456 (0.359, 0.558)	0.456	9.1

The sex distribution of the virtual populations was 72/28 (female/male) for the healthy population and 80/20 for the moderate renal impairment population

BSA body surface area, *%CV* coefficient of variation expressed as a percent, *GFR* glomerular filtration rate, *Max* maximum, *Min* minimum, *SD* standard deviation

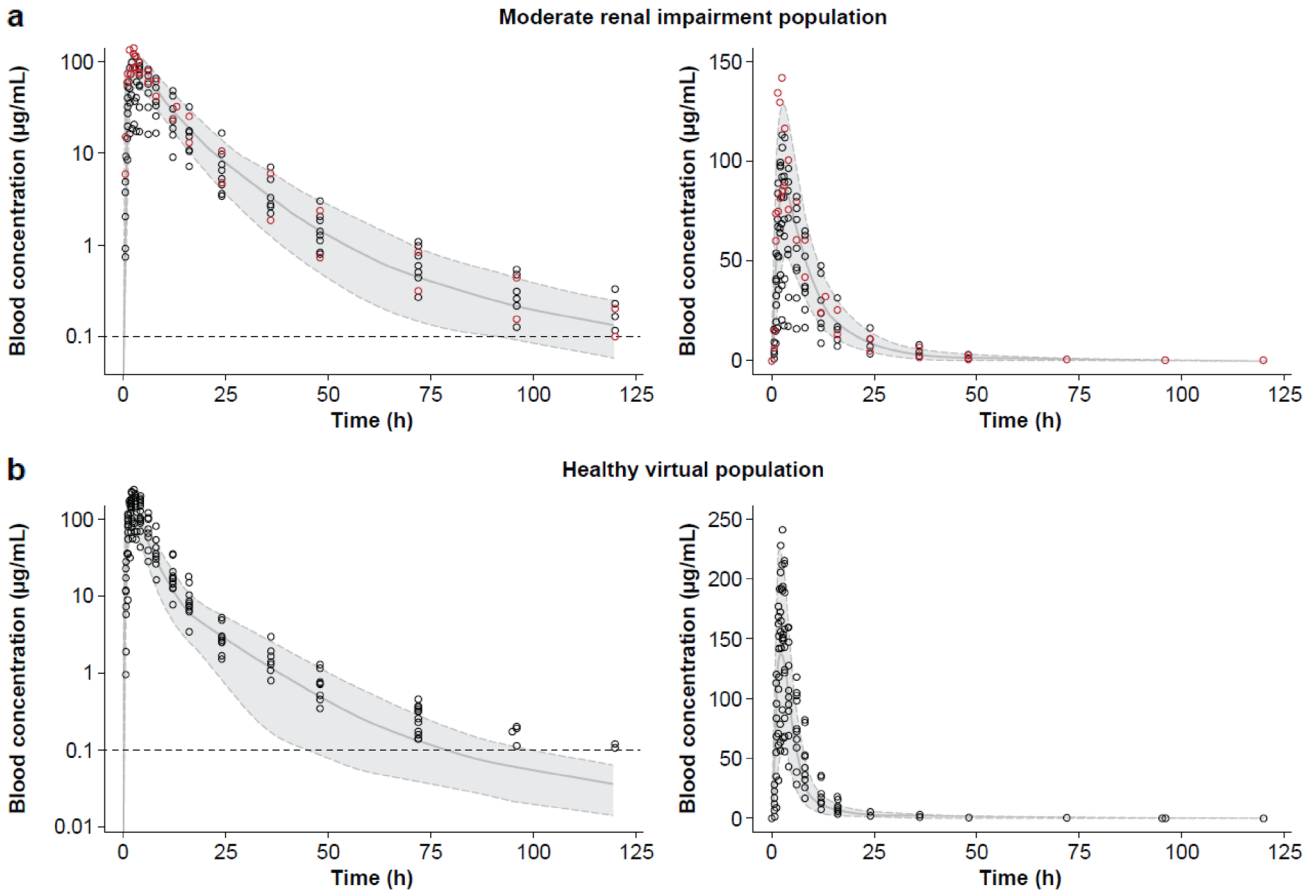

Fig. 4 Stochastic simulation output for the mean trofinetide blood concentrations versus time with overlay of observed trofinetide blood concentrations (left panel shows logarithmic y-axis and right panel shows linear y-axis): **a** moderate renal impairment population; **b** healthy virtual population. The solid and dashed grey lines represent the mean with 5th and 95th percentiles of predicted trofinetide blood concentration of 100 simulated subjects. The black circles represent observed concentration from the phase 1 study. The red circles represent observed concentrations normalized to a 6-g dose from the two participants who received the 8-g doses. The horizontal line represents the lower limit of quantification (0.1 µg/mL)

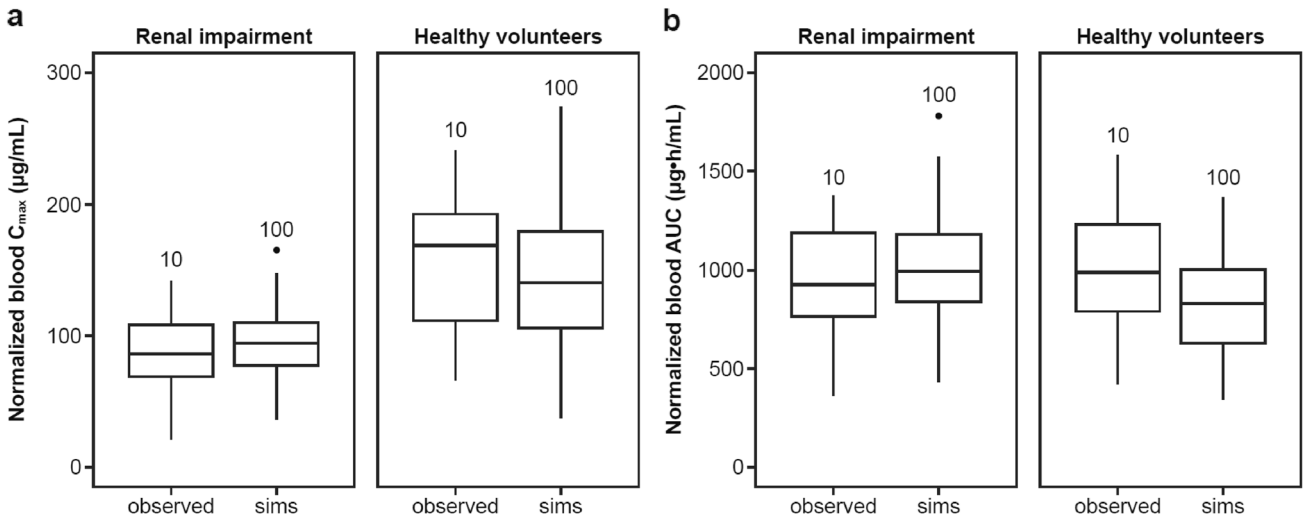

Fig. 5 Boxplots of dose-normalized trofinetide C_{max} **a** and AUC_{inf} **b** in participants from the moderate renal impairment and matched healthy control groups compared with the PBPK stochastic simulation output in the corresponding virtual population. AUC_{inf} area under the blood concentration–time curve from time 0 to infinity; C_{max} maximum drug concentration, *sims* simulations. Boxes represent the 25th and 75th percentiles, and the lines within the boxes represent the median (50th percentile); whiskers extend to the most extreme point within 1.5 interquartile ranges; filled circles show data points outside this range. The *number* of study participants or virtual subjects is above each box

50% dose reduction in the moderately impaired renal population to achieve the target exposure range. The 50% dose reduction in the clinical study and the model resulted in a lower C_{max} and a comparable AUC_{inf} in the moderate renal impairment population compared with the matched healthy controls. When the exposure parameters were dose-normalized, a process that assumes dose equivalency, the observed C_{max} values were comparable between the moderate renal impairment group and matched healthy controls. In contrast, the dose-normalized AUC_{inf} values were approximately 2-fold higher in the moderate renal impairment group compared with matched healthy controls. These results are consistent with the PBPK model prediction, where 50% reduction of dose (6 g vs. 12 g) in the moderate renal impairment group resulted in comparable AUC_{inf} and lower C_{max} when compared with matched healthy controls. This discrepancy between the C_{max} and AUC_{inf} was reflected by the significant negative correlation between AUC and CL_{cr}, which was not evident between the C_{max} and Cl_{cr}, suggesting that the AUC is a more reliable measure of impaired renal clearance. This is expected since the AUC is a function of both the extent of absorption and clearance of a drug over time, whereas the C_{max} is more indicative of the rate and extent of absorption.

The agreement between the predicted stochastic simulations and observed trofinetide concentration distributions and pharmacokinetic parameters related to exposure provided good validation of the predictive performance of the PBPK model, including the pediatric physiologies, and supported the recommended 50% dose reduction for trofinetide in individuals with moderate renal impairment. Dosing adjustments were not considered necessary for mild renal impairment since the pediatric simulation predicted only a negligible increase in exposure in the mild renal impairment group versus the matched healthy controls. Trofinetide is not recommended for use in severe renal impairment and ESRD because it primarily undergoes renal elimination and the PBPK model predicted exposure levels for the AUC prior to any dose adjustments that were up to 10-fold higher compared with a healthy subject (ACP-2566-MS-003 study; unpublished data).

In consideration of the deterministic simulations based on pediatric physiologies, although there is uncertainty regarding the pathophysiologic changes present in pediatric patients with renal impairment, the extrapolation of similar pathophysiologic changes in adult renal impairment to pediatric PBPK physiologies has been shown to be predictive in reported PBPK simulations of other renally excreted drugs [18–20], thus providing support for the clinical relevance of these simulation results.

Trofinetide administered orally as a single dose of 6 g or 8 g in participants with moderate renal impairment and a 12-g dose in healthy matched controls was well tolerated and the incidence of any TEAEs did not differ meaningfully between the two groups.

The single-dose design that was employed in the phase 1 study is standard for pharmacokinetic studies of drugs with a short half-life (trofinetide's effective half-life is ~ 2.6 h [6]) with no significant accumulation upon multiple dosing as has been demonstrated for trofinetide [8]. Study limitations include the small sample size and the fact that the study

Table 8 Virtual pediatric physiology characteristics in matched healthy and mild and moderate renal impairment subjects aged from 2 to 13 years

Renal function status	Age (year)	Body-weight (kg)	BMI (kg/m^2)	Hematocrit	Trofinetide R_{bp}	GFR (mL/min/kg)	Age (year)	Body-weight (kg)	BMI (kg/m^2)	Hematocrit	Trofinetide R_{bp}	GFR (mL/min/kg)
Healthy (n=14)	2	9.3	14.7	0.4083	0.5690	3.37	7	27.5	17.5	0.4236	0.5528	2.76
	2	11.6	16.3	0.4083	0.5690	3.37	8	31.3	18.2	0.4267	0.5496	2.64
	2	12.0	16.2	0.4083	0.5690	3.37	9	35.3	18.8	0.4298	0.5464	2.52
	3	14.5	16.1	0.4114	0.5658	3.36	10	39.4	19.5	0.4328	0.5431	2.40
	4	17.3	16.2	0.4144	0.5625	3.20	11	43.6	20.3	0.4359	0.5399	2.28
	5	20.4	16.5	0.4175	0.5593	3.00	12	47.7	21.0	0.4390	0.5366	2.18
	6	23.8	17.0	0.4206	0.5561	2.85	13	51.7	21.7	0.4420	0.5334	2.08
Mild (n=14)	2	9.3	14.7	0.4083	0.5690	2.81	7	27.5	17.5	0.4236	0.5528	2.30
	2	11.6	16.3	0.4083	0.5690	2.81	8	31.3	18.2	0.4267	0.5496	2.20
	2	12.0	16.2	0.4083	0.5690	2.81	9	35.3	18.8	0.4298	0.5464	2.10
	3	14.5	16.1	0.4114	0.5658	2.80	10	39.4	19.5	0.4328	0.5431	2.00
	4	17.3	16.2	0.4144	0.5625	2.67	11	43.6	20.3	0.4359	0.5399	1.90
	5	20.4	16.5	0.4175	0.5593	2.50	12	47.7	21.0	0.4390	0.5366	1.81
	6	23.8	17.0	0.4206	0.5561	2.37	13	51.7	21.7	0.4420	0.5334	1.74
Moderate (n=14)	2	9.3	14.7	0.3944	0.5837	1.69	7	27.5	17.5	0.4092	0.5680	1.38
	2	11.6	16.3	0.3944	0.5837	1.69	8	31.3	18.2	0.4122	0.5649	1.32
	2	12.0	16.2	0.3944	0.5837	1.69	9	35.3	18.8	0.4152	0.5618	1.26
	3	14.5	16.1	0.3974	0.5806	1.68	10	39.4	19.5	0.4181	0.5587	1.20
	4	17.3	16.2	0.4003	0.5774	1.60	11	43.6	20.3	0.4211	0.5555	1.14
	5	20.4	16.5	0.4033	0.5743	1.50	12	47.7	21.0	0.4241	0.5524	1.09
	6	23.8	17.0	0.4063	0.5712	1.43	13	51.7	21.7	0.4270	0.5493	1.04

BMI body mass index, *GFR* glomerular filtration rate, R_{bp} blood to plasma concentration ratio

population did not include individuals with RTT but instead included subjects with moderate renal impairment who are otherwise healthy; however, in these types of studies, the pharmacokinetic findings are routinely extrapolated to the clinical setting. It is also unfeasible to recruit RTT patients with renal impairment given the fact that renal impairment has never been reported as a comorbid condition in the RTT population. Given the ethical and clinical challenges of studying individuals with severe renal impairment or ESRD, who would be dependent on frequent dialysis, the clinically observed results were restricted to moderate renal impairment. Nevertheless, once validated, the PBPK model allows the extrapolation of the findings in moderate renal impairment to the other stages of renal impairment.

Plasma protein binding is usually reduced in subjects with renal impairment, which often results in a larger apparent volume of distribution and shorter elimination half-life [21]. Although there was no assessment of the percentage of unbound drug in this phase 1 study or the inclusion of the fraction of unbound drug in the PBPK modeling, trofinetide has previously been shown to have minimal protein binding in human plasma and in human serum albumin solution (Data on file; CEREP Report Study No. 10907). Therefore, we would not expect reduced plasma protein binding to have any meaningful effect on trofinetide pharmacokinetic parameters.

The trofinetide PBPK model was developed using trofinetide clinical studies to validate pharmacokinetic parameter settings and included one phase 2 study in RTT [22], though the majority of studies were in healthy volunteers. Pediatric populations were absent from the studies included in the PBPK model, which meant there was no observed pharmacokinetic data to validate the predicted exposures in the virtual pediatric physiologies. In general, considering the small sample size of 10 study participants compared with the 100 virtual subjects from the stochastic simulations, there is substantial overlap and agreement between the clinically observed trofinetide exposure measures and the predicted exposures from the PBPK stochastic simulations.

6 Conclusion

In conclusion, the observed and predicted systemic exposure results confirm that a 50% dose reduction in individuals with moderate renal impairment is sufficient to achieve the same target exposure range associated with the recommended dose in individuals with normal renal function and provides validation of the PBPK model of renal impairment.

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Declarations

Funding This trial was sponsored by Acadia Pharmaceuticals Inc.

Conflicts of Interest Mona Darwish, Rene Nunez, James M. Youakim, and Di An are employees of and hold stock in Acadia Pharmaceuticals Inc. Kathie M. Bishop is a consultant for Acadia Pharmaceuticals Inc., and former employee. Inger Darling and Viera Lukacova are employees of and hold stock in Simulations Plus, Inc. Thomas C. Marbury is an employee and equity owner of Orlando Clinical Research Center.

Ethics Approval The phase 1 clinical study was approved by an Institutional Review Board and was performed in accordance with Health Insurance Portability and Accountability Act regulations, Food and Drug Administration Good Clinical Practice Regulations, and International Council for Harmonisation Good Clinical Practice Guidelines. All participants provided written informed consent.

Consent to Participate All study participants provided written informed consent.

Consent for Publication Not applicable.

Data Availability The datasets generated during and/or analyzed during the current study are not publicly available due to data confidentiality but are available from the corresponding author on reasonable request providing a confidentiality agreement is signed.

Code Availability Not applicable.

Author Contributions Conceptualisation: MD, JMY, and KMB; Methodology: MD, RN, JMY, DA, ID, VL, and KMB; Data curation, formal analysis, investigation, visualisation: MD, TCM, RN, DA, ID, and VL; Writing (original draft/previous versions/approval): MD, TCM, RN, JMY, DA, ID, VL, and KMB.

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