25 June 2026
EMADOC-1829012207-50893
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Daybu

International non-proprietary name: Trofinetide

Procedure No. EMEA/H/C/006482/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

BID	twice daily
DIPEA	Diisopropylethylamine
DMAPA	3-Dimethylaminopropylamine
DOE	Design of experiments
DS	Drug substance
DP	Drug Product
EDC.HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide HCl
EtOAc	Ethyl acetate
FID	Flame ionization detector
GC	Gas chromatography
Glu-OH	L-glutamic acid
HMDSO	Hexamethyldisiloxane
HPLC	High-performance liquid chromatography
ICH	International Council for Harmonisation
ICP	Inductively coupled plasma
IPCs	In-Process Controls
IR	Infrared Spectroscopy
KF	Karl Fischer
$KHSO_4$	Potassium bisulfate
LOQ	Limit of quantitation
MePro	(S)-2-methylproline hydrochloride
$NaHCO_3$	Sodium Bicarbonate
NDMA	N-nitrosodimethylamine
NMR	Nuclear magnetic resonance
NMT	Not more than
NOAEL	No observed adverse effect level
NOR	Normal Operating Range
NR	Not reported
OFAT	One factor at time
PAR	Proven Acceptable Range
PCI	potentially clinically important
Pd/C	Palladium on carbon

ppm	Parts per million
RSM	regulatory starting materials
TMA	N-methyl-N-(trimethylsilyl)acetamide
TMS	Trimethylsilyl
TRF	Trofinetide-related fragments
TRF-1	Z-Gly-MePro-OH
TRF-2	Z-Gly-MePro-Glu-OH
TRF-3	Trofinetide
UHPLC	Ultra high-performance liquid chromatography
USP	United States Pharmacopeia
UV	Ultraviolet
VWD	Variable wavelength detector
Z-Gly-OH	Z-glycine

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Acadia Pharmaceuticals (Netherlands) B.V. submitted on 20 December 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Daybu, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 14 December 2023.

Daybu was designated as an orphan medicinal product EU/3/15/1534 on 10 August 2015 in the following condition: Treatment of Rett syndrome.

The applicant applied for the following indication:

'treatment of Rett syndrome in adults and paediatric patients 2 years of age and older'.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

1.3. Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) *P/0188/2024* on the agreement of a paediatric investigation plan (PIP) for Treatment of Rett syndrome.

At the time of submission of the application, the PIP had been completed.

The PDCO issued on 6 September 2024 a positive opinion on compliance for PIP EMEA-C-003587-PIP01-24.

All studies/measures contained in the PIP were checked for compliance:

Agreed studies/measures **(study identifier)**	**Description**
Study 1 (0621-16031)	Repeat-dose oral dosage study in juvenile rats to evaluate the toxicity, and toxicokinetic profile and reversibility of possible toxicity or effects on development of trofinetide
Study 2 (0621-16032)	Repeat-dose oral dosage study in juvenile dogs to evaluate the toxicity, and toxicokinetic profile and reversibility of possible toxicity or effects on development of trofinetide.
Study 3 (Neu-2566-RETT-001)	Randomised, double-blind, placebo-controlled, dose-escalation study of trofinetide in female paediatric patients

	from 16 to less than 18 years of age (and adult females) with RTT.
Study 4 (Neu-2566-RETT-002)	Randomised, double-blind, placebo-controlled, parallel group, dose-ranging study of the safety, pharmacokinetics and efficacy of trofinetide in female paediatric patients aged from 5 to less than 16 years diagnosed with post-regression, classic Rett syndrome.
Study 5 (ACP-2566-003)	Randomised, double-blind, placebo-controlled, parallel group study to evaluate efficacy, safety and pharmacokinetics of trofinetide in female subjects aged from 5 to less than 18 years diagnosed with post-regression, classic Rett syndrome.
Study 6 (ACP-2566-004)	40-week open-label extension study to evaluate the long-term safety, tolerability, efficacy, and pharmacokinetics of trofinetide in paediatric and adult patients with RTT who completed PIP Study 5 (ACP-2566-003).
Study 7 (ACP-2566-005).	32-month, multicentre, open-label extension study to evaluate long-term safety and tolerability of trofinetide in paediatric and adult patients with RTT who completed PIP Study 6 study (Study ACP-2566-004).
Study 8 (ACP-2566-009).	Open-label study to evaluate safety and tolerability, pharmacokinetics, and efficacy of trofinetide in female paediatric subjects aged 2 to less than 5 years with Rett syndrome.
Study 9 (ACP-2566-MS-007, ACP-2566-MS-008, ACP-2566-MS-010, ACP-2566-MS-009).	Population pharmacokinetic model to establish the dose of trofinetide to be used in paediatric patients and exposure-response (E-R) model to confirm appropriateness of target exposure and support extrapolation of efficacy in patients below 5 years of age

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report, addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Daybu as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/daybu

1.5. Applicant's request(s) for consideration

1.5.1. New active Substance status

The applicant requested the active substance trofinetide (hydrate) contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Protocol assistance

The applicant did not seek Protocol assistance from the CHMP.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Peter Mol Co-Rapporteur: Ewa Balkowiec Iskra

The application was received by the EMA on	20 December 2024
The procedure started on	23 January 2025
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	14 April 2025
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	15 May 2025
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	25 April 2025
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	22 May 2025
The applicant submitted the responses to the CHMP consolidated List of Questions on	13 August 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	24 September 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	02 October 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues	10 October 2025

to all CHMP and PRAC members on	
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	16 October 2025
The applicant submitted the responses to the CHMP List of Outstanding Issues on	19 December 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	14 January 2026
SAG-N was convened to address questions raised by the CHMP on The CHMP considered the views of the SAG-N as presented in the minutes of this meeting.	16 January 2026
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	23 January 2026
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	27 January 2026
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues (after Oral Explanation) to all CHMP and PRAC members on	11 February 2026
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues (after Oral Explanation) to all CHMP and PRAC members on	18 February 2026
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	26 February 2026
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a marketing authorisation to Daybu on	26 February 2026

1.8. Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Christian Gartner Co-Rapporteur: Selma Arapovic Dzakula

The Applicant submitted written notice to the EMA, to request a re-examination of Daybu CHMP opinion of 26 February 2026, on	16 March 2026
The CHMP appointed Christian Gartner as Rapporteur and Selma Arapovic Dzakula as Co-Rapporteur on	26 March 2026
The Applicant submitted the detailed grounds for the re-examination on	28 April 2026
The re-examination procedure started on	29 April 2026
The CHMP Rapporteur's re-examination assessment report was circulated to all CHMP and PRAC members on	28 May 2026
The CHMP Co-Rapporteur's assessment report was circulated to all CHMP and PRAC members on	28 May 2026
SAG/Expert group/ Working Party experts (as appropriate) were convened to address questions raised by the CHMP on The CHMP considered the views of the SAG/Expert group/ Working Party (as appropriate) as presented in the minutes of this meeting	17 June 2026
The CHMP, in the light of the scientific data available and the scientific discussion within the Committee, re-examined its initial opinion and in its final opinion concluded that the application satisfied the criteria for authorisation and recommended the granting of the marketing authorisation on 25 June 2026.	25 June 2026

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The claimed indication is "treatment of Rett syndrome in adults and paediatric patients 2 years of age and older."

2.1.2. Epidemiology

Rett syndrome (RTT) is a seriously debilitating neurodevelopmental disorder whose incidence is reported as approximately 1 in 10,000 female live births worldwide (Petriti 2023). While the great majority of patients with RTT are females, males who meet the criteria for RTT have also been identified (Neul et al. 2019).

2.1.3. Biologic features, aetiology and pathogenesis

In approximately 96% to 98% of patients diagnosed with classic RTT, the disease is caused by mutations in the X-linked methyl-CpG–binding protein 2 gene (MECP2) (Percy et al. 2018). MECP2 encodes human methyl-CpG binding protein 2 (MeCP2), which modulates gene expression by binding to methylated CpG dinucleotides, primarily by activating but also by repressing transcription (Hite et al. 2009; Ip et al. 2018; Kriaucionis et al. 2003; Neul et al. 2008). The activity of the MeCP2 protein is diminished in RTT in both neurons and astrocytes (Yasui et al. 2013).

2.1.4. Clinical presentation and diagnosis

In patients with typical RTT, there is seemingly normal psychomotor development for the first 6 months of life, but thereafter the failure to reach normal developmental milestones can be observed, followed by a period of developmental regression in which there is a loss of normal use of the hands and of spoken language (Neul et al. 2014; Samaco et al. 2011). Rett syndrome is defined by characteristic clinical features, which typically include loss of verbal and non-verbal communication and voluntary motor function, characteristic repetitive hand stereotypies, and gait problems. Loss of purposeful hand use is a defining aspect of RTT during the onset of regression, and at least 30% of individuals remain without any type of purposeful hand use (Downs et al. 2010).

Other commonly observed symptoms include seizures, awake breathing disruptions, scoliosis, and intense eye communication is also a hallmark (Neul et al. 2010). Seizures have a lifetime prevalence in RTT of around 90%. The course of seizure occurrence and remission is highly variable, with the most common pattern being waxing and waning over the lifespan. The age of first seizure onset in classic RTT ranges from immediately after birth to approximately 45 years (Tarquinio et al. 2017). Gastrointestinal dysfunction, including constipation, gastroesophageal reflux disease, and chewing and swallowing difficulties are observed in most patients with RTT (Baikie et al. 2014; Motil et al. 2012). Autonomic manifestations, which include abnormalities in cardiac and respiratory function, as well as in peripheral circulation, are considered to be predominantly of central nervous system (CNS) origin. Rett syndrome is also characterized by impaired growth affecting the brain and other organ systems.

2.1.5. Management

There are no therapies approved for the treatment of RTT in Europe. Treatment focuses on the management of each patient's symptoms and, even in that regard, it is often unsatisfactory and has only a limited effect on functional improvement. Accordingly, the burden on families and other caregivers is substantial (Palacios-Ceña et al., 2018).

Most medical management is symptomatic, targeting the previously mentioned symptoms of the disorder and do not directly treat the pathophysiology of the disease (Fu et al., 2020). When medicines are prescribed to individuals with RTT, they are intended to relieve the severity of some of the associated features rather than to modify either the disease course or to eliminate the disease itself.

Considering the multi-systemic involvement in RTT, a coordinated multidisciplinary approach to medical care and management is the preferred option. At an early age, shortly after the diagnosis, intensive early intervention as applied to other neurodevelopmental disorders is recommended.

2.2. About the product

Pharmacotherapeutic group: Other nervous system drugs, ATC code: N07XX24

DAYBU (trofinetide) has a claimed indication for the treatment of Rett syndrome in adults and paediatric patients 2 years of age and older.

trofinetide is a synthetic analogue of the N-terminal tripeptide of insulin-like growth factor 1. The mechanism of trofinetide in the treatment of Rett syndrome is unclear. However, trofinetide is thought to improve neuronal synaptic function and morphology.

trofinetide should be taken twice daily, morning and evening, according to patient weight:

Table 1: Dosing of trofinetide

Patient weight	DAYBU dosage	DAYBU volume
9 kg to less than 12 kg	5 g twice daily	25 mL twice daily
12 kg to less than 20 kg	6 g twice daily	30 mL twice daily
20 kg to less than 35 kg	8 g twice daily	40 mL twice daily
35 kg to less than 50 kg	10 g twice daily	50 mL twice daily
50 kg or more	12 g twice daily	60 mL twice daily

2.3. Type of Application and aspects on development

trofinetide was initially developed by Neuren Pharmaceuticals Limited (Neuren). Acadia Pharmaceuticals Inc. (Acadia, current Sponsor) entered into a license agreement with Neuren in August 2018 and obtained exclusive North American rights to trofinetide for the treatment of RTT and other indications. In July 2023, Acadia expanded its existing license agreement for trofinetide with Neuren to include rights to market the drug in Europe.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as oral solution containing 200 mg/ml of trofinetide as active substance. The product contains the active substance amorphous form or the hydrate.

Other ingredients are: maltitol (E965), sucralose (E955), Allura Red AC (E129), strawberry flavour, sodium methyl parahydroxybenzoate (E219), sodium propyl parahydroxybenzoate (E217) and purified water. The strawberry flavour contains propylene glycol (E1520) and artificial flavours.

The product is available in translucent high-density polyethylene (HDPE) multi-dose bottle containing 450 mL oral solution with a white polypropylene child-resistant closure. The product is co-packaged with 30 mL and 110 mL CE marked polypropylene gradated dosing cups.

2.4.2. Active Substance – trofinetide

2.4.2.1. General information

The chemical name of trofinetide is (2S)-2-{[(2S)-1-(2-aminoacetyl)-2-methylpyrrolidine-2-carbonyl] amino}pentanedioic acid corresponding to the molecular formula $C_{13}H_{21}N_3O_6$. It has a molecular mass of 315.33 g/mol and the following structure:

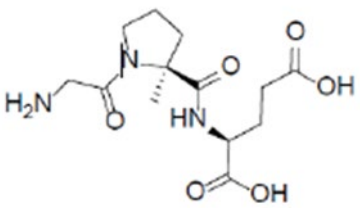


Figure 1: Trofinetide structure

The chemical structure of trofinetide amorphous was elucidated by a combination of proton and carbon nuclear magnetic resonance spectroscopy (1H, 13C, COSY, NOESY, TOCSY, HSQC, HMBC, H2BC), mass spectrometry and infrared spectroscopy. The solid-state properties of trofinetide were measured by single crystal X-ray crystallography, XRPD, TGA and DSC. trofinetide is consistently obtained as the amorphous form in the manufacturing process and does not change upon storage.

trofinetide amorphous is a white to off white solid, highly soluble (> 500 mg/mL) in aqueous media across the physiologically relevant pH range. Trofinetide amorphous is hygroscopic.

trofinetide exhibits stereoisomerism due to the presence of two chiral centres. The stereocentres are already present in the starting materials. As these starting materials are of the S configuration, trofinetide exists as a single stereoisomer with the S,S configuration. The enantiomeric purity is controlled in the starting materials by chiral HPLC.

2.4.2.2. Manufacture, characterisation and process controls

The active substance is manufactured at two manufacturing sites for which satisfactory GMP documentation has been provided.

Trofinetide amorphous is synthesized in several steps using multiple well defined starting materials with acceptable specifications.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The active substance manufacturing process is adequately described and the overall control strategy, including in process controls, starting material, intermediate and reagent specifications, and process parameter controls (including proven acceptable ranges (PARs)) ensures that the process consistently produces active substance of the intended quality.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

The initial discussion on mutagenic impurities was not complete since reagents and intermediates in the synthesis were not included and, also the mutagenic potential of identified impurities was not sufficient. Therefore, the CHMP raised a major objection (MO1) which was partly addressed by submitting an updated section on mutagenic impurities which included also the reagents and intermediates of the synthesis. The conclusion that all materials are non-mutagenic was not supported for a specific reagent. The Applicant resolved the MOs by Ames test results showing negative potential for the reagent. Consequently, this impurity is treated as a non-mutagenic impurity and the control with a limit of NMT 500 ppm in TRF-2 is acceptable.

A risk of formation of N-nitrosodimethylamine (NDMA) is indicated, due to the carry-over of nitrite in a reagent and the presence of a tertiary amine in 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide HCl (EDC-HCl) and the urea by-product (EDU). The Applicant performed a purge factor calculation and confirmatory testing with a suitably validated analytical procedure. The test results for NDMA in trofinetide hydrate were less than 10% of the acceptable intake (AI). No further control is therefore necessary.

Three synthetic routes were used in the clinical development program. The changes introduced have been presented in sufficient detail and justified. It has been demonstrated that the change(s) did not

have a significant impact on the quality of the product as the phase 3 clinical trials were performed with material manufactured with the proposed commercial process.

The manufacturing process has been developed using a combination of conventional univariate studies and elements of Quality by Design (QbD) such as risk assessment, design of experiment (DOE) studies. PARs have been defined for some manufacturing steps. The development data, the proposed control strategy and batch analysis data from commercial scale batches fully support the proposed PARs.

The active substance is packaged in double low-density polyethylene (LDPE) bags that are placed inside an aluminised bag along with desiccant and heat sealed. The primary packaging complies with Commission Regulation (EU) 10/2011, as amended.

2.4.2.3. Specification

The active substance specification includes tests for appearance, identification, assay, related substances, water content, residual solvents, elemental impurity palladium, residue on ignition, and microbial purity.

Impurities present above the ICH Q3A qualification threshold were qualified by toxicological and clinical studies, and appropriate acceptance criteria have been set. The limits for specified impurities have been supported by batch analysis and stability data. The limits for residual solvents and palladium are compliant with ICH Q3C and ICH Q3D respectively.

The analytical methods used have been adequately described and (non-compendial methods) appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

Batch analysis data from ten production scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

2.4.2.4. Stability

Stability data from seven commercial scale batches of active substance from the proposed manufacturer, stored in the intended commercial package for up to 36 months under long term conditions (2-8°C) and for up to 6 months under accelerated conditions (25ºC / 60% RH) according to the ICH guidelines were provided. The following parameters were tested: appearance, assay, related substances, water content, microbial testing. The analytical methods used were the same as for release and were stability indicating.

Since out-of-specification results for unspecified impurities are observed at accelerated conditions the storage precaution 'store at 2-8°C' is justified. At long-term conditions, an increase in total impurities and a decrease in water content is observed but all results are within proposed specification limits.

The post-approval stability commitments are acceptable in view of the already provided stability data and ICH Q1A. "Any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA".

Forced degradation testing, and photostability testing following the ICH guideline Q1B, were performed on one batch sample as part of method validation. Results on stress conditions (80°C up to 48 hrs, photolytic stress, HCl 1N for 24 hrs, NaOH 1N for 24 hrs, H2O2 for 24 hrs) show that trofinetide is not significantly degraded. For thermal and basic conditions, a more pronounced degradation was observed. Trofinetide (as powder) is not sensitive to light.

The stability results indicate that the active substance manufactured by the proposed manufacturer is sufficiently stable. The stability results justify the proposed retest period of 36 months when stored refrigerated (2-8°C) in the proposed container.

2.4.3. Active substance – Trofinetide hydrate

2.4.3.1. General information

The chemical name is (2S)-2-{[(2S)-1-(2-aminoacetyl)-2-methylpyrrolidine-2-carbonyl]amino}pentanedioic acid as a hydrate. The Applicant has stated the molecular formula as C13H27N3O9, which is depicting the presence of 3 water molecules. Trofinetide hydrate has a molecular mass of 369.3 g/mol and the following structure:

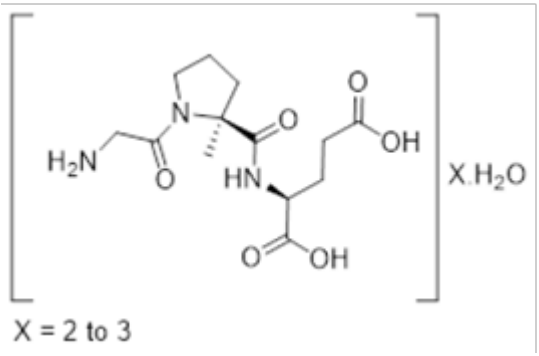


Figure 2: Trofinetide hydrate structure

The chemical structure of trofinetide hydrate was elucidated by a combination of proton and carbon nuclear magnetic resonance spectroscopy (1H, 13C, COSY, NOESY, TOCSY, HSQC, HMBC, H2BC), mass spectrometry, infrared spectroscopy, and single crystal X-ray crystallography. The solid-state properties of trofinetide hydrate were measured by single crystal X-ray crystallography, XRPD, TGA and DSC. The stable crystalline nature (Form A) as produced by the proposed manufacturing process has been shown. Two other polymorphs (B and C) can be produced under specific conditions.

The hydrous nature of the substance has been shown, although the exact amount of water in the crystal lattice is not constant. Therefore, Form A is not a true trihydrate and so it is designated as a hydrate.

Trofinetide hydrate is a white to off white solid, highly soluble (> 500 mg/mL) in aqueous media across the physiologically relevant pH range. Trofinetide hydrate is slightly hygroscopic above 80%RH. Trofinetide hydrate exhibits stereoisomerism due to the presence of two chiral centres (see trofinetide discussion above).

2.4.3.2. Manufacture, characterisation and process controls

The active substance is manufactured by two manufacturers for which satisfactory GMP documentation has been provided.

Trofinetide hydrate is synthesized in several main steps. The synthesis of the first few steps is identical to trofinetide, although an additional supplier is used for the starting material. The manufacturing process uses the same starting materials and generate the same isolated intermediates as for trofinetide. The same processing parameters are used. Minor variations of some charges/setpoints or

normal operating ranges may exist between sites, but all parameters are operated within the proven acceptable range.

The active substance manufacturing process is adequately described and the overall control strategy, including in process controls, starting material, intermediate and reagent specifications, and process parameter controls (including proven acceptable ranges (PARs)) ensures that the process consistently produces active substance of the intended quality.

For related substances, reagent related impurities, and potential mutagenic impurities, see discussion for trofinetide.

The development of the trofinetide hydrate manufacturing process is based on the trofinetide process which removes impurities but increases the levels of residual solvent. In addition to the development studies as described for trofinetide, a Design of Experiments (DOE) study was used to characterise the crystallisation process. No critical parameters were identified and the settings for the parameters in the crystallisation step have been laid down with sufficient control.

Phase 3 clinical trials have also been performed with trofinetide hydrate manufactured with the proposed commercial process.

The active substance is packaged in double low-density polyethylene (LDPE) bags that are placed inside an aluminised bag along with desiccant and heat sealed. The primary packaging complies with Commission Regulation (EU) 10/2011, as amended.

2.4.3.3. Specification

The active substance specification includes tests for appearance, identification, assay, related substances, water content, residual solvents, elemental impurity palladium, residue on ignition, and microbial purity.

The same analytical methods as for trofinetide are used, except for residual solvents. The in-house method for residual solvents has been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standard used for trofinetide hydrate has been presented.

Batch analysis data from eleven batches (3 registration stability, 3 process validation, five commercial) of the trofinetide hydrate are provided. The results are within the specifications and consistent from batch to batch.

2.4.3.4. Stability

Stability data from seven batches (3 registration stability/phase 3 batches, 3 validation/commercial batches, one annual stability batch) of trofinetide hydrate from the proposed manufacturer, and stored in the intended commercial package for up to 36 months under long term conditions (2-8°C) and for up to 36 months under accelerated conditions (25ºC / 60% RH) according to the ICH guidelines were provided. The following parameters were tested: appearance, assay, related substances, water content, microbial testing. The analytical methods used were the same as for release and were stability indicating. In addition, melting point (DSC) and crystallinity (XRPD) were monitored.

Since out-of-specification results for assay at accelerated conditions were observed, the storage precaution 'store at 2-8°C' is justified. At long-term conditions all tested parameters were within the specifications and no significant trends were observed.

When compared with trofinetide anhydrous, it is shown that higher water content in trofinetide hydrate does not have any negative impact on stability. It was observed that crystalline trofinetide hydrate exhibited a substantially slower rate of degradation over time at the accelerated 25°C/60% RH storage condition compared to the amorphous trofinetide.

The post-approval stability commitments are acceptable in view of the already provided stability data and ICH Q1A. Any confirmed out-of-specification result, or significant negative trend, should be reported to the Rapporteur and EMA".

For photostability testing following the ICH guideline Q1B, see trofinetide section above.

The stability results indicate that the active substance manufactured by the proposed manufacturer is sufficiently stable. The stability results justify the proposed retest period of 36 months when stored refrigerated (2-8°C) in the proposed container.

2.4.4. Finished Medicinal Product

2.4.4.1. Description of the product and pharmaceutical development

The proposed finished product is presented as a pink to red oral solution with strawberry flavour, containing 200 mg/mL of trofinetide as an active substance. 450 mL of product is filled in the 500 mL bottle presentation.

The pharmaceutical development of the finished product contains QbD elements. A quality target product profile (QTPP) as per ICHQ8(R2) was defined for the finished product to be used in Phase 3 clinical studies and commercial manufacture. The aim was to develop a safe and efficacious immediate oral solution that is ready to use and easy to administer to adult and paediatric patients.

Table 2: Quality Target Product Profile for the finished product

QTTP Elements	Target	Justification
Intended Use	Treatment of Rett Syndrome	Seriously debilitating neurodevelopmental disorder for which there is currently no approved treatment.
Dosage Form	Solution	Solution designed for ease of administration to adult and pediatric patients.
Delivery System	Solution (target pH 4)	Trofinetide is highly water-soluble at refrigerated and room temperature conditions. The oral solution composition is designed to deliver an appropriate dose. Trofinetide solutions self-buffer near pH 4
Route of Administration	Oral[a]	Ease of administration for the patient population. For palatability flavor and sweetener will be included.
Dosage Strength	200 mg/ mL	Up to 60 mL dosed twice daily based upon body weight.
Container Closure System	Multidose child resistant container closure system	Designed to hold an appropriate volume of trofinetide oral solution to provide flexibility for multiple dose levels and avoid inadvertent access by children in the household.
Purity	Impurities and degradation products	Process impurities and degradation products will be controlled in the drug substance used for manufacture of the DP according to ICH guidelines. Trofinetide oral solution will be manufactured at controlled temperature conditions and will be stored refrigerated. Trofinetide is known to degrade upon storage as a function of temperature. Degradation products of trofinetide oral solution will be controlled according to ICH guidelines by storing the drug product appropriately.
	Microbial purity	Anticipating majority component will be water, the product will be formulated to include preservatives to ensure compliance with USP and Ph. Eur. microbial purity criteria for oral solutions including *Burkholderia cepacia* complex.
	Elemental impurities	The raw materials will be controlled for the elemental impurities. The drug product process is not anticipated to introduce any elemental impurities. A risk assessment will be performed to verify compliance with ICH requirements.
	Extractable and leachable impurities from container closure system	Once the product composition and primary package is identified, an extractables study will be conducted. Leachable compounds will be monitored during the stability study.
Pharmacokinetics	Immediate release	Product will be designed as a solution and no release modification is needed.
Product Stability	At least 12-month shelf-life under refrigerated conditions	To provide adequate stability of trofinetide and maintain preservative effectiveness throughout the shelf-life.

[a] While developed as an oral solution, the product can be administered via gastrostomy tubes or other devices.

The characteristics of the active substance that influence the performance of the finished product have been adequately discussed. Because the amorphous and crystalline active substance are both freely soluble in aqueous solution, trofinetide oral solution can be produced using each solid-state form by the same finished product manufacturing process. Active substance particle size is not considered relevant as the substance is dissolved in the finished product.

Excipient compatibility studies during finished product formulation development have shown compatibility with trofinetide amorphous. These studies were not repeated with trofinetide hydrate since the previous excipient compatibility studies were done with dissolved active substance.

The formulation development has been adequately described, with information about the various prototype formulations. The impact of different flavours, sweeteners, colourants and preservatives has been investigated and the final formulation is justified. The formulation used during Phase 3 clinical studies is the same as that intended for marketing.

The proposed formulation was initially not acceptable since the preservative efficacy results did not comply with the Ph. Eur. 5.1.3 requirements for reduction of *A. brasiliensis*. The CHMP raised a MO to investigate or change the formulation to comply with Ph. Eur. 5.1.3 requirements (MO2). The Applicant explained that alternative preservatives tested were not efficacious and/or raised safety concerns (e.g. potassium sorbate, benzoates and individual parahydroxybenzoate esters, addition of propylene glycol). Furthermore, it is difficult for this product to meet all preservatives efficacy Ph. Eur. requirements. Because of the high daily dose volume (up to 120 mL) it is a significant constraint to keep the total preservative intake (volume x concentration) within the permissible daily exposure (PDE). Also, the active substance exhibits self-buffering behaviour which maintains the solution at a sub-optimal pH range for preservative efficacy. Still, the currently proposed levels of preservatives are well below the maximal permitted daily exposure, even in the youngest patients. Based on the justifications provided for the observed out-of-specification result for the test organism *A. brasiliensis* and the selected preservatives, the proposed formulation was accepted. The applicant has committed to investigate the possibility to lower the preservative concentration and the applicant is advised to address this point in any further marketing authorisation application.

The inclusion of the colouring agent Allura Red AC was not accepted initially since EMA quality guidance requirements on excipients (to be used in formulations for the paediatric population) were not addressed since colouring agents with documented safety risks should not be used in medicinal products for paediatric use. Therefore, the CHMP raised this as a MO, in particular because of safety concerns due to the use of azo dyes in formulations for paediatric patients (MO3). The Applicant stated that the colourant Allura Red AC is included to mask the natural discoloration of the product, which is not a sign of poor quality, and that alternative natural pigments investigated (carmine, capsorubin, lycopene) were not suitable. The maximal exposure to Allura Red AC would correspond to 1.5% of the acceptable daily intake as defined by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Therefore, the use of this colourant was considered acceptable and the MO was resolved.

A risk assessment has been performed on the potential presence of ethylene glycol and diethylene glycol in the excipients maltitol and propylene glycol (main component of the Strawberry flavour), and these contaminants are sufficiently controlled in the excipients themselves.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. or EC standards.

The manufacturing process consists of simple steps (compounding the bulk solution, mixing, filtration, filling). The development of the manufacturing process is described with sufficient details, with description of the process and control strategy applied for the various development batches. The manufacturing process development has been performed at a different manufacturing site not intended for the EU market, and only very limited number of batches have been manufactured in the finally proposed commercial manufacturing site. However, as the process at the commercial manufacturing site is fully validated and the batch results from the two sites are comparable, this is accepted.

The primary packaging is a white translucent round high-density polyethylene (HDPE) multi-dose bottle with a polypropylene child-resistant closure. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. A dosing device was not included in the initial commercial packaging and the initial SmPC statement that a CE marked measuring device should be obtained from the pharmacy

to measure and deliver the prescribed dose accurately was not accepted. The CHMP raised this as MO requesting to include suitable dosing devices in the commercial pack for measuring all possible dosages as per the posology in the SmPC (MO4). The Applicant agreed and the product was co-packaged with 30 mL and 110 mL CE marked polypropylene gradated dosing cups. The MO was resolved including follow-up responses to demonstrate compliance with Ph. Eur. requirements for uniformity of delivered doses.

2.4.4.2. Manufacture of the product and process controls

The finished product is manufactured by the manufacturing sites. For all sites involved in the manufacture, control and batch release of the finished product sufficient evidence of GMP compliance has been provided.

The manufacturing process consists of three main steps: compounding of ingredients in water, filtration of the bulk solution and filling. The process is considered to be a standard manufacturing process.

Major steps of the manufacturing process have been validated on three batches of the proposed commercial batch size at the proposed commercial manufacturing site. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this pharmaceutical form. There are no intermediates in the manufacturing process. A bulk holding time of 5 days (from start of compounding to completion of fill) is justified.

2.4.4.3. Product specification

The finished product release and shelf life specifications include appropriate tests for this dosage form: appearance (visual), identification trofinetide, identification methyl & propyl paraben, assay, degradation products, methylparaben assay, propylparaben assay, pH, microbiological tests and uniformity of delivered dose in 30 mL & 110 mL cups.

The proposed specification is justified with reference to current EMA and ICH guidelines (ICHQ6A, ICHQ3B, ICHQ3D), compendial requirements and supportive data from development batches, registration stability batches and commercial production batches. In accordance with Ph. Eur. requirements for liquid preparations for oral use (0672) supplied in multidose containers, a test for uniformity of mass of delivered doses from multidose containers (2.9.27) is performed on both dosing cups for the minimal and maximal volume to be measured. Omission of a test for deliverable volume (Ph. Eur.) has been adequately justified based on in-process results, development and stability data.

The major degradation products in the finished product have been characterised. One degradation product, with acceptance limits above the ICH Q3B qualification threshold, has been qualified by toxicological studies. The initially proposed specification limit of NMT 0.15% for unspecified impurities was not in line with the ICH Q3B identification threshold and a MO was raised by the CHMP (MO5). According to the Applicant batch results were observed above 0.10%, probably diluent related or because of sample artifacts but this was not fully accepted. Finally, the Applicant agreed to tighten the limit to NMT 0.10% as per ICH Q3B and the analytical method description for related substances was updated to reflect an improved sample preparation procedure.

The potential presence of elemental impurities in the finished product has been assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Batch analysis data on seven batches using a validated ICP-MS method was provided, demonstrating that each relevant elemental impurity was not detected above 30% of the respective PDE. Based on the risk assessment

and the presented batch data it can be concluded that it is not necessary to include any elemental impurity controls in the finished product specification.

The risk assessment concerning the potential presence of nitrosamine impurities in the finished product concluded that no risks are identified, and therefore confirmatory testing was not performed. However, as described under the active substance section, the report indicated a risk of N-nitrosodimethylamine (NDMA) formation in the active substance due to nitrite carry-over from a coupling reagent and the presence of a tertiary amine EDC-HCl and its urea by-product EDU. Further, the overall risk assessment for the finished product only referred to trofinetide amorphous and risk assessment template and conclusions were not taking all potential root causes into consideration and a MO was raised. The CHMP requested confirmatory testing and an updated complete risk assessment (MO6). The Applicant provided confirmatory testing results for NDMA on three commercial scale batches of each active substance source showing results well below 10% of the acceptable intake. Also, a complete risk assessment report was presented considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided, it is accepted that there is no risk of nitrosamine impurities in the active substance or the related finished product, and the major objection was resolved.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay of active substance, assay of preservatives, and impurities testing has been presented.

Batch analysis results are provided for all batches manufactured at the manufacturing sites for both clinical and commercial supply. They include three registration batches, three process validation batches and one commercial batch from the proposed commercial site. All these batches were manufactured with both trofinetide amorphous and trofinetide hydrate. The submitted batch results confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

2.4.4.4. Stability of the product

Stability studies have been performed on several finished product batches, from both manufacturing sites and with both forms of active substance. Stability data from 14 batches (3 registration and 3 process validation batches manufactured at Patheon Whitby using amorphous active substance, 3 US registration batches manufactured at CoreRx using amorphous active substance, 3 US registration batches manufactured at CoreRx using crystalline active substance, and 2 supporting batches manufactured at CoreRx at 450L commercial scale using amorphous active substance) of finished product stored for up to 36 months under long term conditions (25 ºC / 60% RH) and for up to 6 months under accelerated conditions (40 ºC / 75% RH) according to the ICH guidelines were provided. The finished product stability batches are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, assay, related substances, assay of methylparaben, assay of propylparaben, pH of solution, water loss and microbial purity. Registration batches manufactured with amorphous trofinetide have also been tested for deliverable volume. The analytical procedures used are stability indicating.

The accelerated stability results show significant changes from the 3 months' time point on, with decrease of assay and increase of specified, unspecified and total impurities. Therefore the proposed temperature storage restriction (store in the refrigerator) is considered justified and acceptable.

No significant changes have been observed at long-term storage conditions for batches from the commercial manufacturing site. Although the results for assay show a decreasing trend and significant changes (decrease larger than 5%) at the 36 months' time point for several clinical batches, these batches are considered only supportive for the shelf life establishment.

During long-term stability studies, unspecified impurities have been detected above the identification threshold of 0.10%. However, these have been justified as artifacts, visible also in the blank. The UHPLC method has been adjusted to avoid this issue.

In addition, one batch sample was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The finished product is not sensitive to light. Freeze-thaw studies demonstrated that the quality of the product is still adequate after four freeze-thaw cycles, however the label statement 'do not freeze' is accepted to avoid the use of partially unthawed (and potentially not uniform) solution.

In-use stability studies up to 30 days after first opening have been performed on stability samples stored for 36 months at 2-8°C. At controlled room temperature, significant degradation is observed. The results confirm that the finished product after first opening should be stored refrigerated (at 2-8°C) when in-use by the patient.

Based on available stability data, the proposed shelf-life of 3 years when stored in a refrigerator (between 2°C and 8°C) for the unopened product, and of 30 days after first opening of the bottle are acceptable.

2.4.4.5. Adventitious agents

No excipients derived from animal or human origin have been used.

2.4.5. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

During the procedure 6 MOs were raised by the CHMP related to 1) mutagenic impurities in the active substance, 2), preservative efficacy of the finished product formulation, 3) inclusion of Allura Red AC in the finished product formulation, 4) absence of measuring device(s) in the commercial pack, 5) proposed limit for unspecified impurities in the finished product, 6) risk assessment and request for confirmatory testing of nitrosamines.

All the MOs were satisfactorily resolved by: 1) presenting a complete discussion on mutagenic impurities (including reagents and intermediates) together with a discussion on their mutagenic potential, 2) additional justification for inclusion of Allura Red AC which will not expose patients above 1.5% of the acceptable daily intake as stated by the JECFA, 3) additional justifications for inclusion of the selected preservatives and out-of-specification result for one test organism, 4) co-packaging the finished product with 30 mL and 110 mL CE marked polypropylene gradated dosing cups, and demonstrating compliance with the Ph. Eur. requirements for the uniformity of delivered doses, 6) tightening the release and shelf life specification limit for any unspecified impurity in accordance with

ICH Q3B identification threshold, 7) submission of a complete nitrosamines risk assessment report considering all suspected and actual root causes in line with EMA , along with confirmatory test results for NMDA in both active sources showing levels well below 10% of the acceptable intake for NMDA.

The applicant has applied QbD principles in the development of the active substance manufacturing process. However, no design spaces were claimed for the manufacturing process of the active substance.

2.4.6. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the proposed SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. In conclusion, the documentation submitted for the active substances and finished product is acceptable from a quality point of view.

2.4.7. Recommendation(s) for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

Trofinetide is a synthetic analogue of glycine-proline-glutamate (also known as glypromate or GPE), a peptide that occurs naturally in the brain. GPE is the N-terminal tripeptide of the insulin-like growth factor 1 (IGF-1) protein.

Trofinetide has been evaluated in a comprehensive program of pharmacology, pharmacokinetic (PK) and toxicology studies. Proof of concept has been studied in MeCP2 mutant mice, and the absorption, distribution, metabolism, and excretion (ADME) profile and potential drug transporter interactions of trofinetide have been characterized. The toxicity potential of trofinetide was assessed in a battery of *in vitro* and *in vivo* non-clinical safety studies. Standard rodent (rat) and non-rodent (dog) species were used for toxicity and safety studies based on PK and similarity to human metabolism profiles; trofinetide does not undergo Phase 1 metabolic transformation. Juvenile-aged and/or adult-aged animals (rats, dogs, rabbits) were used for single dose, repeat dose, and reproductive and developmental studies. The TgHras2 model was used to evaluate carcinogenicity.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Many of the neurological features (cognitive and motor deficits) seen in Rett syndrome patients may be due to deficits in synaptic maturation and function in the brain due to mutations in MeCP2. MeCP2 mutant mice have sparse dendritic spines and reduced post-synaptic density protein 95 (PSD-95), a postsynaptic scaffold protein at excitatory synapses that promotes synapse maturation and exerts a major influence on synaptic strength and plasticity in motor cortex pyramidal neurons. Brain slices

from Mecp2 mutant mice show overt changes in synaptic function including reduced synaptic plasticity (Asaka et al. 2006; Moretti et al. 2006; Guy et al. 2007; Nelson et al. 2008; Weng et al. 2011).

The rationale for trofinetide as a therapeutic agent to treat Rett syndrome was indicated to come from studies with GPE (glycine-proline-glutamate), a naturally occurring tripeptide in brain associated with neuroprotective activity including in Mecp2 mutant mice, a non-clinical model of Rett syndrome. Treatment with GPE (0.01 mg/g [intraperitoneal (IP)] once daily [QD]) partially restored spine density and synaptic amplitude, increases PSD-95, and stabilized cortical plasticity to wild-type levels (Tropea et al. 2009)

Trofinetide (glycyl-L-2-methylprolyl-L-glutamic acid) is a synthetic, chemically modified analogue of GPE, resulting from α-methylation of the proline moiety. It is designed to prolong the plasma half-life of trofinetide compared to GPE, while retaining similar *in vivo* efficacy, making it much better suited for use as a therapeutic agent (Bickerdike et al. 2009).

In vitro, two assays were conducted in which the neuroprotective effect of trofinetide (1 nm – 10 µM) was demonstrated against agents inducing apoptosis (okadaic acid) and oxidative stress (3-nitropropionic acid) in neuronal striatal and cerebellar cultures, respectively. These results show some evidence that trofinetide may be a neuroprotective agent, but the assays are not convincing. Although statistically significant, there appears to an incomplete recovery in cell viability compared to the injured cells, and a clear dose-response seems lacking as the lowest concentrations seem similar effective as the highest concentration. The effect of trofinetide on synaptic function and neuronal morphology were evaluated by examining ex vivo hippocampal slices taken from Rett syndrome knockout mice. On this basis, the Applicant concluded that an *in vitro* analysis of the action of trofinetide in isolated neurons is unnecessary.

Trofinetide has been tested in an established genetic mouse model of RTT in which exon 3 of the MeCP2 gene is deleted, a similar model as used for study of GPE (Tropea et al, 2009). In line with GPE, trofinetide is thought to improve neuronal synaptic function and morphology. The effects of intraperitoneally (IP) administered trofinetide (20 mg/kg) on electrophysiology and dendrite morphology were investigated following a 5 week treatment initiated in 4-week old mice. Trofinetide significantly improved hippocampal long-term potentiation (LTP) and dendritic length. It is understood that LTP was studied using acute hippocampal slice preparations due to technical reasons and the fact that it is an often used model system to investigate electrophysiological processes in the brain. The neurological symptoms in patients with Rett syndrome primarily arise from subtle defects in dendrites, axons, synaptic structures or the electrophysiological processes these structures participate in, such as forms of synaptic plasticity. Several studies indicated that synaptic defects are also observed in forebrain regions in Rett syndrome. This includes the brain analysis of Rett syndrome patients revealing structural abnormalities of synapses in both the hippocampus and in discrete cortical areas. Also in Mecp2 mouse models, defects in synaptic plasticity have been described in different brain regions including forebrain/cortex and hippocampus. Given that trofinetide protected both the cortex and striatum against middle carotid artery occlusion-induced infarct, trofinetide's effects are likely not limited to the hippocampus and it can therefore be agreed that synaptic plasticity defects like LTP observed in the hippocampus are most likely generalizable to other cortical brain areas.

In a second cohort of animals that were administered 20 mg/kg trofinetide, overall survival, the number of apneas at various time points, aspects of autonomic (cardiovascular and respiratory) function at 9 weeks and motor function (tremor score, locomotor activity, gait and general condition) at 13 weeks were examined. Overall survival was statistically significantly increased when animals were treated with trofinetide, showing 50 percent survival at 15.5 weeks while vehicle treated animals showed 50 percent survival at 13.5 weeks. Tremor score was statistically significantly increased following administration of trofinetide.

Several *in vivo* studies of other neurological disease models were provided to show that trofinetide has neuroprotective and neuroregulatory capacity in related disorders. These included models of fragile x syndrome, stroke and traumatic brain injury. Although the underlying cause of the disease and the primary affected injury regions differ compared to RTT the Applicant explained that they share the clinical phenotypes including cognitive impairment and motor dysfunction, as well as underlying signalling deficits and pathophysiological mechanisms including impaired synaptic plasticity, neuroinflammation, and oxidative stress.

The effect of trofinetide (100 mg/kg, IP for 28 days), compared with vehicle control, was investigated in a knockout model for fragile x syndrome (Fmr1) mice and wild-type (WT) mice. The data on FXS showed that trofinetide reversed abnormalities in spine density and in phosphorylation of Akt/ERK intracellular signalling, indicative of restored signaling in the PI3K/Akt/mTOR and MAPK pathways—systems involved in neuronal function and survival that are also disrupted in RTT due to MeCP2 dysfunction.

Based on studies evaluating trofinetide in stroke, the Applicant aimed to further provide evidence that trofinetide has a similar profile to GPE, and could be an effective therapeutic agent to treat RTT. In animal models of ischemic injury, including the endothelin-1 (ET-1)–induced middle cerebral artery occlusion model, trofinetide demonstrated dose-dependent neuroprotection when administered intravenously or orally, suggesting that trofinetide is able to reduce oxidative stress-induced damage. Oxidative stress plays a significant factor in pathogenesis of RTT. Although it was stated that trofinetide exerts antioxidant effects likely through IGF-1–mediated pathways, this was not further supported by data and rather unlikely as it was shown that trofinetide does not bind to the IGF-1R.

In multiple rodent models of traumatic brain injury (TBI), trofinetide demonstrated anti-inflammatory effects (reduction of pro-inflammatory cytokine expression and microglia activation). Trofinetide did not significantly reduce injury volumes. Also following cerebral ischemia, trofinetide reduced both astrocytic and microglial activation. Chronic neuroinflammation, as observed by elevated pro-inflammatory cytokines in both MeCP2-deficient mice and patient-derived biospecimen, as well as glial cell activation, are significant features of RTT pathology and contribute to neuronal dysfunction and degeneration.

Across all evaluated models including FXS, ischemic stroke and TBI, trofinetide improved functional outcomes. In FXS, improvements were seen in hyperactivity, learning and memory, social behaviour and anxiety. In ischemic stroke studies, trofinetide was stated to improve sensory-motor function and other behavioural tests indicative of a neurological deficit score, although no adequate figures were provided. Functional outcome improvements in TBI included some improvement in beam walking, rotarod and water maze.

In aged rats, trofinetide improved working memory. At the cellular level, trofinetide increased the number of proliferating cells in the subventricular zone in the brain, indicative of adult neurogenesis, and reduced astrogliosis in the hippocampus, indicative of reduced neuroinflammation.

2.5.2.2. Secondary pharmacodynamic studies

The activity of trofinetide was tested in a competition radioligand binding assay consisting of over 80 common targets (GPCRs, ion channels, enzymes, receptors and transporter systems). Trofinetide tested at 50 nM did not show activity (>50%) at any of the tested targets.

In a broad panel of CNS molecular targets (GPCRs, ion channels and transporters), trofinetide (at 10 µM) exhibited activity at the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDA) and kainate receptors (81.8%, 74.6% and 36.4% inhibition of radioligand

binding, respectively). Binding to kainate receptor was weak to moderate. For the AMPA and kainate receptors, binding of the agonist radioligand was inhibited, for NMDA the antagonist radioligand. In a follow-up radioligand binding study, the Ki values for the AMPA, NMDA, and kainate receptors were determined to be 1.9 µM, 3.4 µM and 5.9 µM respectively. Patch clamp electrophysiology determined that trofinetide did not activate (at 10 and 30 µM) or block (at 10 µM) NMDA receptor mediated currents in rat cortical neurons, suggesting no functional activity of trofinetide at neuronal NMDA receptors at the tested concentrations. No electrophysiology was conducted for the AMPA and kainate receptors.

Since trofinetide contains both glycine and glutamate moieties, the potential for binding to metabotropic glutamatergic receptors (mGluRs) was evaluated. Trofinetide displaced L-[3H]-glutamate binding, that acts on all mGluR subtypes, with an IC50 of 90.6 ± 6 µM. In C6 glioma cells, endogenously expressing only the mGluR1 subtype, trofinetide did not displace L-[3H]-Glutamate binding at concentrations up to 10 mM (data not shown). Subsequent radioligand competition binding assays demonstrated that trofinetide displaced [3H]-LY341495, specific for group II mGluRs, with an IC50 of 33.1 µM (Ki 16.7 ± 4.0 µM). In addition, trofinetide (at 100 nM) did not act as a positive or negative allosteric modulator for L-Glutamate on group II mGluRs.

In a screen of 55 kinases, trofinetide tested up to 1 µM had no notable inhibitory activity on any of the tested kinases. Trofinetide (up to 100 µM) did also not act on the histone-deacetylase enzymes, HDAC1, HDAC2, HDAC3 and HDAC8. In a second screen with 1 mM trofinetide, significant results were observed at the NMDA receptor (103.9% inhibition of binding). Trofinetide has no effect through the IGF-1R.

2.5.2.3. Safety pharmacology programme

The safety pharmacology package comprised GLP-compliant cardiovascular (dogs), CNS (rats) and respiratory (rats) safety studies. *In vitro* studies comprised two GLP-compliant hERG inhibition assays.

In the first hERG inhibition study, less than 2% hERG suppression was observed at 10.65 µM and 115.1 µM and therefore no IC50 was calculated. In the second hERG inhibition study, there was 6% hERG current inhibition at 20 mM trofinetide. The IC50 for the effect of trofinetide on hERG potassium current could not be determined but is estimated to be > 20 mM, which is approximately 40-fold the clinical exposure at the MRHD (free Cmax: 495 µM), suggesting that no meaningful effects on hERG channel currents by trofinetide are expected clinically.

In the GLP-compliant cardiovascular safety study in dogs, there were no effects on arterial blood pressure, heart rate, RR interval, PR interval, QRS duration and QT interval at doses up to 400 mg/kg/h. QTc interval was prolonged at 90 to 120 minutes and 60 to 90 minutes after the administrations of 200 and 400 mg/kg/h, respectively. QTc interval was increased up to 18 ms (up to 8%) of the QT interval and was observed at mean peak plasma levels of 573 µg/mL and higher. At the QTc NOAEL of 240 mg/kg, a Cmax of 335 µg/ml was observed, which is approximately 1.7-fold the clinical exposure at the MRHD, suggesting that based on these data, QTc prolongation cannot be excluded to occur in the clinic. In addition, trofinetide infusions of 60, 120, 200 or 400 mg/kg/h was associated with clinical signs of lip licking and tremors. Subdued behaviour and pupil dilation were also noted at 400 mg/kg/h. The dosing solutions were hyperosmotic which may have contributed to some of the clinical signs observed.

In the GLP-compliant CNS rat study, there were no effects on neurobehavioural effects as measured by the Irwin test at trofinetide doses up to 350 mg/kg. Therefore, the NOAEL for CNS effects is 350 mg/kg.

In a GLP-compliant respiratory rat study, there were no apparent effects of trofinetide on respiratory rate and tidal volume up to the highest tested dose of 610 mg/kg (actual dose level based on formulation analysis).

Notably, only male animals (dogs and rats) were tested in the *in vivo* studies, without adequate justification by the Applicant. However, no apparent gender differences were noted regarding pharmacokinetics/toxicokinetics in dogs and rats (see section 2.5.3) and the use of one gender is therefore accepted.

2.5.2.4. Pharmacodynamic drug interactions

No dedicated non-clinical studies on pharmacodynamic drug interactions were provided. Any potential interactions are covered in the clinical overview and in the labelling/SmPC.

2.5.3. Pharmacokinetics

Trofinetide (NNZ-2566) was investigated in a range of *in vitro* and *in vivo* pharmacokinetic (PK) and toxicokinetic (TK) studies in mice, rats, rabbits and dogs. Sprague Dawley (SD) rats and Beagle dogs, were the nonclinical safety species selected. These studies were conducted to define the absorption, distribution, metabolism, and excretion (ADME) of trofinetide. Oral administration, which is the route of clinical administration, was selected for the pharmacokinetic studies in animals. Data from these studies were used to characterize the PK and TK properties of trofinetide to support nonclinical toxicology evaluations and to support its intended clinical use.

2.5.3.1. Analytical methods

The bioanalytical methods used in support of GLP toxicity and safety studies in rat and dog were validated and compliant with European Medicines Agency (EMA) and ICH guidelines on bioanalytical method validation and study sample analysis, but not conducted under GLP compliance.

Whole blood was treated with a protease inhibitor to reduce instability in biological matrices. A protease inhibitor cocktail was added to whole blood at the ratio of 1:100 (Sigma. Product Number P 8340). The protease inhibitor cocktail contains six individual components for inhibiting serine, cysteine, and acid proteases, and aminopeptidases; AEBSF, Aprotinin, Bestatin, E-64, Leupeptin and Pepstatin.

A non-GLP LC-MS/MS method, using acetonitrile to precipitate out the protein, was qualified to support the quantification of trofinetide in incubation matrices of *in vitro* assays. Morphine 3-β-D-glucuronide-d3 (M3G-d3) was used as internal standard. The method was qualified over the range of 5 to 2000 nM (~ 1.6 – 631 ng/mL) and evaluated for accuracy, precision, selectivity, carryover, reinjection reproducibility, stability (6h at RT), carryover, reinjection reproducibility, accuracy and precision. The methods met the acceptance criteria and is considered fit for purpose.

A non-GLP LC-MS/MS method of analysis was qualified for measurement of trofinetide EDTA mouse plasma. Trofinetide (NNZ-2566) was extracted from stabilized animal whole blood by protein precipitation followed by solid phase extraction at alkaline pH using an Oasis MAX plate. The acidic eluant was collected and evaporated to dryness. The residue was reconstituted with 90:10:0.1 water:ACN:formic acid. Analysis was performed by LC-MS/MS using a Restek Ultra PFPP column with a gradient water/acetonitrile/formic acid mobile phase. The method is considered valid within the investigated concentration range of 0.100 to 100 µg/mL. All acceptable results were within the validation criteria specified in this project. This included selectivity/specificity, linearity, sensitivity, accuracy, precision, recovery, dilution, and stability experiments. Short term stability EDTA plasma at

room temperature was 97 hrs in mouse. Long term stability at -80°C 89 d in mouse. Freeze/thaw stability at -80°C was determined for 4 times for mouse. The method met the acceptance criteria and is considered fit for purpose.

Three other non-GLP LC-MS/MS methods of analysis were qualified for measurement of trofinetide in lithium heparinized rat, rabbit, and dog whole blood. These methods were validated in accordance with applicable SOPs, the validation workplan, and the FDA document "Guidance for Industry, Bioanalytical Method Validation", May 2001 and European Medicines Agency (EMA) Guideline on Bioanalytical Method Validation, but not conducted under GLP compliance. Trofinetide (NNZ-2566) was extracted from stabilized animal whole blood by protein precipitation followed by solid phase extraction at alkaline pH using an Oasis MAX plate. Before the extraction, methanol is added to the samples, then isotope labelled NNZ-2566-IS was added as an internal standard. The acidic eluate is collected and evaporated to dryness. The residue is reconstituted with mobile phase. Analysis was performed by LC/MS/MS using a Sciex API 3000™ mass spectrometer with electrospray ionization source and Analyst™ control software and a Restek PFP Propyl column with a water/acetonitrile/formic acid mobile phase. The methods were valid within a concentration range of 0.100 (LLOQ) -100 µg/mL (ULOQ) for trofinetide. All acceptable results were within the validation criteria specified and defined in ICH_M10. This included selectivity/specificity, linearity, sensitivity, accuracy, precision, recovery, dilution, and stability experiments. Short term stability in stabilized lithium heparinized whole blood at room temperature was 24 hours in dog, 26 hrs in rabbit, 24 hrs in rat and 97 hrs in mouse. Long term stability at -80°C was 324 days in dog, 7 d in rabbit, 961 d in rat and 89 d in mouse. Freeze/thaw stability at -80°C was determined for 4 times for rabbit and mouse and 3 times for rat and not determined for dog samples**.**

The method for measurement of TK samples in Rabbit qualified a stability period at -80°C of 7 days. Study 20222417 was performed between 17022020 and10042020, whereas the TK report draft report was dated 11122020. No raw data TK measurements and measurement time were included in the report, and it is questionable if samples have been analyzed within the stability period. However, long stability in samples from dog and rat were much longer and long-term stability of rabbit samples is expected to be longer than the reported 7 days. Therefore, no consequences for the conclusions of the TK measurements in rabbit studies are expected.

None of the methods were validated in compliance with GLP. Although validation of the methods used for the analysis of TK in the pivotal studies should be performed in compliance with GLP, no consequences for the conclusions of these studies are expected.

Incurred Sample Reanalysis (ISR) is the repeated measurement of an analyte's concentration from study samples to demonstrate reproducibility. For mice and rat ISR was performed and in both studies 30/30 (100%) of the samples demonstrated a percent difference within ±20.0% (Study 0706-22576 and Study 2329-001, respectively), for dog ISR was successful for 49/60 (81.7%) of the samples (Study 0621-16032) and for rabbit for 31/ 36 (86%) of the samples (Study 2022417); all met the acceptance criteria (>66.7%).

Metabolism of trofinetide is very low and as no major metabolites were identified, no specific assays are required.

2.5.3.2. Absorption

PK studies with trofinetide were conducted after single dose and continuous intravenous (IV) administration in Sprague Dawley (SD) rats, after 14 days repeated oral (PO) administration in CD-1 and TgHRAS mice (2-13 wks), and as toxicokinetic studies under GLP compliance in juvenile (26 wks), pregnant or lactating SD rats, pregnant New Zealand White (NZW) rabbits (3 wks) and beagle dogs

(39 wks). PK analyses were performed in plasma for rat single dose IV and studies in mice, all other PK and TK studies were conducted in whole venous blood samples that was treated with a protease inhibitor to reduce instability in biological matrices.

A single dose PK study using an intravenous (IV) solution (in 0.1 M succinate buffer) was conducted in male (M) SD rats by a bolus injection and male and female (F) rats were exposed to continuous 24-hour infusion. After intravenous bolus administration, plasma concentration declined rapidly with an terminal elimination half-life ($t_{½}$) of 20.7 minutes and clearance (CL) of 30.1 mL/hr. The IV PK study in rats has not been extensively analysed and relevant parameters are missing e.g. distribution volume. In addition, when combined with PO PK data in rat, bioavailability could be determined, but that data has not been provided. A single dose PK study using an intravenous bolus injection solution was conducted dogs (M+F) as part of a dose range finding study at 350, 750 and 1500 mg/kg, resulting in $AUC_{0\text{-}24}$ of 1007 µg.h/ml, 2377 µg.h/ml and 4361 µg.h/ml, respectively. The elimination half-life was determined at 45.5 minutes, but no other PK parameters such as clearance and distribution volume were calculated. Following 7-day intravenous infusions of 20, 60 and 150 mg/kg/hr trofinetide to male and female dogs over 4 cycles, the systemic exposure (Concentration at steady state and AUC_{0_96}) increased in proportionally with infusion rate and trofinetide did not accumulate with repeated treatment cycles, which is consistent with the short estimated half-life and indicates that the kinetics of trofinetide were linear. The terminal half-life of trofinetide was estimated between 0.8 to 1.4 hr, the systemic clearance (CL) of trofinetide from blood was moderate, on average, 442 to 832 mL/hr/kg (M and F, resp., ~25-50% of liver blood flow) and the distribution volume was high in dogs, 571 to 1240 mL/kg (M, F, resp.), which approximates or is greater than total body water in dogs (604 mL/kg), indicating extensive distribution or binding of trofinetide to tissue.

In two non-GLP 14-day oral dose range-finding studies in CD-1 mice (study 0706-22574) and TgHRAS mice (study 0706-22575), trofinetide (solution in water for injection) was dosed at 300, 600, or 2000 mg/kg/day in male and female animals (as BID doses of 150, 300, or 1000 mg/kg/dose given 6 hours apart). In toxicokinetic studies under GLP compliance, a 4 week study in TgHRAS mice (study 0706-22576) and a 13 week study in CD-1 mice (study 0706-22577) trofinetide was dosed at 400, 800, or 2000 mg/kg/day in male and female animals (as BID doses of 200, 400, or 1000 mg/kg/dose). Comparable data were obtained in all 4 PK studies in mice. Upon a single oral administration, trofinetide was rapidly absorbed into systemic circulation with time to peak concentrations occurring 1.5 hours (T_{max}) after dosing. The average (M/F) peak exposure level C_{max} was 12-17 µg/mL at 300 mg/kg, 18-54 µg/mL at 400 mg/kg, 31-40 µg/mL at 600 mg/kg, 58-74 µg/mL at 800 mg/kg and 142-296 µg/mL at 2000 mg/kg. The average (M/F) $AUC_{0\text{-}24}$ was 99-211 h*µg/mL at 300 mg/kg, 160-556 h*µg/mL at 400 mg/kg, 187-399 h*µg/mL at 600 mg/kg, 338-800 h*µg/mL at 800 mg/kg and 800-1480 h*µg/mL at 2000 mg/kg. There were no significant sex differences observed. In study 0706-22574 trofinetide exposure calculated by dose normalized (dn) Cmax and $dnAUC_{0\text{-}24}$, was slightly greater than dose proportional for C_{max} (2-3 fold increase at higher dose), but not for $AUC_{0\text{-}24}$. In general, in mice a dose proportional increase of exposure of trofinetide was observed under the conditions of the studies. There was no evidence of accumulation upon multiple dosing when comparing exposures (C_{max} and $AUC_{0\text{-}24}$) on Day 1 vs Day 14, 28 or 91.

The PK/TK of trofinetide upon repeat oral dosing was studied in male rats with a 3 day dosing study to evaluate the effect of 2 (BID, total dose 60 and 600 mg/kg/day) or 3 (TID, total dose 90 and 900 mg/kg/day) times daily dosing (study 0621-11118), in a 26 week juvenile BID study in male and female animals (0621-16031), in pregnant rats (study 20222415) and in lactating rats (study 20222418). All TK studies were GLP compliant. In rats, trofinetide was rapidly absorbed into systemic circulation with time to peak concentrations occurring between 1-2 hours (T_{max}) after repeated dosing. In the 3 day repeat dose study C_{max} and T_{max} were comparable for both BID and TID oral dosing regimens, and dnC_{max}, values increased 2-4 fold with increasing dose in a greater than dose

proportional manner, the effect on AUC_{0-24} was less pronounced (1.5 fold increase). Comparable data were obtained in all 4 PK studies in rats after oral dosing BID. The average (M/F) peak exposure level C_{max} was 2-3 µg/mL at 60 mg/kg.d (M only), 3-4 µ/mL at 90 mg/kg.d (TID, M only), 16-69 µg/mL at 300 mg/kg.d, 62-108 µg/mL at 600 mg/kg.d, 68-115 µg/mL at 900 mg/kg.d (TID, M only) and 147-405 µg/mL at 2000 mg/kg.d. The average (M/F) AUC_{0-24} was 23 h*µg/mL at 60 mg/kg.d (M only), 38 h*µg/mL at 90 mg/kg.d (TID, M only), 112-668 h*µg/mL at 300 mg/kg.d, 223-1330 h*µg/mL at 600 mg/kg.d, 424-671 h*µg/mL at 900 mg/kg.d (TID, M only) and 860-5080 h*µg/mL at 2000 mg/kg.d. There was no evidence of accumulation upon multiple dosing when comparing exposures (C_{max} and AUC_{0-24}) on day 1 vs Day 3, Gestation Day 20, Lactation Day 20, week 9, or week 26. There were no significant sex differences observed. In general, in rats a dose proportional increase of exposure of trofinetide was observed under the conditions of the studies.

In rabbits, oral absorption was variable with peak concentrations occurring between 1 and 18 hours (T_{max}) after repeated dosing. The rapid plasma clearance observed in mice and rats is not seen in rabbits, but an IV study to quantify the plasma clearance was not performed. In female pregnant rabbits, following PO administration from GD 7 through GD19 trofinetide was dosed in a dose range finding study at 0, 150, 300, or 500 mg/kg (300, 600, or 1000 mg/kg/day total dose, study 20222416) and in the definitive study at 0, 75, 150, or 300 mg/kg BID trofinetide (150, 300, or 600 mg/kg/day total daily dose, study 20222417). Both studies were GLP compliant. The peak exposure level C_{max} was 1-3 µg/mL at 150 mg/kg, 1-13 µg/mL at 300 mg/kg.d, 3-21 µg/mL at 600 mg/kg.d and 4-24 µg/mL at 1000 mg/kg.d. The average AUC_{0-24} was 13-58 h*µg/mL at 150 mg/kg.d, 20-129 h*µg/mL at 300 mg/kg.d, 52-425 h*µg/mL at 600 mg/kg.d and 79-470 h*µg/mL at 1000 mg/kg.d. Trofinetide accumulation upon multiple dosing was observed on GD 19 relative to GD 7. A 4 to 9 fold accumulation of exposure was seen based on C_{max} and AUC_{0-24}. Accumulation was not observed in other toxicity studies in mice, rat and dog despite higher doses administered over longer time periods and higher exposures. It is noted that the exposures obtained on GD19 in these rabbit studies were 2 fold (AUC_{0-24}) to 6 fold (C_{max}) lower, than the exposures obtained in repeat dose toxicity studies in mouse, rat and dog, indicating that the absorption of trofinetide in rabbits is different compared to other species. The trofinetide accumulation appears specific to rabbits and does not appear to represent the typical PKs of trofinetide. In rabbits a dose proportional increase in exposure (C_{max} and AUC) of trofinetide was observed under the conditions of the studies.

In a GLP compliant 39-week repeat dose toxicity study in dogs, trofinetide was administered PO BID at 25, 150, or 500 mg/kg BID trofinetide (50, 300, or 1000 mg/kg/day total dose, study 0621-16032). Trofinetide was rapidly absorbed with a T_{max} between 1 or 2 hours after dosing. The average (M/F) peak exposure level C_{max} was 2-4 µg/mL at 50 mg/kg.d, 18-44 µg/mL at 300 mg/kg.d and 110-201 µg/mL at 1000 mg/kg.d. The average AUC_{0-24} was 10-15 h*µg/mL at 50 mg/kg.d, 66-122 h*µg/mL at 300 mg/kg.d, 391-614 h*µg/mL at 1000 mg/kg.d. There were no significant sex differences observed. In general, in dogs a dose proportional increase of exposure of trofinetide was observed under the conditions of the studies. There was no evidence of accumulation upon multiple dosing when comparing exposures (C_{max} and AUC_{0-24}) on Day 1 vs week 12 and week 38.

In summary, the pharmacokinetic profile of trofinetide upon oral BID dosing is similar in mice, rats and dogs with the highest exposure after oral administration of 2000 mg/kg.d measured in rats C_{max} 301-405 230g/mL and AUC 4340-5080 h*µg/mL. In rabbits the exposure at comparable doses is 2-5 fold lower compared to the other species tested. After intravenous administration, the clearance (CL) of trofinetide was low in rats, 30.1 mL/hr (~ 5% of liver blood flow) and moderate in dogs, 442 to 832 mL/hr/kg (M and F, resp., ~25-50% of liver blood flow). The terminal elimination half-life (t½) was short in rats and dogs, 20.7 and 45.5 minutes, respectively. The distribution volume was high in dogs (571 to 1240 mL/kg, M and F, resp.), which approximates or is greater than total body water in dogs (604 mL/kg), the distribution volume (V_d) in rat has not been determined. Following oral

administration, trofinetide was rapidly absorbed into systemic circulation with time to peak concentrations occurring around 2 hours (T_{max}) after dosing. Bioavailability data for rat and dog is lacking. There were no significant sex differences and a dose proportional increase of exposure of trofinetide was observed. There was no evidence of accumulation upon multiple dosing when comparing exposures (C_{max} and AUC_{0-24}) on Day 1 up to 39 weeks, except after 3 weeks in rabbits.

2.5.3.3. Distribution

The binding of trofinetide to rat, dog, and human plasma proteins was evaluated at different concentrations using ultrafiltration or equilibrium dialysis. Using ultra filtration at concentrations of 400 µM (126 µg/mL) and 2000 µM (631 µg/mL), plasma protein binding of trofinetide was 4.0 and 5.5%, respectively in human plasma. Protein binding in HSA solution at 400 and 2000 µM was 4.2% and 9.5%. respectively. Using a more sensitive assay, equilibrium dialysis, at 200 fold lower concentrations (75 ng/mL (0.24µM), 750 ng/mL (2.4 µM), and 3,000 ng/mL (9.5 µM), plasma protein binding of trofinetide was low in rat (1.5%), dog (0.5%), and human (1.1%). In the protein plasma binding assays, no saturation of binding sites was observed up to the highest tested concentration (2000 µM), however, given the 2-5 fold difference in % plasma protein binding between the 2 methods, a concentration dependency cannot be excluded.

The *in vivo* tissue distribution of [^{14}C]-Trofinetide was investigated in the male albino SD rat (PO, 200 mg/kg, 100 µCi/kg = 3.7 MBq/kg, n=1/timepoint) and in male partially pigmented Long Evans rats (IV, 20 mg/kg, 100 µCi/kg = 3.7 MBq/kg, n=1/timepoint) using quantitative whole body autoradiography (QWBA) at 0.25 up to 168 h for PO and 0.08 to 24 h for IV. A certificate of analysis of Trofinetide, [methyl-14C] Lot no. TRF-19-001 has been provided and is compliant. The LLOQ for tissues analysed by QWBA was 4,824 ng equiv./g for tissues, 335 ng equiv./g for plasma and 363 ng equiv./g whole blood. In addition to quantification of blood and tissue levels of radioactivity by QWBA, whole blood, plasma, expired air, urine, bile, faeces, cage rinse, cage wash, activated carbon, brain, and CSF samples were analysed by total radioactivity by liquid scintillation counting (LSC). The quantitation limit (AQL) for CSF was 203 ng-equiv./g and 72 ng-equiv./g for brain. The LLOQ for tissues analysed by LSC was approximately 67-fold lower (QWBA 4,824 ng equiv./g for tissues, LSC 72 ng-equiv./g for brain) than the LLOQ for tissues analysed by QWBA.

After a single oral (gavage) dose of 200 mg/kg [^{14}C]-Trofinetide to male SD rats, [^{14}C]-Trofinetide and/or its [^{14}C]-trofinetide related radioactivity was broadly distributed and measurable by QWBA in all tissues for at least 1 time point, except the spinal cord, eye (lens), and brain, and were generally less than those in plasma. Maximum concentration was detected at 6 h (T_{max}) for liver and large intestine but at 1 h, or less for all other tissues. For most tissues radioactivity was BLQ at 24 hrs after oral dosing. Tissues with highest exposure (tissue to blood ratio (T/B) based on AUC_{last}) were kidney (all parts, T/B 7-24) and walls of the gastrointestinal tract (T/B 9-19), and T/B ~1.5 in liver, followed by T/B ~ 0.5 in stomach, lung and adrenal gland. T/B ratio of the remaining tissue was low with 0.3 and 0.2 in skin, spleen, thyroid gland and bone marrow and below 0.2 in other tissues measured. Although in the pigmented Long-Evans rat only four tissues were investigated at 5 min, 1 h and 24 hrs, no preferential association with pigmented tissues such as skin and uveal tract was observed., Radioactivity also did not appear to partition appreciably into blood cells with a blood:plasma ratio of 0.53, indicating trofinetide is distributed mainly in plasma. Penetration into the central nervous system was detected but at levels that were generally much lower than in other tissues, CSF was 1.4% (1,039/71,900 ng equiv./g) and in brain 0.65% (467/71,900 ng equiv./g).

Distribution of trofinetide in brain was further evaluated in micro-dialysis samples of SD rats following a single PO (30 mg/kg) or IV (90 mg/kg, 30 mg/kg/hour during 3 hours) dose. The mean maximum trofinetide concentrations in brain micro dialysis samples was ~400 ng/mL, occurred from 150 to 210

minutes after the start of the 3-hour IV infusion and declined thereafter with a half-life of ~75 minutes. After oral dosing, the average micro-dialysis concentration, showed a peak at 30 min of 20 ng/ml and an extended exposure of ~3 - 9 ng/ml. Micro-dialysis concentration was very variable between individual animals. Maximum trofinetide concentrations were low (10-53 ng/mL) and occurred at 30 minutes post dosing in 2 animals, at 120 minutes in 1 animal and at 360 and 0 minutes post dosing in the remaining 2 animals. From 1 to 6 hours, trofinetide was still detectable in brain micro-dialysis samples, which is not in line with the plasma concentration exposures. No explanation was provided for this variability, nor for the proposed extended brain exposure upon oral dosing. However, no details about the method were provided in the report and though LLOQ seems below 1 ng/mL, in basal samples (predose) concentrations trofinetide were 7-11 ng/mL in 2 animals. It cannot be excluded that the observations between 150 and 360 min are within the assay variability. In addition, the low dose may not represent relevant exposure of trofinetide in the brain obtained after much higher dosing.

For metabolite profiling of [^{14}C]-Trofinetide-derived radioactivity plasma, bile, urine, and brain and faecal homogenate samples were collected, but these are not reported in study 00616032 (see section 2.5.3.4 Metabolism).

Tissued distribution after repeated dosing has not been evaluated, but given the estimated short half-life of 75 minutes, accumulation is not anticipated.

Studies evaluating placental transfer and excretion in milk have not been conducted.

2.5.3.4. Metabolism

In vitro stability has been determined in rat, dog and human blood (Study 10117). A half-life of >60 minutes was calculated in human blood based on individual data in Table 2-2 (see a copy below). At all selected time points (except t=0), a huge variability was observed up to the measurement of 191% remaining parent compound. The Applicant has not given any explanation for this conflicting data set, raising questions about the reliability of the measurements in this study, however, consequences for the conclusions of these studies are expected.

The role of human CYP enzymes in the metabolism of trofinetide (0.1 and 1 µM) has been studied in human liver microsomes and after incubation with a panel of recombinant human CYP enzymes (rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6 and rCYP3A4). Low trofinetide turnover was observed in human liver microsomes and with a panel of recombinant human CYP enzymes suggests minor CYP involvement in the metabolism of trofinetide. *In vitro* examination of trofinetide metabolism in rat, dog, and human liver microsomes and liver S9, showed a single metabolite M1 (formed via dehydration) across species in liver microsomes and liver S9 incubations, but not in hepatocytes.

The *in vivo* metabolism of trofinetide was investigated in intact and bile duct-cannulated Sprague Dawley rats by analysing bile, brain, faeces, plasma, and urine samples collected after a single 200 mg/kg oral (PO) dose of [^{14}C]-Trofinetide (100 µCi/kg = 3.7 MBq/kg).
Qualitative examination of the metabolic fate of trofinetide revealed no identified metabolites in the samples. Trofinetide was the only drug-related component in plasma, urine and faeces. Trofinetide was also the only drug-related component identified in brain, but total radioactivity in brain was too low to enable radiochemical analysis. These data indicate that metabolism is not an important route of elimination of trofinetide in rat.

In the mass balance study (Study 00616032) plasma, bile, urine, and brain and faecal homogenate samples were collected for metabolite profiling of [14C]-trofinetide-derived radioactivity and shipped to the Principal Investigator at sponsor-designated test site. A report addressing their assigned phase of

the study, which was supplied upon request showed that no metabolites were present in the samples, indicating that metabolism is not an important route of elimination of Trofinetide in rats.

The data in rat is comparable to human *in vitro* data indicating that trofinetide was not significantly metabolized by human CYP enzymes. In a clinical study of oral [^{14}C]-trofinetide, there was minimal metabolism of trofinetide. Unchanged trofinetide was the major component in all samples. Only 2 minor metabolites identified, M168/1 and M186/1, accounted for less than 3% of the total dose in urine and faeces. A third metabolite, M129/1, which was not detected in plasma, blood, or urine, accounted for less than 1% of the total dose in faeces.

2.5.3.5. Excretion

The excretion of trofinetide was evaluated in Sprague Dawley rats following a single PO [^{14}C]-trofinetide dose in a pilot study and in a subsequent excretion mass balance study. The overall recovery 168 h post-dose (including cage washings and tissues) in the mass balance study was >97% what is acceptable. Stability of [^{14}C]- trofinetide during the study was demonstrated by predose and post dose analyses. As indicated by pre and post dose radiopurity results of 100% for all formulations, the radio-labelled test article was stable over the dosing period.

Trofinetide Equivalents (parent compound + metabolites) were measured in urine and faeces and it is not known if this detected radiolabeled compound is unchanged parent, biotransformation products or for faeces unabsorbed compounds, biliary excreted compounds (parent or metabolites).

In the mass balance study in rats following a single PO gavage dose of 200 mg/kg [^{14}C]-trofinetide, approximately 99.0% of the dose administered was recovered over 168 hours post dose in urine, faeces, and cage rinse from rats following a single oral (gavage) dose of [^{14}C]-Trofinetide at 200 mg/kg. The recovered radioactivity was divided between the urine (55.4 % of the administered dose, including cage rinse) and faeces (43.6%). [^{14}C]-Trofinetide equivalents in the faeces are likely excreted without absorption. In bile duct cannulated rats 46.5% of the administered dose [^{14}C]-trofinetide, was recovered in the urine (including cage rinse) and 49.6% faeces. Total recovery of radioactivity was 96.6% over 48 hours post dose. Less than 0.2% was excreted in the bile.
A pilot study established that < 0.1% of the administered dose of [^{14}C]-Trofinetide was excreted in the expired air over 48 hours following a single 200 mg/kg oral dose. Recovery of radioactivity in the excreta and cage rinse represented > 98% of the radiolabeled dose within 96 hours post dose.

In human, trofinetide was primarily excreted unchanged in urine. The recovery of trofinetide after an oral administration of [^{14}C]-trofinetide was complete (99%), with 15% and 84% of the total radioactivity excreted in faeces and urine, respectively.

In conclusion, following oral administration, [^{14}C]-trofinetide was rapidly but incompletely absorbed, within 1 hour and primarily cleared via renal clearance in rat as it is in human. The remaining [^{14}C]-trofinetide equivalents in the faeces of rat were likely excreted without absorption. Biliary excretion of [^{14}C]-trofinetide (and/or metabolites) appears to play a minor role.

2.5.3.6. Pharmacokinetic drug interactions

The results of the pharmacokinetic Drug-Drug Interactions (DDI) studies with trofinetide will be evaluated in the clinical assessment report.

2.5.3.7. Other pharmacokinetic studies

No other pharmacokinetic studies with trofinetide have been conducted, which is agreed.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

Table 3: Single dose toxicity

Study details Species Duration + recovery (weeks) Route GLP status (Study ID)		No:Sex/ Group	**Dose** (mg/kg)	Exposure		Major (alt. Salient) findings
				Cmax µg/ml	AUC µg.h/ml	
	Single-dose toxicity studies (MTDs highlighted)					
Rat (SD) IV slow bolus over ~2 min GLP (0621-05316)		Main: 5M/5F	0	ND	ND	None
			60	ND	ND	
			200	ND	ND	
			350	ND	ND	
Dog (beagle) Oral Non-GLP (2084-004)		Main: 2M/2F	Phase A 2000	ND	ND	≥600: skin discoloration (red ears) =2000: panting, emesis, red/soft feces, loss of skin elasticity, salivation
			Phase A lowered to **600** (300 BID)	ND	ND	
			Phase B **600** (300 BID)	M: 232 F:283 (t=2 hours post-dose)	ND	

Dog (beagle) IV slow bolus over ~1 min GLP (0621-06037)		Main: 2M/2F	350	1848	1007	≥ 350: conjunctival hyperemia, eyelids partially closed ≥ 750: dilated unresponsive pupil =1500: Mortality (1F), hyperemia in lung and stomach. ↓ activity, salivation, emesis, rapid breathing, tremors, and tenesmus; first degree AV block (1F) <u>ECG</u>: ≥ 350: affected atrioventricular conduction and/or ventricular repolarization ↑ QT/QTc intervals. deeply negative T-wave in lead II =1500: ↑ QT/QTc intervals ≥ 30 msec
			750	4255	2377	
			1500	7310	4361	
Dog (beagle) IV slow bolus over ~2 min GLP (0621-05317)		Main: 2M/2F	**175**	M: 751 F: 686	418	None ECG was performed, no findings

2.5.4.2. Repeat dose toxicity

Table 4: Pivotal studies

Species Duration + recovery (weeks) Route GLP status (Study ID)	**Number of animals**	**Dose (mg /kg/ day)**	**Exposure**		**Major findings & NOAEL**
			Cmax µg/ml	**AUC µg.h/ml**	
Repeat-dose toxicity studies (NOAELs highlighted)					
Mouse (CD-1) 13 W PO GLP (0706-22577)	Main: 10M/F TK satellites: 42F/M	0	-	-	≥400: ↓ monocytes **NOAEL: 2000**
		400	33.7	306	
		800	74.4	639	
		2000	194	1630	
Rat (SD) 26 W + 4 W recovery PO GLP (0621-16031)	Main: 15M/F Recovery: 15F/M TK satellites: 6F/M	0	-	-	≥300: ↑ body weight and food consumption, ↓ testes weight, ↑ kidney (F) weight =2000: soft feces (M), ↑ (slight) ALP and ALT (M), ↑ triglycerides, ↓ Urea N and creatinine (F), ↑ somatropine (M), ↑ liver (M), kidney (M), heart weight, ↓ uterus weight **NOAEL: N.D.** <u>Recovery:</u> ≥600: ↑ APTT, =2000: ↓ testes weight, ↑ kidney, heart, thymus weight
		300	34.6	208	
		600	85.7	449	
		2000	174	1700	
Dog (Beagle) 39 W + 4 W recovery PO GLP (0621-16032)	Main: 4M/F Recovery: 2F/M	0	-	-	≥300: ↑ kidney, liver weight (M), ↓ uterus weight =1000: diarrhoea, liver Kupffer cell pigmentation, thyroid hyperplasia ECG performed: no findings **NOAEL: 50** <u>Recovery:</u> =1000: ↓ uterus weight
		50	M: 4.3 F: 2.4	M: 43.7 F: 28.7	
		300	M: 17 F: 48	M: 198 F: 343	
		1000	M: 136 F: 142	M: 1475 F: 1500	

Dog (Beagle) 28 Days (4x7 day cycle) = 2 weeks recovery IV GLP (0621-06038)	Main: 3-5M/F Recovery: 1-2F/M	0	-	-	≥480: thyroid hyperplasia =3600: decreased appetite, soft feces, limping (M) ECG performed: no findings **NOAEL: N.D.** <u>Recovery:</u> no findings
		480	M: 42 F: 43	M: 1042 F: 1068	
		1440	M: 117 F: 144	M: 2471 F: 3228	
		3600	M: 272 F: 305	M: 5586 F: 6271	

Table 5: Non-pivotal studies

Species Duration + recovery (weeks) Route GLP status (Study ID)	**Number of animals**	**Dose (mg /kg/ day)**	**Exposure**		**Main findings**
			Cmax µg/ml	**AUC µg.h/ml**	
Repeat-dose toxicity studies (NOAELs highlighted)					
Mouse (CD-1) 14 days PO Non-GLP (0706-22574)	Main: 8M/F	0	ND	ND	None **NOAEL: 2000**
		300	ND	ND	
		600	ND	ND	
		2000	ND	ND	
Mouse (TgHRAS) 14 days PO Non-GLP (0706-22575)	Main: 8M/F	0	-	-	≥600: ↓ body weight (F) **NOAEL: 300**
		300	ND	ND	
		600	ND	ND	
		2000	ND	ND	
Mouse (TgHRAS)	Main: 12M/F	0	-	-	=2000: ↑ BW gain

4 w PO GLP (070-622576)	TK satellites: 40F/M	400	M: 97.9 F: 22.4	M: 887 F: 225	**NOAEL: 2000**
		800	M: 90.6 F: 56.7	M: 534 F: 387	
		2000	M: 501 F: 177	M: 1700 F: 971	
Rat (SD) 4 w + 2w recovery PO GLP (2329-001)	Main: 10-15M/F Recovery: 5F/M TK satellites: 9F/M	0	-	-	≥1200: ↑ ALT **NOAEL: 2100**
		450	M: 42.7 F: 54.5	M: 348 F: 306	
		1200	M: 113 F: 171	M: 768 F: 900	
		2100	M: 181 F: 258	M: 1191 F: 1344	
Rat (SD) 7 days + 2w recovery IV GLP (0621-08120)	Main: 8-12M/F Recovery: 4F/M TK satellites: 6F/M	0	-	-	≥2784: ↓ BW gain and food consumption, ↓ neutrophils, monocytes, ↑ serum phosphorus, ↓ T4 and TSH, ↑ kidney weight, dilatation of kidney cortex tubules, thyroid hypertrophy **NOAEL: N.D.**
		2784	M: 97 F: 87	M: 2750 F: 2490	
		5544	M: 210 F: 183	M: 6000 F: 5280	
Rat (SD) 14 days IV GLP (0621-05314)	Main: 10-15M/F TK satellites: 6F/M	0	-	-	≥120: ↑ neutrophils, monocytes **NOAEL: 700**
		120	9.87	ND	
		500	22.0	ND	
		700	42.6	ND	
Dog (beagle) 7 days PO Non-GLP (0621-15160)	Main: 2M/F	1000	ND	ND	Diarrhea

Dog (beagle) 7 days PO Non-GLP (0621-15233)	Main: 2M/F	2000	ND	ND	Diarrhea

2.5.4.3. Genotoxicity

No GLP compliant genotoxicity studies were conducted. However, based on the minimal modification of the methylated proline which does not contain a structural alert for genotoxicity, and the lack of carcinogenicity findings in the transgenic mouse study, it can be concluded that trofinetide has no genotoxic potential.

2.5.4.4. Carcinogenicity

Table 6: Carcinogenicity

Study details Species Duration (weeks) Route GLP status (Study ID)	No.Sex/ Group	Dose (mg/kg/ day)	Exposure AUC µg.h/ml	Major (alt. Salient) findings
Carcinogenicity studies (NOAELs highlighted)				
Mouse (TgHRAS) 26 w PO GLP (00616083)	Main: 25M/F TK satellite: 39-40M/F	0	- 0-last	=2000: ↑ Body weight gain
		300	M: 258 F: 245	
		600 (F)	487	
		800 (M)	602	
		2000	M: 1440 F: 1630	

A 2-year rat carcinogenicity study is currently ongoing.

2.5.4.5. Reproductive and developmental toxicity

Table 7: Fertility and Early Embryofetal Development

Rat (SD) Male 1 month before cohabitation – Day 66 (euthanization) Female 2 weeks before cohabitation – GD7 Euthanization GD13 Oral Gavage GLP status (20202588)	22M 22F	**Dose** mg/kg/ day	Cmax	AUC	M None F None M+F fertility endpoints **NOAEL 2000 mg/kg/day**
		0	-	-	
		300	Not measured	Not measured	
		900	Not measured	Not measured	
		2000	Not measured	Not measured	

Rat (CRL:CD[SD][CD® IGS) PND13 through Day 70 Oral Gavage GLP (0621-16030)	20M 20F	0	-	-	M None F None M+F fertility endpoints **NOAEL 2000 mg/kg/day**
		300	Not measured	Not measured	
		600	Not measured	Not measured	
		2000	Not measured	Not measured	

Rat CD [Crl:CD(SD)] Male	30M Recovery 20M	0	Cmax (ug/mL)	AUC 0-4 (ug.h/mL)	M General endpoints: ≥300: Swelling, ulcerated lesion, rough coat, thin, redness around eyes, bw↓ (findings related to jugular vein catheter and local
		300	Not measured	Not measured	
		600	46.4	347	

1 month before cohabitation – 4 days of mating (euthanization) Intravenous infusion GLP (2282-001)		1200 (2100 recovery phase)	88.6	703	irritation due to the dosing formulation. Similar findings were observed in control animals, but of a lesser severity). Fertility endpoints: None M fertility endpoints **NOAEL 1200 mg/kg/day**
Rat CD [Crl:CD(SD)] Female 2 weeks before cohabitation – GD7 (C-section GD13) Intravenous infusion GLP (2282-002)	25F	0	Cmax (ug/mL)	AUC 0-4 (ug.h/mL)	F General endpoints: ≥300: Swelling, redness around eyes ≥600: rough coat Fertility endpoints: None F fertility endpoints **NOAEL 1200 mg/kg/day**
		300	30.5	110	
		600	54.2	49.5	
		1200	94.9	106	

Table 8: Embryo Fetal Development

Rat (CD®IGS [Crl:CD(SD)]) GD 7-17 C-section GD18 PO GLP (20222415)	20F	0	Cmax ug.mL (GD17)	AUClast ug.h/mL (GD17)	Maternal and Fetal findings: None Fetal and maternal NOAEL: 2000
		300	29.8	182	
		900	106	671	
		2000	214	1420	

Rabbit Crl:KBL(NZW) GD 7-19 C-section GD29 PO GLP (20222417)	20F	0	Cmax ug.mL (GD17)	AUClast Ug.h/mL (GD17)	Maternal: ≥300: FC↓, bw gain↓ soft feces, ungroomed fur 600: BW↓, Fetal: None Fetal NOAEL: 600 Maternal NOAEL: 150
		150	2.71	57.5	
		300	13	29.3	

Pre- and Postnatal Development

Rat (SD) GD 7-PND20 PO GLP (20222418)	22F	mg/kg/day	Cmax	AUC	<u>Maternal</u> No findings <u>F1</u> No findings NOAEL maternal and F1 2000
		300	15.9	112	
		900	68.1	424	
		2000	147	860	

2.5.4.6. Toxicokinetic data

See PK section.

2.5.4.7. Local Tolerance

No local tolerance studies were conducted.

2.5.4.8. Other toxicity studies

A 28-day mechanistic study was performed in rats, to evaluate the impact of trofinetide on circulating and brain IGF-1, IGFBP-2, and IGFBP-3 levels, since trofinetide is an analogue of GPE, the terminal tripeptide of IGF-1. No effects were seen on IGF-1 and IFGBP-3 levels, and only a transient effect on IGFBP2 at one time point only. There no clinically relevant effects were seen on levels of IGF-1, IGFBP-2, and IGFBP-3.

2.5.5. Ecotoxicity/environmental risk assessment

Trofinetide is not a PBT substance.

Both the default and the refined PECSW exceeds the action limit of 0.01 µg/L. Phase II assessment is warranted but has not been provided yet.

Table 9: Summary of main study results

Substance (INN/Invented Name):			
CAS-number (if available):			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	$logD_{ow}$ at pH 5: -4.2 $logD_{ow}$ at pH 7: -5.3 $logD_{ow}$ at pH 9: -5.7	Potential PBT: N
PBT-statement :	Trofinetide is considered to be not PBT, nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC_{sw}, default	120	µg/L	≥ 0.01 threshold: Y
Other concerns (e.g. chemical class)			Y/N

2.5.6. Discussion on non-clinical aspects

Pharmacodynamics
The potential mechanisms of action in Rett syndrome specifically, were not demonstrated *in vitro*. *In vivo*, trofinetide has been tested in an established genetic mouse model of RTT in which exon 3 of the MeCP2 gene is deleted. Treatment with trofinetide was associated with improvements in neuronal connectivity as evidenced by significantly improved hippocampal LTP measured ex vivo, which was suggested to be due to improved dendritic morphology. While the Applicant describes an apparent increase in hippocampal dendritic arborization, in the study report it was described as a trend towards increased dendritic complexity and only dendritic length appeared clearly increased upon treatment with trofinetide.

In a second cohort of animals that were administered 20 mg/kg trofinetide, overall survival, the number of apneas at various time points, aspects of autonomic (cardiovascular and respiratory) function at 9 weeks and motor function (tremor score, locomotor activity, gait and general condition) at 13 weeks were examined. There were no effects on motor function and autonomic function with trofinetide, although these parameters were reduced in Mecp2 mutant mice at 13 weeks relative to wild type controls, and is in contrast to what was seen with GPE (Tropea et al., 2009). In addition, GPE treatment led to a partial rescue of PSD-95 concentrations and cortical circuit plasticity. Unfortunately, for trofinetide itself this was not evaluated, while according to literature, PSD-95 has a major influence on synaptic strength and plasticity, and trofinetide is thought to improve neuronal synaptic function. It is therefore not agreed with the Applicant that the effects of trofinetide treatment in preclinical models of Rett syndrome are consistent with published literature with GPE. Furthermore, only one dose (20 mg/kg) was administered, therefore no dose-response relationship could have been established.

The totality of non-clinical data on FXS, TBI and ischemic stroke is supportive for the conclusion that trofinetide modulates pathophysiological mechanisms underlying RTT, including synaptic dysfunction, oxidative stress and neuroinflammation, which may underlie the observed characteristic cognitive, motor, and breathing impairments in these other models. In Mecp2 knockout (KO) mice, GPE improved locomotor activity, breathing regularity, and autonomic tone (Tropea et al., 2009), reflecting amelioration of core RTT symptoms. As described above, efficacy regarding motor function and autonomic function in Mecp2 KO mice when treated with trofinetide was not demonstrated.

Overall, the mechanism of action in Rett syndrome is not clearly discussed or demonstrated. The Applicant indicated that no single receptor and/or mechanism of action has been conclusively identified, but did not provide a thorough discussion on the potential mechanisms of action including the involved targets and signalling pathways of trofinetide in Rett syndrome. Trofinetide is thought to improve neuronal synaptic function and morphology and this is stated in section 5.1 of the proposed SmPC, but it was not sufficiently discussed nor demonstrated how this works. In an animal model of RTT (Mecp2 KO mice), trofinetide particularly improved synaptic plasticity and dendritic length, with a trend towards increased dendritic complexity. Although these effects were stated to underly the learning difficulties, epileptic episodes and behavioural regression observed in the disorder, it remains unclear how this would result in a clinically meaningful effect given the very limited or absent changes in functional effects with trofinetide in Mecp2 KO mice. From a non-clinical perspective, the proof of concept of trofinetide to improve RTT is not convincing and should be established clinically.
With respect to the potential window of opportunity for treatment from a non-clinical perspective, evidence from Mecp2 deficient mice suggests the window for GPE to impact synaptic plasticity extends from juvenile to young adult mice. Deficits in synaptic maturation or stabilization persisted into adulthood following MeCP2 deficiency, which was prevented upon GPE treatment.

Regarding secondary pharmacodynamics, in the competition radioligand binding assay trofinetide tested at 50 nM showed inhibitory activity (31.47%) at the neurotensin receptor (brain/gut peptide) which is below the standard 50% threshold to be considered significant. Thus, for this experiment it can be agreed that there was no relevant neurotensin inhibition. However, in light of the high clinical mean Cmax (495 µM), a potential link between of neurotensin and the clinically observed gastrointestinal effects cannot be definitely excluded as this receptor was not included in the newly conducted Safety Screen 44 Tier 1 panel.

Given that the mean clinical Cmax/ss of 155.518 µg/ml, corresponding to 495 µM, results in 3.2 µM (0.65%) brain exposure it is not agreed that the trofinetide levels reaching the brain in humans are considerably lower than the IC50 for the AMPA, NMDA and kainate receptors which were determined to be 2.1 µM, 4.2 µM and 7.4 µM. In addition, the *in vitro* setting in which IC50 values were determined

may not be representative for the *in vivo* situation since IC50 values are dependent on the substrate concentration used and therefore may result in overestimation. Actual trofinetide levels that are needed to antagonize NMDA receptors in the *in vivo* situation may be lower. The low oral dose used in the rat study (200 mg/kg) may also not necessarily represent the exposure of trofinetide in the brain obtained after higher or repeated dosing. In addition, the highest clinical Cmax observed in Study ACP-2566-003 was 268.61 µg/mL, equalling 852 µM and 5.5 µM brain exposure. While it can be agreed that it is unlikely that NDMA antagonism is part of the mechanism of action based on the provided discussion on predicted occupancy levels that are needed to produce behavioural effects in humans, for patients with these high plasma levels, transient effects shortly after dosing (Tmax ~2 hours) cannot be excluded. Especially when taking into account the significant NDMA inhibition at 1mM in study 100077245.

The Applicant was therefore asked to discuss potential toxicological consequences of NDMA antagonism. The Applicant suggests that functional measurements are more relevant than *in vitro* binding data and that in this assay, there were no physiological effects via activation of NMDA receptors at free drug concentrations up to 30 µM or inhibition of NMDA receptors at free drug concentrations up to 10 µM, while the clinical brain exposures could reach a concentration of 5.5 µM. Only based on the *in vitro* binding assays, a theoretical risk at transient high trofinetide brain concentrations cannot be completely excluded. Although it is agreed that non-clinical data on repeat-dose toxicity and CNS safety pharmacology studies are not suggestive of NMDA-related findings such as adverse neurobehavioural signs or cognitive impairments, it should be noted that the clinical exposures were hardly reached in these studies. From a clinical perspective, Rett syndrome is a complex neurodevelopmental disorder characterized by seizures and behavioural impairments, which complicates the interpretation of neurological safety signals. Within these limitations, the available clinical data from the phase 3 study do not indicate clear or consistent impact of trofinetide on neuronal excitability. Taking into account the overall data, the risk for clinically relevant NDMA antagonism is low.

The Applicant was also asked to further discuss the potential consequences of kainate and AMPA receptor binding. Clinical effects associated with AMPA and kainate receptor (KAR) activation include excitotoxicity, seizures, or cognitive impairment. The available clinical data from the phase 3 study do not indicate clear or consistent impact of trofinetide on these aspects, although interpretation is complicated, and seizures are included as ADR for trofinetide. Given that the approved AMPA antagonist perampanel has a substantially higher affinity, in the nM range, compared to trofinetide, further suggests that significant clinical effects mediated by trofinetide activity at AMPA/KAR receptors are unlikely. There is no drug approved specifically for KARs, but perampanel demonstrates cross-reactivity with certain KAR subunits at concentrations below those relevant to trofinetide. While a theoretical risk at transient supratherapeutic concentrations cannot be completely excluded, also taking into account the absence of glutamatergic-associated toxicity in non-clinical studies despite the limited exposure margins, there are no clear indications that trofinetide will induce a clinical safety risk related to AMPA/kainate receptor activity.

Overall, the secondary pharmacodynamics, and thus potential off target effects of trofinetide, were not adequately evaluated in the first round. A new secondary pharmacodynamics screen (Study 100077245) was conducted with 1 mM trofinetide. This is in the range of the highest observed clinical free unbound Cmax of 852 µM. The Safety Screen 44 Tier 1 panel was selected since it includes well-established targets and pathways as contributors to clinical adverse drug reaction risks. At 1 mM trofinetide, significant results were observed at the NMDA receptor (103.9% inhibition of binding) and COX-2 (86.2% inhibition of activity). The Applicant was asked to discuss possible adverse effects in patients of COX-2 inhibition. However, the hit for COX-2 appeared incorrect. In a statement provided

by the CRO, it is stated that the COX-2 assay, which relies on fluorescence detection, should have been flagged for interference with the fluorescent signal. In a subsequent retest (Study 1000774240 V2), trofinetide was not active at COX-2 up to concentrations of 300 µM. At 1 mM, two samples resulted in no inhibition at any test concentration, while the other two samples showed an inhibition of around 42% and around 59%. It cannot be concluded with confidence whether this signal is due to interference, and therefore they should be taken as true values. Based also on clinical and non-clinical data, the applicant considers the risk of COX-2 inhibition by trofinetide low. Regarding the clinical data is should be noted that these are of limited value, since the data on QT prolongation are not related to the mechanism by which COX-2 inhibition results in cardiovascular adverse effects, and these effects can be rare and not identified as yet in the clinical trials. The non-clinical toxicology data are also of limited value, since the exposure margins were very low. The *in vitro* data on potential inhibition is therefore of importance in identifying a potential risk. Taken together, there is only one sample that might have an IC50 in the region of the clinical Cmax, and three samples that have IC50 values clearly below the Cmax. Therefore, it is agreed with the applicant that the risk of clinically meaningful COX-2 inhibition is considered low.

As requested, the Applicant evaluated potential binding of trofinetide to the IGF-1 receptor. No binding of trofinetide to the IGF-1 receptor was observed up to the highest tested concentration of 1 mM. Together with the fact that there was no effect on IGF binding proteins (IGFBP-2 and -3), and growth hormone (GH), it can be concluded that trofinetide has no effect through the IGF-1R.

Safety pharmacology studies indicate that trofinetide may cause cardiovascular effects, including QT prolongation, at clinically relevant exposures. Also, in the single dose toxicity study in dogs, the target organ is the cardiovascular system. However, from a clinical point of view the risk of serious consequences due to cardiac repolarization is considered minimal. Exposure margins in the QT study are suboptimal. Yet, no risk of QT prolongation was identified in the RTT clinical program or post-marketing in the US. Further, RTT patients are routinely monitored by ECG, due to the inherent risk of QT prolongation (see clinical AR). CNS and respiratory safety studies suggest a low risk for adverse effects, but it should be noted that the clinical exposures, based on Cmax, were not reached in these studies (estimated based on the TK data derived from the 26-week rat study).

Pharmacokinetics
Trofinetide (NNZ-2566) was investigated in a range of *in vitro* and *in vivo* pharmacokinetic (PK) and toxicokinetic (TK) studies in mice, rats, rabbits and dogs. Sprague Dawley (SD) rats and Beagle dogs, were the nonclinical safety species selected. These studies were conducted to define the absorption, distribution, metabolism, and excretion (ADME) of trofinetide. Oral administration, which is the route of clinical administration, was selected for the pharmacokinetic studies in animals. Data from these studies were used to characterize the PK and TK properties of trofinetide to support nonclinical toxicology evaluations and to support its intended clinical use.
Following oral (PO) administration, trofinetide was rapidly absorbed into systemic circulation with peak concentrations occurring around 2 hours (T_{max}) after dosing. Bioavailability data for rat and dog is lacking. Although requested, the Applicant could not deliver the requested PK parameter as dedicated studies were not conducted. The Applicant has not made clear why preclinical ADME studies were not designed in such a way that relevant PK parameters can be extracted. The pharmacokinetic profile of trofinetide upon oral twice a day dosing is similar in mice, rats and dogs with the highest exposure after oral administration of 2000 mg/kg.d measured in rats (AUC_{0-24} 4340-5080 h*µg/mL). In rabbits the exposure at comparable doses is 2-5 fold lower compared to the other species tested. After intravenous (IV) administration, the clearance (CL) of trofinetide was low, ~ 5% of liver blood flow in rats and moderate, ~25-50% of liver blood flow in dogs. The terminal elimination half-life ($t_{½}$) was

short in rats and dogs, 20.7 and 45.5 minutes, respectively. The distribution volume (V_d) was high in dogs which approximates or is greater than total body water in dogs.
There were no significant sex differences and a dose proportional increase of exposure of trofinetide was observed. There was no evidence of accumulation upon multiple dosing when comparing exposures (C_{max} and AUC_{0-24}) on Day 1 up to 39 weeks, except after 3 weeks in rabbits. The trofinetide accumulation appears specific to rabbits and may not represent the typical PKs of trofinetide. Plasma protein binding of trofinetide was low in rat (1.5%), dog (0.5%), and human (1.1%). After oral administration trofinetide was broadly distributed with highest exposure in kidney, walls of the gastrointestinal tract and liver and low distribution in the remaining tissues. Penetration into the central nervous system was limited, the percentage of the systemic plasma dose trofinetide present in CSF was 1.4% and in brain 0.65%. The blood:plasma ratio indicated that trofinetide is distributed mainly in plasma. Distribution of trofinetide in brain was evaluated in micro-dialysis samples of rats following a low PO dose. After PO administration maximum trofinetide concentrations were low in brain (2-5 fold the pre-dose concentration) and very variable between individual animals. The low oral dose used in this study may not represent relevant exposure of trofinetide in the brain obtained after higher dosing.
The metabolite profiling of trofinetide has not been reported in much detail. A single metabolite "M1" formed via dehydration was observed *in vitro* in liver microsomes and according to the Applicant no metabolites were identified *in vivo* indicating that metabolism of trofinetide is limited, however, a metabolic profiling study report is lacking. Upon request, the Applicant has submitted an updated report including Appendix 10 reporting the metabolite analysis. Analysis of samples from *in vivo* studies of [^{14}C]-Trofinetide in both intact and bile duct-cannulated rats revealed that no metabolites were detected in any sample. Parent Trofinetide was the only drug-related component detected in all samples analyzed. These data indicate that metabolism is not an important route of elimination of Trofinetide in rats. Following oral administration, trofinetide was rapidly but incompletely absorbed, within 1 hour and primarily cleared via renal clearance (~50%) in rat as it is in human. The remaining trofinetide equivalents in the faeces (~50%) of rat were likely excreted without absorption. Biliary excretion of trofinetide (and/or metabolites) appears to play a minor role. Studies evaluating placental transfer and excretion in milk have not been conducted. The results of the pharmacokinetic Drug-Drug Interactions (DDI) studies with trofinetide will be evaluated in the clinical assessment report.

Based on PK/PD analysis MS-010 based on data from Study 003 and paediatric Study 09, the highest weight banded therapeutic dose (i.e. 5 g BID for patients weighing 9 kg to less than 12 kg) resulted in mean trofinetide Cmax of up to 203 µg/mL and AUC0-12h of up to 1108 hr.µg/mL (calculated AUC0-24h [2 x AUC0-12h] of 2216 hr.µg/mL). These values are used for the calculation of the exposure and safety margins.

Toxicology

Single dose toxicity studies were performed in rats (IV dosing) and dogs (oral and IV dosing). No effects were seen rats at doses up to 350 mg/kg. Although no TK analysis was performed, it can be assumed that these doses were too low to see any effect, since rats in another IV study given much higher doses had exposures still below the clinical exposure.

In dogs, the target organ is the cardiovascular system. In several studies, safety pharmacology, single dose and repeated dose toxicology studies, ECGs were performed. Increased QT intervals were seen from exposure margins (EM) of 2.8 based on Cmax, with a safety margin on 1.7, in a safety pharmacology study. In a single dose study in dogs, effects were seen at an EM of 9.1. There was no safety margin in this study as effects were seen at the lowest dose tested. The effects consisted of increased QT/QTc interval, deeply negative T-waves in lead II and hyperemia. At the high dose with an EM of 36 one female had an AV block. In a second single dose dog study with lower doses, no effects

on ECG were seen up to an EM of 3.4. ECGs were also performed in 2 repeated dose dog studies (see below), with even lower doses. No effects were seen at EMs up to 1.3 based on Cmax. Since cardiovascular effects were already seen at low exposures of 2.8-fold the human Cmax, clinical relevance cannot be excluded and should be further evaluated in a clinical study (see clinical AR).

Repeated dose toxicity of trofinetide was evaluated in mouse, rats and dogs. Overall, trofinetide was well-tolerated in all species, however due to the high clinical dose, the exposure margins in the animals at the highest oral doses tested are low with exposures in the range or below clinical exposure. In rats, IV dosing did not appear to increase the exposure significantly, but in dogs higher exposures of 2.7-fold the human exposure were reached in a 28-day IV study. Thus, the exposure margins in the general toxicity studies are too low for adequate risk assessment. In addition, there is a limited clinical safety database. Long-term clinical safety data are available (up to 4 years), but in a limited number of patients, with a limited patient exposure duration and in an uncontrolled setting.

In the mouse, trofinetide was tested up to 13 weeks up to 2000 mg/kg/day resulting in an exposure margin <1, and with no findings of concern. In juvenile rats dosed up to 2000 mg/kg/day for 26 weeks resulting in an exposure margin <1, body weight gain and food consumption was increased, which might be relevant for the intended juvenile population. Other target organs in the juvenile rat are kidney, liver and heart, which were increased in weight (also relative to body weight), although in none of these organs any histopathological effects were seen. These effects on organ weight could be related to the increase in body weight and earlier attainment of adult weight. However, in dogs increases in kidney and liver weight was also seen, without an increase in body weight. It is not known what the cause is of these increased weights, but due to the lack of any histopathological findings in these organs, the effect is not considered adverse. However, at higher exposure margins, histopathological effects may become evident, and therefore an adverse effect on liver and kidney cannot be ruled out. Other effects were decreases in testes and uterus weights. These effects could be related to an effect on fertility. Although no effects were seen in the dedicated fertility studies (see section 2.5.4), the exposure in the fertility studies was below the human exposure, and therefore an effect on fertility cannot be ruled out. For the current indication of RETT syndrome however, this is of limited relevance. IV dosing for 7 days up to 5544 mg/kg/day resulting in an exposure margin of <2.5 in rats revealed an opposite effect on body weight, and identified the thyroid as an additional target organ, where hyperplasia was seen along with decreases in T4 and TSH. It is not known if the effect on body is a result of the thyroid changes, or that other pathways are involved, especially considering the difference between the two rat studies in terms of body weight changes. Another effect that was seen in the 26-week rat study only was an increase in APTT. This could have been a chance finding, however, this effect was also seen in the 3-month impurity study in rats. However, the observed prolongations in APTT in rats administered trofinetide are minimal, non-adverse, and lie within the established physiological range for the species. They are not temporally or dose-dependently associated with liver changes, which themselves represent adaptive responses to increased food consumption rather than direct hepatotoxicity. There were no pathological indicators of impaired hepatic function or bleeding risk. No clinically meaningful coagulation risk is expected based on these data.

In dogs, tested up to 39-weeks with oral administration up to 1000 mg/kg/day, resulting in an exposure margin of <1 and up to 3600 mg/kg/day in four cycles of 7 day IV administration, resulting in an exposure margin of 2.5-fold, trofinetide was well-tolerated. As discussed before, non-adverse increases in kidney and liver weights were observed, along with a decrease in uterus weight, which is of limited relevance in the current treatment population. Also seen was thyroid hyperplasia, without any other effects on thyroid hormones. It is acknowledged that there are differences in thyroid function between both rats and dogs, and human. In the animal species they are likely an adaptive response due to high thyroid hormone turnover and therefore HPT axis perturbation. Rats are particularly sensitive to this effect which is a consequence of increases in hepatic metabolism. This is likely to be a

species-specific mechanism, not relevant for human risk assessment, as hepatic enzyme inducers have so far not been associated with thyroid neoplasia in humans. It can therefore be concluded that the thyroid effects observed in rats and dogs are caused by a species-specific mechanism not relevant for human safety.

The applicant argues that the genotoxicity studies should be considered as GLP compliant. However, as stated previously, there is no record of a GLP status, as also indicated by the applicant, and the FDA has no record of the facility ever having been inspected. Therefore, the studies cannot be considered GLP compliant, as indicated in the first round of the procedure, and therefore they cannot be used in the assessment.

However, further arguments are put forward that genotoxicity testing of the compound is not necessary due to the nature of the product. Trofinetide is a modified peptide, consisting of the naturally occurring amino acids glycine and glutamate and proline modified to methylproline. It is acknowledged that glycine and glutamate have no genotoxic potential, and any hypothetical concern is due the methylated proline. To address this concern, the applicant has conducted an *in silico* analysis which indicates that methylproline does not contain any structural alerts indicating genotoxic potential. Although an *in silico* method is generally only accepted for impurities for which the daily intake does not exceed 1mg, the nature of this modified amino acid does allow for some lenience in the evaluation approach, and the provided QSAR analysis can be seen as supportive evidence. Further, it is acknowledged that the transgenic mouse carcinogenicity study did not reveal any increase in tumours, which would be expected for a genotoxic compound. Although the mouse carcinogenicity study is of limited value for evaluation of non-genotoxic mechanisms due the low exposure margins compared to human, the doses were considered sufficiently high to rule out a potential genotoxic mechanism.

Overall, although the non-GLP genotoxicity studies cannot be taken into consideration in the assessment of genotoxic potential, it can be concluded that based on the minimal modification of the methylated proline which does not contain a structural alert for genotoxicity, and the lack of carcinogenicity findings in the transgenic mouse study, trofinetide has no genotoxic potential.

Carcinogenicity of trofinetide was evaluated in a 26-week study in TgHRAS mice, at doses up to 2000 mg/kg/day. There were no neoplastic findings in the trofinetide dosing groups, and no non-neoplastic findings apart from a slight increase in body weight in the high dose animals. Despite the high doses in terms of mg/kg, the exposure did not reach the clinical exposure at the intended dose. Therefore, the negative outcome of this study is of limited value.

The 2-year carcinogenicity study in rats is ongoing. In the protocol provided, a high dose of 2500 mg/kg/day (1250 mg/kg/day BID) is reported. From the general toxicity studies, it can be extrapolated that this dose may result in exposures just above or at clinical exposure. The submission of the final study report post-approval is agreed due to the seriously debilitating nature of the disorder and the unmet medical need. The final study report will be submitted by July 2026. To evaluate the risk in the meantime, a Weight of Evidence for carcinogenic potential was submitted. Unmodified GPE has not been associated with carcinogenic potential. However, the mode of action of trofinetide is not completely clear, and therefore some uncertainties remain about possible pathways involved in tumorigenesis. One particular and important pathway known to be involved in tumorigenesis, potential binding to IGF-1R, has been ruled out, which provides reassurance.

With regard to histopathology findings, it is agreed that there are no pre-neoplastic effects in the chronic studies. The only exception is the thyroid hyperplasia, however this has been concluded to be not relevant for humans. Further, no signs of hormone perturbation or immune modulation have been observed. It should be noted that due to the high clinical dose, exposures were below the clinical exposure which introduces some uncertainty.

The genotoxicity studies cannot be considered as GLP compliant, and they are thus unreliable and cannot be used in genotoxicity assessment. However, the negative mouse carcinogenicity study and the nature of modification of the GPE tripeptide provide sufficient evidence to conclude that trofinetide has no genotoxic potential.

It can therefore be concluded that from the data generated so far, there are no indications that trofinetide has carcinogenic potential. However, due to the largely unknown mechanism of action and the low exposure margins in the chronic toxicology studies, there is some uncertainty.

Developmental and Reproductive Toxicity (DART)
The applicant performed both an oral and dedicated IV Fertility and Early Embryofetal Development (FEED) studies in male and female rats, and in addition, an oral FEED study starting exposure in juvenile rats from PND13 until PND70. No adverse findings on male and female fertility were observed in any of the studies up to a dose of 2000 and 1200 mg/kg/day, for the oral and IV studies respectively. This corresponded to exposure margins of approximately 1.3 and 0.6-0.8 fold exposure at MRHD, in the oral and IV studies, respectively.

A rat and rabbit Embryo-Fetal Developmental (EFD) toxicity study were performed, both showing no adverse effects on fetal development up to the highest dose tested. Maternal toxicity was only observed in rabbits, consisting of reduced food consumption with associated bw loss and reduced body weight gain and findings on decreased general wellbeing of the dams (ungroomed fur, soft feces). The exposure margin at the fetal NOAEL was 1.3 and 0.7 fold exposure at MRHD.

A pre- and postnatal developmental (PPND) toxicity study in rats did not show any signs of developmental or maternal toxicity up to the highest dose tested (2000 mg/kg/day) with an exposure margin of approximately 1 fold maternal exposure at MRHD.

Across all DART studies, exposure margins at the highest dose tested were at or lower than exposure in human at the MRHD. Therefore, according to ICHS5(R3), there is still an increased risk, and the DART studies provide limited value for human risk assessment. This is reflected in the proposed SmPC sections 4.6 and 5.3.

No local tolerance studies were conducted. As the intended clinical route of administration is oral, this is agreed. No immunotoxicity, antigenicity or dependence, studies were performed. This risk is considered low and therefore the lack of studies is agreed.

Two 13-week rat studies were performed with spiked batches containing impurities in trofinetide. The toxicity findings did not differ significantly between batches containing impurities and batches without. When slight differences were observed between the two groups (e.g., increased incidence of chronic progressive nephropathy in the kidney in males in the batch with impurities) then there was no difference with the control group. Therefore, the impurities are qualified for general toxicity in these studies. Further, QSARs have been performed and submitted to rule out genotoxicity.

No phototoxicity studies are performed. Since there is no absorption of trofinetide in the UV-visible wavelength range, there is no concern for phototoxicity and the lack of studies is agreed.

Environmental Risk Assessment:
Trofinetide is not a PBT substance.
Both the default and the refined PECsw exceeds the action limit of 0.01 µg/L. Phase II assessment is warranted but has not been provided yet. The Applicant committed to submitting the final ERA,

including a Phase I and II assessment, within 6-12 months post approval. According to the Applicant this ERA will also include a dedicated subsection on endocrine-related effects.

2.5.6.1. Assessment of paediatric data on non-clinical aspects

Paediatric data has been discussed in the repeated dose toxicology section.

2.5.7. Conclusion on the non-clinical aspects

In general, the non-clinical pharmacology dossier lacks a clear demonstration on the mode of action of trofinetide. Binding studies were lacking. Functional *in vitro* assays show some evidence that trofinetide may be a neuroprotective agent. An *in vivo* study in MeCP2 mutant mice showed the potential of trofinetide to improve synaptic plasticity, dendritic morphology and longevity, however a dose-response was lacking and functional parameters were not improved. Trofinetide showed some beneficial effects in non-clinical models of fragile X syndrome, stroke and traumatic brain injury. Together the data do not provide a convincing proof of concept for the treatment of trofinetide for the multiple body system symptoms of Rett syndrome.

The results on secondary pharmacodynamics suggest potential binding to NMDA, AMPA and kainate receptors, although the potential for clinically relevant effects is low.

Safety pharmacology evaluation revealed the cardiovascular system as a potential target. No CNS or respiratory effects were observed. However, the lack of a meaningful exposure margin in most non-clinical toxicology studies may have masked potential effects.

The absorption, distribution, metabolism, and excretion (ADME) of trofinetide was adequately evaluated in *in vitro* systems (protein plasma binding, metabolism) and using *in vivo* PK studies (PO) in mouse and rabbit and (PO, IV) in rat and dog. In addition, the *in vivo* multiple-dose TK of trofinetide was evaluated via oral administration to TgHRAS wild type and CD-1 mice, Sprague-Dawley rats, New Zealand White rabbits and Beagle dogs, which were used as part of the non-clinical safety program. Trofinetide was well-tolerated in mice, rats and dogs, when tested via oral or IV administration, in mice up to 13 weeks, in rats up to 26 weeks and in dogs up to 39 weeks. Although the maximal feasible dose was tested, exposure margins were in the range or below clinical exposures.

In addition, across all DART studies, exposure margins at the highest dose tested were at or lower than exposure in human at the MRHD. Therefore, according to ICHS5(R3), there is still an increased risk, and the DART studies provide limited value for human risk assessment. This is reflected in the proposed SmPC sections 4.6 and 5.3.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

- **Tabular overview of clinical studies**

Table 10: Trofinetide clinical studies supporting clinical pharmacology assessments

Study number Study title Section	Study design	Objectives	Test product, dosage, regimens, duration of treatment	Subjects
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	Phase 1, double-blind, randomized, dose-escalation study.	To obtain evidence of the safety of trofinetide in healthy subjects compared to placebo when administered orally. To determine the blood PK of trofinetide in healthy subjects when administered orally.	Trofinetide single oral dose of 6 or 30 mg/kg or placebo. Trofinetide oral dose 100 mg/kg BID or placebo BID for one day. Following a safety assessment, the same subjects received 100 mg/kg oral doses of trofinetide or placebo BID for 5 days.	Healthy adult subjects; 12 male and 12 female.
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	Phase 1, open-label, single-dose study.	The PK objective was to characterize the PK (AME) and total radioactive recovery following the administration of a single 12 g dose of trofinetide containing radiolabeled [^{14}C]-trofinetide to healthy male subjects. To evaluate the safety and tolerability of a single 12 g dose of trofinetide containing radiolabeled [^{14}C]-trofinetide in 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 male.
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, Phase 1a, double-blind, randomized, dose-escalation study.	To determine the safety, tolerability, and PK of a single IV dose of trofinetide in healthy subjects 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 adult subjects; 28 male.

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, Phase 1, double-blind, randomized, dose-escalation study.	To determine the safety and tolerability of a single IV dose of trofinetide in healthy subjects when administered as a 12, 24, or 72 hour infusion. To determine the single-dose PK of trofinetide in healthy subjects when administered as a 12, 24, or 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 male.
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)	Phase 1, double-blind, randomized, dose-escalation study.	To determine the safety and tolerability of an IV dose of trofinetide in healthy subjects when administered as a 10 minute infusion immediately followed by either a 12, 24, 48, or 72 hour infusion. To determine the PK of an IV dose of trofinetide in healthy subjects when administered as a 10-minute infusion immediately followed by either a 12, 24, 48, or 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; 29 male.
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)	Phase 1, double-blind, randomized, dose-escalation study.	To determine the safety and tolerability of an IV dose of trofinetide in healthy female subjects when administered as a 10 minute infusion immediately followed by a 72 hour infusion. To determine the PK of an IV dose of trofinetide in healthy female subjects when administered as a 10 minute infusion immediately followed by a 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 female.

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	Phase 1, randomized, open-label, single-dose study.	To evaluate the PK of a single 12 g oral dose of trofinetide, administered under fed and fasted conditions, in healthy adult subjects. To evaluate the effects of evening dosing administered under fasted conditions on the PK of a single 12 g oral dose of trofinetide in healthy adult subjects. To evaluate the safety and tolerability of a single 12 g oral dose of trofinetide in healthy adult subjects.	Trofinetide 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 male and 13 female.
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	Phase 1, randomized, double-blind, placebo-controlled, ascending dose, nested crossover study.	To assess the safety and tolerability of single oral doses of trofinetide in healthy adult subjects. To evaluate the relationship between trofinetide concentrations in blood and time-matched change in QTcF interval.	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 male and 16 female.
Subjects with moderate Renal impairment Study Report				
ACP-2566-010 (Study 010) A Phase 1, Open-label, Single-dose Study to Evaluate the Pharmacokinetics of Trofinetide in Subjects With Moderate Renal Impairment and Healthy Control Subjects With Normal Renal Function	A Phase 1, Open-label, Single-dose study	To evaluate the PK of a single oral dose of trofinetide in subjects with moderate renal impairment compared with matched healthy controls To evaluate the safety and tolerability of a single oral dose of trofinetide in subjects with moderate renal impairment and matched healthy controls	Trofinetide single dose oral solution Healthy control subjects: 12 g Subjects with moderate renal impairment: 6 g (2 subjects: 8 g due to dosing error.	Adult subjects Healthy: 3 male and 7 female Moderate renal impairment: 3 male and 7 female

Healthy Subject – Drug-Drug Interaction Study				
ACP-2566-012 (Study 012) A Phase 1, Open-label, Sequential, Drug-Drug Interaction Study of IMODIUM® (Loperamide Hydrocholoride) Administered Alone and in Combination with Trofinetide in Healthy Subjects	A Phase 1, Open-label, Sequential, Drug-Drug Interaction Study	To assess the effect of trofinetide on the PK profile of loperamide, a substrate to CYP3A4 enzyme and P-gp transporter To evaluate the safety and tolerability of trofinetide when administered in combination with loperamide in healthy participants	Cycle 1: single dose of loperamide 4 mg alone Cycle 2: single dose of loperamide 4 mg concomitantly administered with first dose of trofinetide 12 g; second daily dose of trofinetide 12 g administered at least 8 h (up to 10 h) following the first trofinetide dose Cycle 3: first dose of trofinetide administered a minimum of 2 h prior to loperamide dosing (single dose of 4 mg); second daily dose of trofinetide 12 g administered at least 8 h (up to 10 h) following the first trofinetide dose	Healthy adult subjects; 14 male and 1 female
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	Phase 2, double-blind, placebo-controlled, dose-escalation study.	To evaluate the safety and tolerability of trofinetide in each dosing cohort by evaluating the incidence of AEs from dosing through to Day 40 post randomization and comparing active with placebo treatment arms. To determine the blood PK of oral trofinetide when administered as 35 or 70 mg/kg BID in adolescent or adult females with RTT.	Trofinetide oral doses as follows: Day 1 10 mg/kg QD, Day 2 20 mg/kg QD, Days 3-14 35 mg/kg BID. 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. 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 female.

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	Phase 3, randomized, double-blind, placebo-controlled, parallel-group study.	To investigate the efficacy of treatment with oral trofinetide versus placebo in girls and women with RTT. To investigate the safety and tolerability of treatment with oral trofinetide versus placebo in girls and women with RTT. To characterize the PK of trofinetide in girls and women with RTT. To assess the PK/PD relationship using safety and efficacy endpoints in girls and women with RTT.	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 female.
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	Phase 2, randomized, double-blind, placebo-controlled, dose-ranging study.	To investigate the safety, tolerability, and blood PK of treatment with oral trofinetide versus placebo in female children and adolescents with RTT.	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 female.
Other Studies				
ACP-2566-004 (Study 004) A 40-Week, Open-label Extension Study of Trofinetide for the Treatment of Girls and Women With Rett Syndrome	Phase 3, multicenter, open-label study.	To investigate the safety and tolerability of long-term treatment with oral trofinetide in girls and women with RTT. To characterize the PK of trofinetide in girls and women with RTT following long-term treatment with oral trofinetide. To assess the PK/PD relationship using safety and efficacy endpoints in girls and women with RTT following long-term treatment with oral trofinetide.	Placebo or 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; 154 female.

ACP-2566-009 (Study 009) An Open-Label Study of Trofinetide for the Treatment of Girls Two to Five Years of Age Who Have Rett Syndrome	Phase 2/3, multicenter, open-label study.	To investigate the safety and tolerability of treatment with oral trofinetide in girls 2 to 5 years of age who have RTT. To characterize the PK of oral trofinetide in girls 2 to 5 years of age who have RTT.	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 to5 years with RTT; 15 female.
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	Phase 2, double-blind, placebo-controlled, parallel-group, fixed-dose study.	To investigate the safety and tolerability of treatment with oral administration of trofinetide versus placebo in adolescent and adult males with FXS. To determine the blood PK of oral trofinetide when administered at 35 or 70 mg/kg BID in adolescent and adult males with FXS.	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 male.
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	Phase 2, randomized, double-blind, placebo-controlled study.	To evaluate the safety, efficacy, and PK of trofinetide compared to placebo, in subjects with TBI.	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 male and 30 female.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Pharmacokinetics

Trofinetide is proposed for treatment of adults and pediatric patients aged 2 years and older with Rett syndrome. A total of 17 clinical pharmacology studies have been conducted: ten studies in healthy subjects, five studies in subjects with RTT, one study in subjects with FXS, and one study in subjects with TBI. The PK profile of trofinetide has been characterized in individual studies using a noncompartmental approach as well as pooled data.

Bioanalytical methods.

Bioanalytical assays, generally applying LC-MS/MS, for measurement of trofinetide in blood and urine have been validated to a fair extent, with requirements with regard to precision and accuracy standard being met. Bioanalytical assays were developed and validated at three different sites.

Incurred Sample Reanalysis was performed in all studies except Studies HV-001, HV-002 and HV-003. For the remainder of the studies, ISR indicated robustness of the analytical assay. Considering the small number and type of studies without ISR, this issue will not be pursued.

Pharmacokinetic data analysis. Standard noncompartmental analyses were used to determine PK parameters in studies where intensive sampling was conducted. PopPK methods were used to characterize exposures of trofinetide in healthy subjects and patients and to assess the effects of demographic characteristics and other covariates on PK.

Evaluation and qualification of models.

PopPK models

To fully characterize the PK of trofinetide, guide dose selection, and confirm the appropriateness of the proposed dosing regimens, popPK modeling was used. For this purpose, an initial preliminary popPK model was initially developed (Oosterholt et al. 2017a[1]). The completed studies were added on an ongoing basis, and the popPK model was refined at different stages through the project development (Study MS-007, Study MS-008 and Study MS-010).

In the popPK model by Oosterholt *et al*. 2017[1] above, population pharmacokinetic modelling and simulation was performed using a nonlinear mixed effects approach, as implemented in NONMEM version 7.3. The studies (only healthy subject) included in the popPK analyses were Studies HV-001, HV-04 and HV-005.

In total, 61 healthy subjects who received trofinetide, and from whom blood samples were collected, were considered evaluable for the purposes of this analysis. Of the 1435 samples available, 20 were excluded as they had concentrations below the limit of detection. In addition, data from 6 individuals receiving doses of 6 mg/kg doses of NNZ-2566 had to be excluded from the analysis due to unexplained variability in drug levels, which seemed to increase throughout the sampling interval. A sensitivity analysis showed that the exclusion of these individuals had no further implications for model development and parameter estimation.

[1] Oosterholt et al. 'Population pharmacokinetics of NNZ-2566 in healthy subjects', *European Journal of Pharmaceutical Sciences,* vol. 109 (2017), pp. S98–S107

First, a base model (no covariates) was built. This was accomplished by first identifying the appropriate structural model parameters. The pharmacokinetics of trofinetide following intravenous administration to healthy subjects was best described by a 2-compartment model with first order elimination (Figure 3).

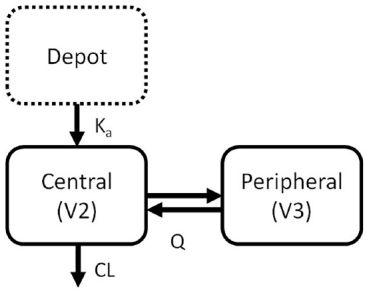


Figure 3: Compartmental pharmacokinetic model structure. In this diagram, Ka is the absorption rate constant, CL the total clearance, Q the intercompartmental clearance, and V2 and V3 represent the central and peripheral volumes of distribution, respectively

PK model building was performed using the FOCE-I estimation method. Covariate analysis was performed to explore potential sources of variability in the pharmacokinetics of trofinetide. Selected covariates were identified using a stepwise forward inclusion backward deletion approach. Covariate effects were considered for the primary model parameters only (e.g., Ka, CL, V2). Biologically plausible factors identified prospectively to be tested in the covariate analysis included age, weight, route of administration, dose and food intake.

In the final model (Oosterholt *et al.* 2017), oral administration of trofinetide was characterised by two parameters (F1AM, F1PM) describing diurnal variation in bioavailability, with lower systemic exposure observed after the afternoon/evening doses, most likely reflecting the impact food effect. No covariate effects were found on any of the model parameters. The residual error was described using an exponential error model.

Table 11: Population pharmacokinetic parameter estimates after administration of trofinetide to healthy subjects (Oosterholt et al, 2017)

Parameter (unit)[a]	Notation	Parameter estimate	RSE (%)	Bootstrap mean (95% CI)
Absorption rate constant, Ka (1/h)	θ1	0.28	15.2	0.29 (0.20–0.39)
Systemic clearance, CL (L/h)	θ2	10.35	2.3	10.36 (9.89–10.86)
Intercompartmental clearance, Q (L/h)	θ3	1.04	15.0	1.06 (0.79–1.41)
Central volume of distribution, V2 (L)	θ4	20.23	2.3	20.22 (19.29–21.24)
Peripheral compartment volume of distribution, V3 (L)	θ5	41.47	8.9	41.43 (33.07–49.24)
Bioavailability morning dose, $F1_{AM}$	θ6	0.63	4.9	0.63 (0.57–0.69)
Bioavailability afternoon dose, $F1_{PM}$	θ7	0.50	5.1	0.50 (0.45–0.55)
Inter-individual variability		**Parameter estimate (CV %)[b]**	**RSE (%)**	**Bootstrap mean (95% CI)**
ηCL	Ω1	0.02 (12.2%)	9.1	0.01 (0.01–0.02)
ηV2	Ω2	0.02 (12.9%)	11.4	0.02 (0.01–0.02)
ηKA	Ω3	0.26 (50.9%)	10.9	0.24 (0.11–0.38)
ηQ	Ω4	0.71 (84.2%)	7.3	0.70 (0.48–0.94)
ηV3	Ω5	0.08 (27.9%)	10.6	0.08 (0.02–0.14)
Residual error		**Parameter estimate (CV %)**	**RSE (%)**	**Bootstrap mean (95% CI)**
Exponential error	σ1	0.03 (17.1%)	7.4	0.03 (0.02–0.04)

The final PK model was validated using GOF plots and a simulation-based, pcVPC methodology to assess concordance between the model-based simulated data and the observed data. pcVPC plots are shown in Figure 4 and Figure 5. In general, the GOF plots and pcVPCs indicated that the model adequately predicted both the central tendency of the concentration data over time, as well as the extent of variability in the observed data. However, based on the IPRED vs OBS plot it is clear that observed data at higher concentrations are underpredicted. This can in theory either be due to the absence of a 3rd compartment in the model, or a miscoding of the exponential residual error (see comments below on Study MS-010).

Figure 4: Goodness-of-fit plots. Upper panels show the observed data (Obs) vs. individual predictions (IPred) (left) and the conditional weighted residuals (CWRES) vs. time (right). Lower panels show the observed data vs. population predictions (Pred) (left) and CWRES vs. population predictions (right) (Oosterholt et al 2017)

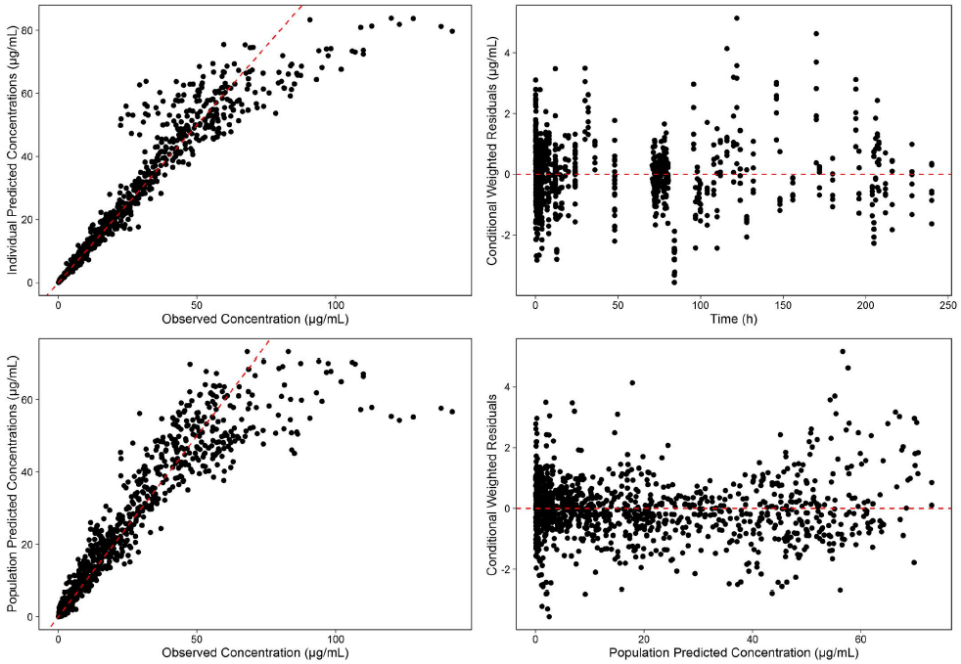
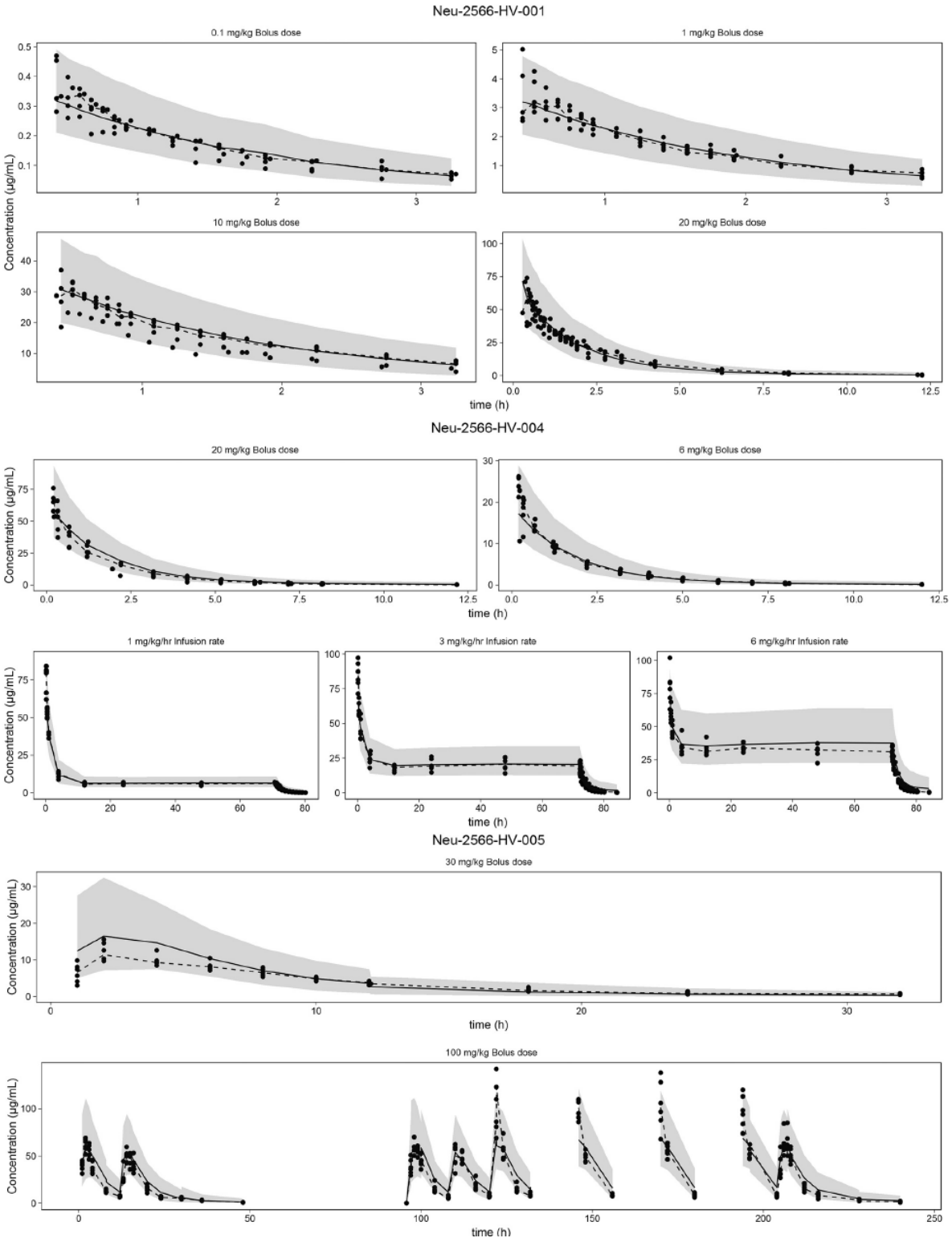


Figure 5: Visual predictive checks. Observed and predicted trofinetide concentration vs. time profiles in healthy subjects. In each panel, the solid and dotted lines represent the median of the simulated profiles along with the corresponding 95% confidence intervals (Oosterholt et al 2017)



The preliminary popPK model by Oosterholt et al 2017 was used to provide a first description of the PK of trofinetide, based on PK data obtained in healthy subjects. The overall performance of the model is considered acceptable. It is to be noted that in later models, the circadian effect (considered to result

from a food effect) was proven to be non-relevant in Study HV-006 and therefore no longer included as covariate.

PopPK model MS-007 was used in preliminary exposure-response analyses in support of the dose to be investigated in Phase 3, based on 9 clinical studies. The model will not be described in detail, since it shows great resemblance with popPK Model MS-008.

PopPK model MS-008 was used to re-estimate the PK parameters from the previous population PK model MS-007 for trofinetide using additional data from pivotal RTT Study 003, and use of the updated population PK model to simulate individual steady-state exposures following proposed dosing regimens for Study 003 to determine whether the 5-20 years old subjects' exposures achieved the target exposure range (area under the concentration time curve from time 0 to 12 hours at steady state [$AUC_{0\text{-}12,ss}$] = 800 - 1200 µg.h/ml). Further, the model was used to investigate intrinsic and extrinsic factors as covariates in the popPK model for trofinetide.

All exploratory data analyses and presentations of data were performed using SAS Version 9.46 and KIWI Version 4 202111.7 Population modeling was performed using the computer program NONMEM, Version 7, Level 3.0.8 NONMEM analyses were performed on an Intel cluster with the Linux operating system.

A total of 3058 trofinetide concentration records collected in 290 subjects were made available from Acadia for potential use in the analysis. A total of 143 pre-first dose BLQ samples and 84 post-dose BLQ samples, affecting 143 subjects and 59 subjects, respectively, were excluded from this analysis.

Key steps for the refinement of the trofinetide population PK model starting from popPK model MS-007 were: 1) exploratory data analysis; 2) base structural model development via application, re-estimation, and refinement of the previous population PK model (developed using data from 9 clinical studies); 3) evaluation of covariate effects using forward selection; 4) full multivariable model evaluation; 5) backward elimination of covariates; 6) final model refinement; and 7) model evaluation.

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. The covariate analysis used a standard forward selection backward = 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 final population PK model for trofinetide was a 2-compartment model with first-order absorption and elimination, and 2 separate exponential error models, 1 for healthy subjects and 1 for subjects with Rett syndrome, FXS or TBI. Interindividual variability was estimated for oral bioavailability (F1), clearance (CL), central volume of distribution (Vc), peripheral volume of distribution (Vp), and intercompartmental clearance (Q) using exponential error models. Effects of fed status, diarrhea occurrence, and supratherapeutic doses (18- and 24-g doses) on F and fed status on k_a were also included in the final model. Following the covariate analysis, effects from body weight, GFR, and both Rett and TBI disease status on CL were included, as well as age and FXS disease status effects on V_c, and both Rett and TBI disease status effects on V_p.

The final PK model parameter estimates and their associated precisions (%RSE) are presented in Table 12. The refined population PK model was similar to the previous population PK model MS-007 developed using data from 9 studies demonstrating consistency of trofinetide PK profile across studies.

Table 12: Parameter estimates for the final PK Model (popPK Study MS-008)

Parameter		Final parameter estimate		Magnitude of variability	
		Population mean	%RSE	Final estimate	%RSE
CL	Central Clearance (L/h)	11.8	2.02	13.6 %CV	17.4
	Exponent of (WTKG/58) for CL	0.443	8.97		
	Proportional Shift in CL for Rett = 1	-0.169	25.6		
	Proportional Shift in CL for TBI = 1	0.235	17.6		
	Exponent of (GFR/124) for CL	0.273	20.2		
V_c	Central Volume of Distribution (L)	24.9	3.89	30.8 %CV	12.8
	Exponent of (AGE/22.4) for V_c	0.549	8.84		
	Proportional Shift in V_c for FXS = 1	1.15	19.9		
Q	Intercompartmental Clearance (L/h)	1.44	6.17	64.4 %CV	18.5
V_p	Peripheral Volume of Distribution (L)	35.3	5.46	28.2 %CV	14.6
	Proportional Shift in V_p for Rett = 1	0.616	35.8		
	Proportional Shift in V_p for TBI = 1	-0.752	4.22		
k_a	First-Order Absorption Rate Constant (1/h)	0.391	4.09	NE	N/A
	Shift in k_a for FED	-0.0949	24.1		
F	Oral Bioavailability	0.828	3.65	20.8 %CV	15.7
	Shift in F for FED	-0.133	7.28		
	Shift in F for 18 g Dose	-0.132	19.9		
	Shift in F for 24 g Dose	-0.284	5.66		
	Shift in F for Diarrhea	-0.148	21.0		
Residual Variability Healthy Subject		0.0789	1.54	28.1 %CV	N/A
Residual Variability Patient		0.137	3.50	37.0 %CV	N/A
Minimum Value of the Objective Function = 25101.786					

Source: Report MS-008 Table 9

Abbreviations: %CV=coefficient of variation expressed as a percent; CL=systemic clearance; FXS=Fragile X syndrome; GFR=glomerular filtration rate; IIV=interindividual variability; N/A=not applicable; NE=not estimated; PK=pharmacokinetic(s); %RSE=relative standard error expressed as a percent; TBI=traumatic brain injury; WTKG=body weight in kilograms

Notes: Shrinkage estimates: 37.1% for IIV in CL, 31.4% for IIV in V_c, 9.5% for IIV in Q, 39.8% for IIV in V_p, and 55.0% for IIV in F.

Goodness-of Fit plots of the final model are shown in Figure 6. The final PK model was validated using a simulation-based, prediction-corrected visual predictive check (pcVPC) methodology to assess concordance between the model-based simulated data and the observed data. The median and 90% PI, derived from the simulated datasets, were overlaid on the observed trofinetide concentration data for the overall population), and separately by study (Figure 8). In general, like in the previous model MS-007, the GOF plots and pcVPCs indicated that the model adequately predicted both the central tendency of the concentration data over time, as well as the extent of variability in the observed data. However, based on the IPRED vs OBS plot it is clear that observed data at higher concentrations are underpredicted. This can in theory either be due to the absence of a 3rd compartment in the model, or a miscoding of the exponential residual error. For the purpose of this popPK Study, i.e., to check if patients 2-20 years achieved exposure within the predefined target exposure of 800-1200 µg.h/ml, it is considered problematic that the model underpredicts the high exposures (see comments on popPK Study MS-010).

Figure 6: Goodness-of-Fit diagnostic plots for the final trofinetide population popPK Model MS-008

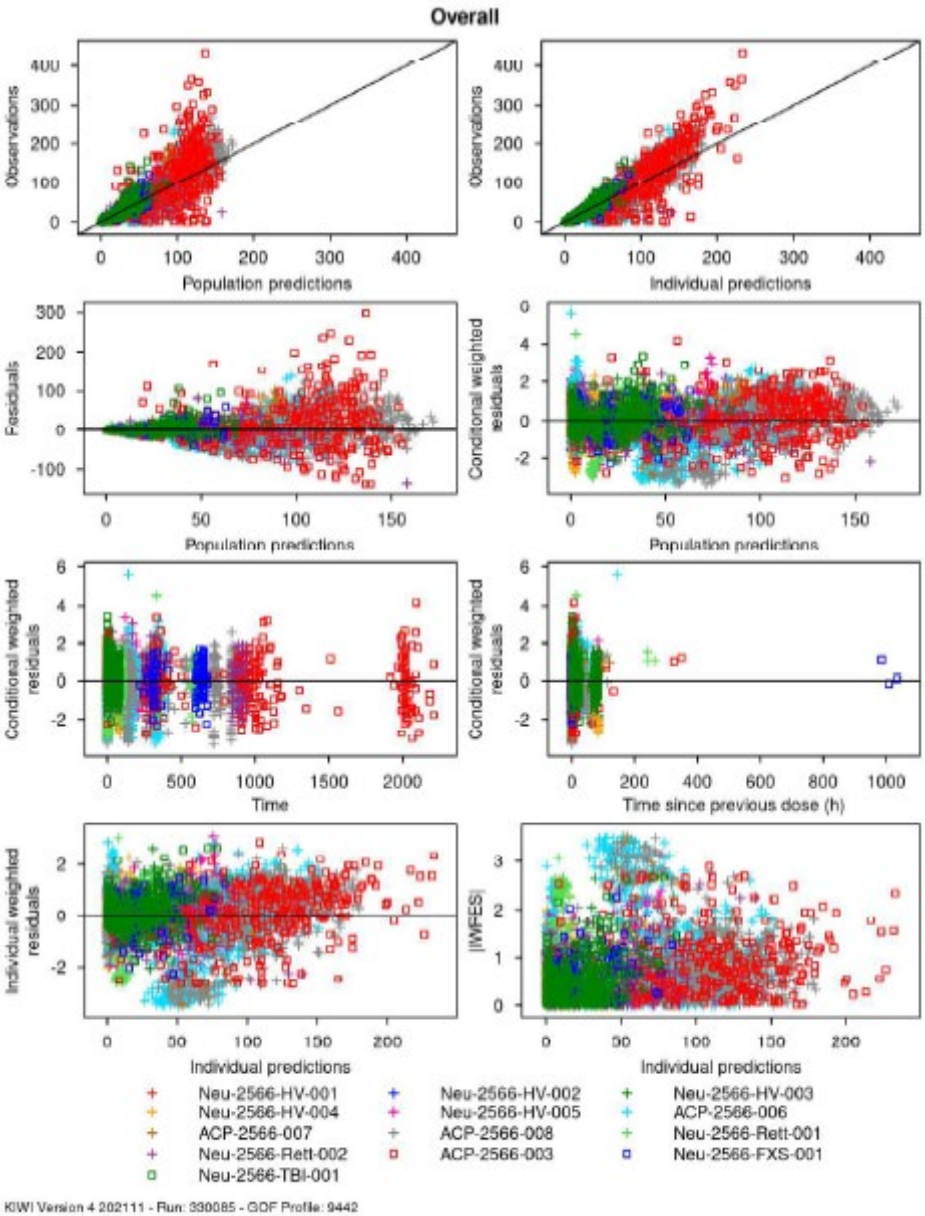


Figure 7: Prediction-corrected visual predictive check for the final trofinetide popPK model (time since first dose, popPK Study MS-008)

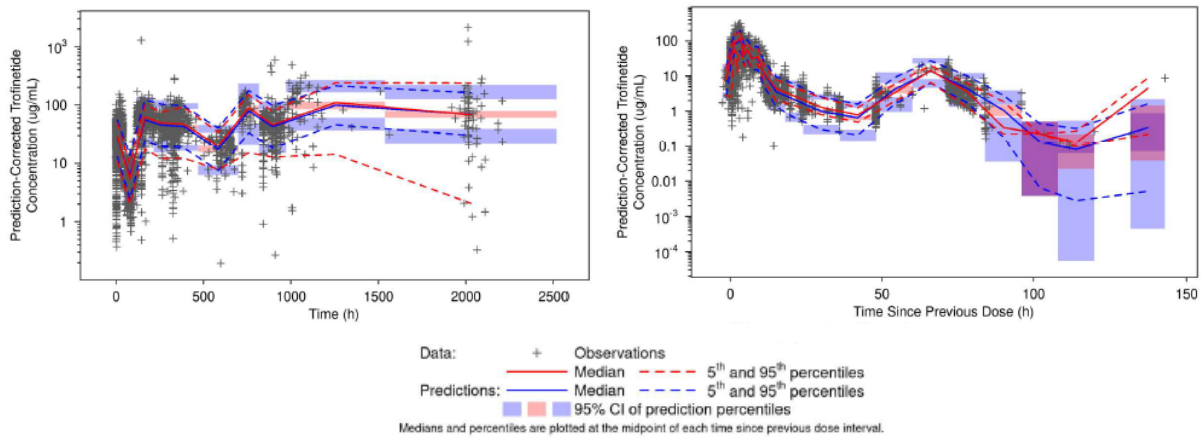
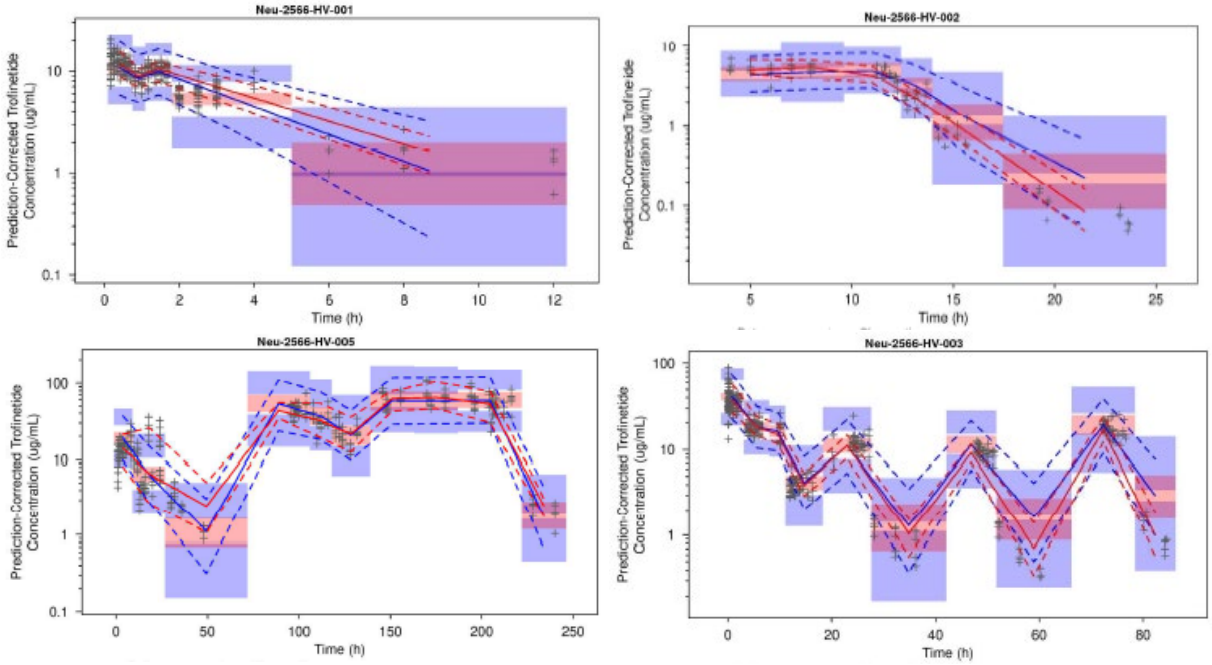
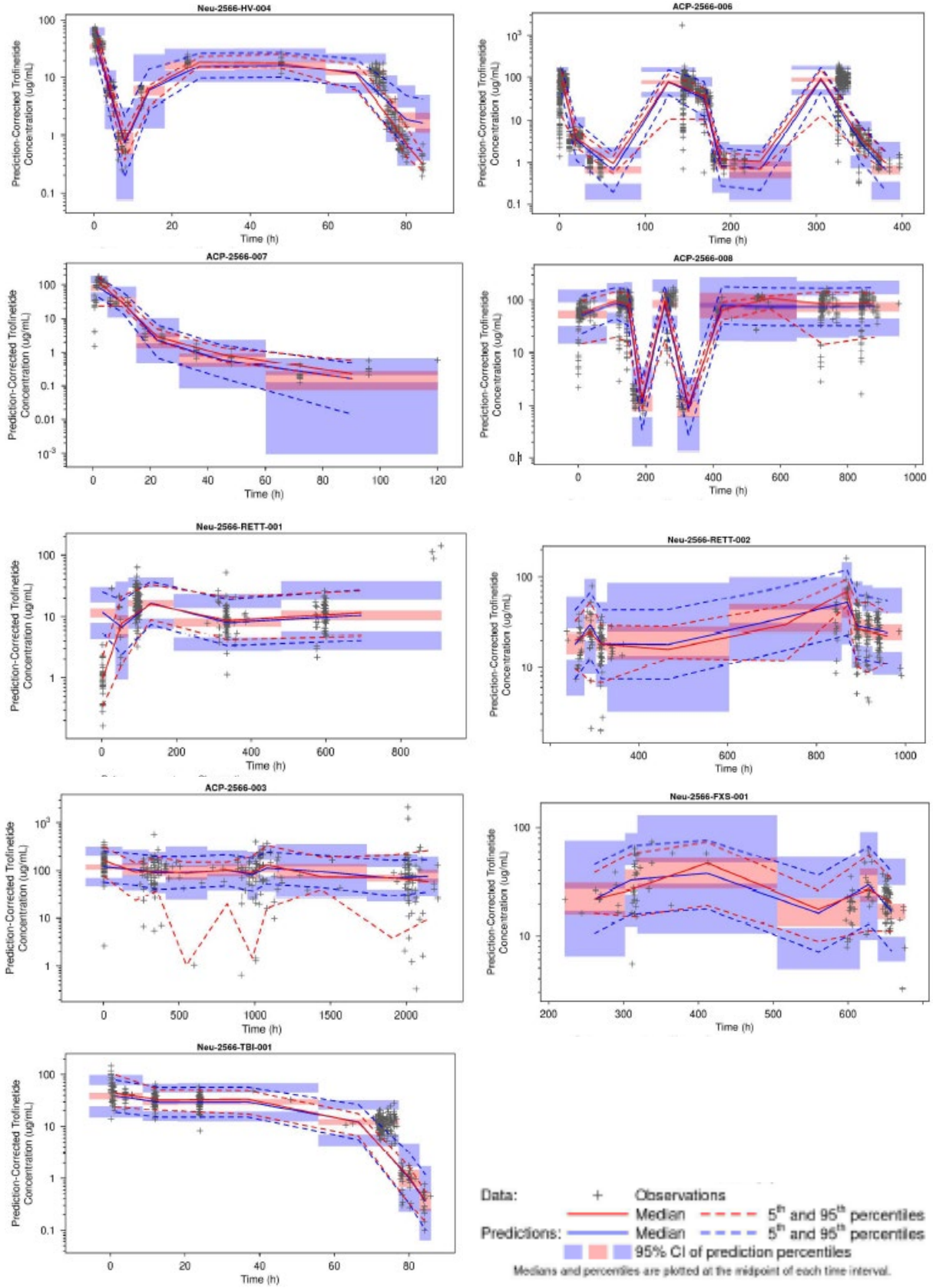


Figure 8: Prediction-corrected Visual Predictive check for the final trofinetide popPK Model, stratified by study (popPK Study MS-008)





The popPK model MS-008 was used to re-estimate the PK parameters from the previous population PK model MS-007 for trofinetide using additional data from pivotal RTT Study 003, and use of the updated population PK model to simulate individual steady-state exposures following proposed dosing regimens

for Study 003 to determine whether the 5-20 years old subjects' exposures achieved the target exposure range (area under the concentration time curve from time 0 to 12 hours at steady state [$AUC_{0-12,ss}$] = 800 - 1200 µg. h/ml). Further, the model was used to investigate intrinsic and extrinsic factors as covariates in the popPK model for trofinetide. With these simulations, it was checked if 5-20 year old patients achieved exposure within the predefined target exposure of 800-1200 µg.h/ml. It was flagged to the Applicant that the model could be further developed in order to improve the prediction of high exposure levels (see next section on popPK Study MS-010).

The aim of popPK Study MS-010 was to re-estimate the PK parameters from the previous population PK model MS-008 for trofinetide using additional data from paediatric Study 009, and use of the updated population PK model to simulate individual steady-state exposures following proposed dosing regimens for Study 009 to determine whether the 2-20 years old subjects' exposures achieved the target exposure range (area under the concentration time curve from time 0 to 12 hours at steady state [$AUC_{0-12,ss}$] = 800 - 1200 µg.h/ml).

All exploratory data analyses and presentations of data were performed using SAS Version 9.46 and KIWI Version 4 202111.7 Population modeling was performed using the computer program NONMEM, Version 7, Level 3.0.8 NONMEM analyses were performed on an Intel cluster with the Linux operating system.

A total of 6196 trofinetide concentration records collected in 452 subjects were available for potential use in the analysis. A total of 291 pre-first dose BLQ samples and 231 post-dose BLQ samples, affecting 291 subjects and 127 subjects, respectively, were excluded from this analysis. Additionally, duplicate samples, pre-first dose measurable concentrations, and subjects with only BLQ samples were excluded, resulting in 5670 records in 450 subjects.

Following re-estimation of the popPK model with the additional paediatric data from Study 009, the final PK parameter estimates were similar to the previous model (see Table 13). The primary PK parameter estimates changed by less than 1.5%, and the majority of the estimates for the covariate effects changed by less than 10% as compared to popPK model MS-008. However, the shift in CL for Rett patients decreased from -16.9% to -14.6% (a 13.6% change), while the shift in V_p for Rett patients increased from 61.6% to 80.5% (a 30.7% change).

Table 13: Parameter estimates for the final PK Model (popPK Study MS-010)

		Final Parameter Estimate		**Magnitude of Variability**	
Parameter		**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		
Vc	Central volume (L)	25.0	3.78	30.8 %CV	12.5
	Exponent of (AGE/22.4) for Vc	0.556	7.98		
	Proportional shift in Vc for FXS = 1	1.16	20.0		

Q	Distribution clearance (L/h)	1.42	5.97	65.3 %CV	17.7
Vp	Peripheral volume (L)	35.4	5.69	30.2 %CV	14.3
	Proportional shift in Vp for RTT = 1	0.805	23.4		
	Proportional shift in Vp for TBI = 1	-0.753	4.43		
ka	First-order absorption rate constant (1/h)	0.394	4.00	NE	NA
	Shift in ka 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. KIWI Run 338586.

Goodness-of Fit plots of the final model are shown in Figure 9. The pcVPCs were generated using the updated population PK model to ensure adequate model performance and to assess the predictive capabilities of the model. The median and 90% PI, derived from the simulated datasets, were overlaid on the observed trofinetide concentration data for the overall population (Figure 10). The median and 80% PI, derived from the simulated datasets, were overlaid on the observed trofinetide concentration data separately for Study 009 (Figure 11). The pcVPCs indicated that the model adequately predicted both the central tendency of the Study 009 concentration data over time, as well as the extent of variability in the observed data. However, based on the IPRED vs OBS plot it is clear that observed data at higher concentrations are underpredicted. This can in theory either be due to the absence of a 3rd compartment in the model, or a miscoding of the exponential residual error.

Figure 9: Goodness-of-Fit diagnostic plots for the updated trofinetide population pharmacokinetic model MS-010

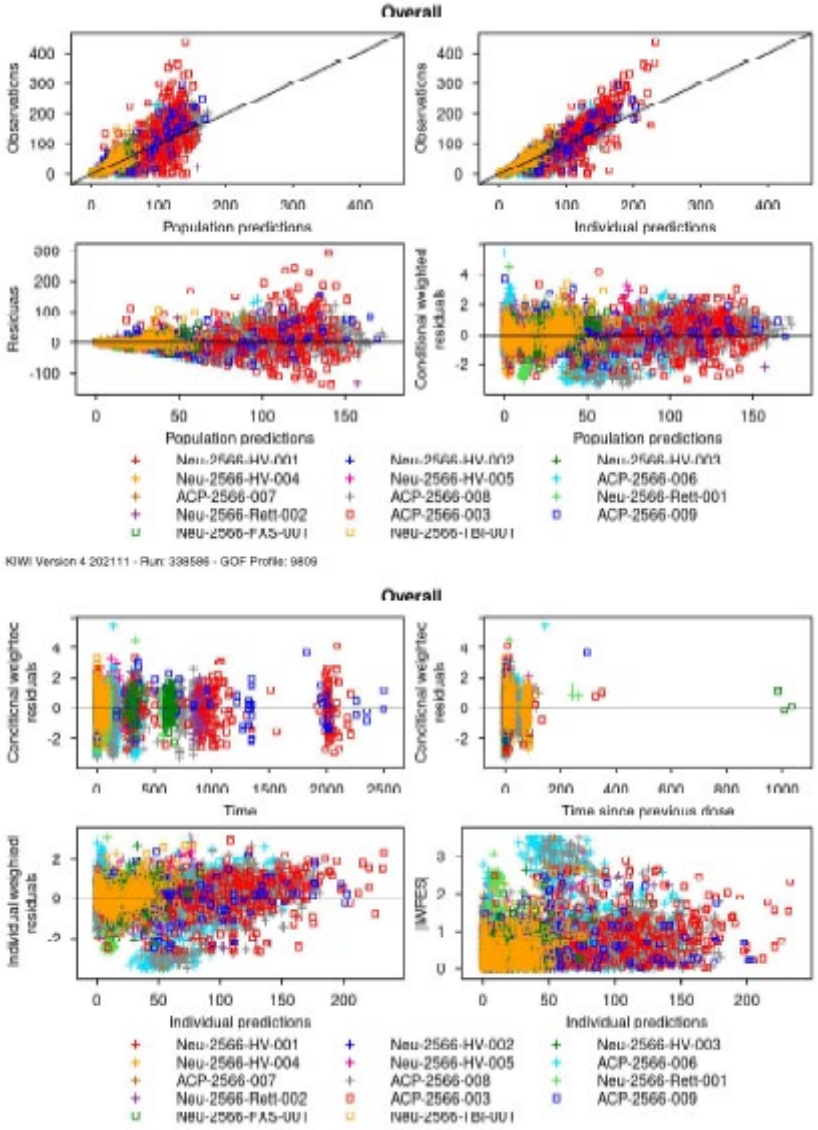


Figure 10: Prediction-corrected visual predictive check for the final trofinetide popPK model (time since previous dose, popPK Study MS-010)

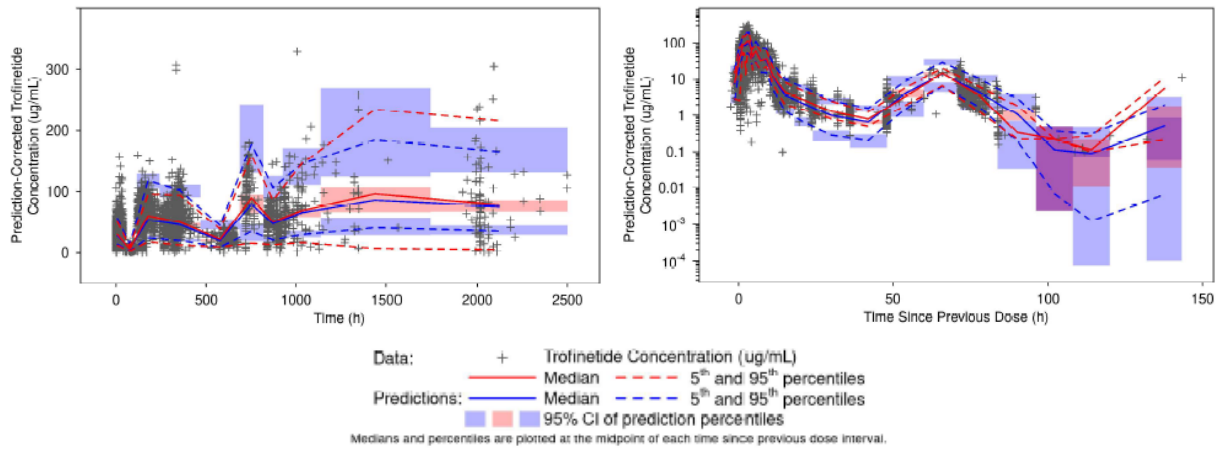
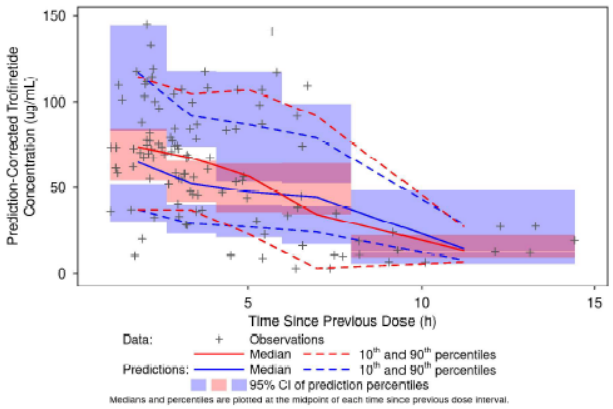


Figure 11: Prediction-corrected visual predictive check for the re-estimated trofinetide popPK model for paediatric Study 009 (popPK Study MS-010)



General conclusion on popPK models

In general, the popPK models from Oosterholt et al, MS-007, MS-008 and MS-010 were validated sufficiently, and provide a reasonable description of the PK data from the actual clinical studies. The GOF plots and pcVPCs indicated that the model reasonably predicted both the central tendency of the concentration data over time, as well as the extent of variability in the observed data. However, higher exposures are underpredicted by all models.

PBPK models

A base PBPK model was developed (PBPK Study MS-002). This model was further used to simulate various scenarios to understand the impact of intrinsic renal impairment PBPK Study MS-003 and MS-011, hepatic impairment (PBPK Study MS-004), age, specifically in paediatric patients aged 2 to 5 years (PBPK Study MS-006;) and extrinsic factors (DDIs (PBPK Study MS-005 and MS-012) on trofinetide PK.

GastroPlus® (Version 9.7),2 along with the PBPKPlusTM module, was used for PBPK simulations. Microsoft® Excel® was used to compile and post-process the GastroPlus simulated output. Graph generation was performed R software (Version 3.6.1).

Initial development and validation of PBPK model MS-002 started using PK data from studies in which trofinetide was administered IV. Pharmacokinetic data following oral administration was subsequently utilized to capture the oral absorption component of the final PBPK model with a final model refinement completed upon availability of full-profile trofinetide concentration data from clinically relevant 12 g trofinetide oral dose administration.

The model incorporated experimental and predicted physicochemical properties along with information on dissolution, absorption, elimination, and excretion pathways, as applicable.

Table 14 provides the physicochemical properties that were included in the PBPK model for trofinetide. All assayed trofinetide concentrations were reported in terms of blood concentrations and were converted to plasma concentrations for modeling purposes using the Rbp of 0.525.

Table 14: Trofinetide physicochemical and biopharmaceutical properties for trofinetide model development (PBPK Study MS-002)

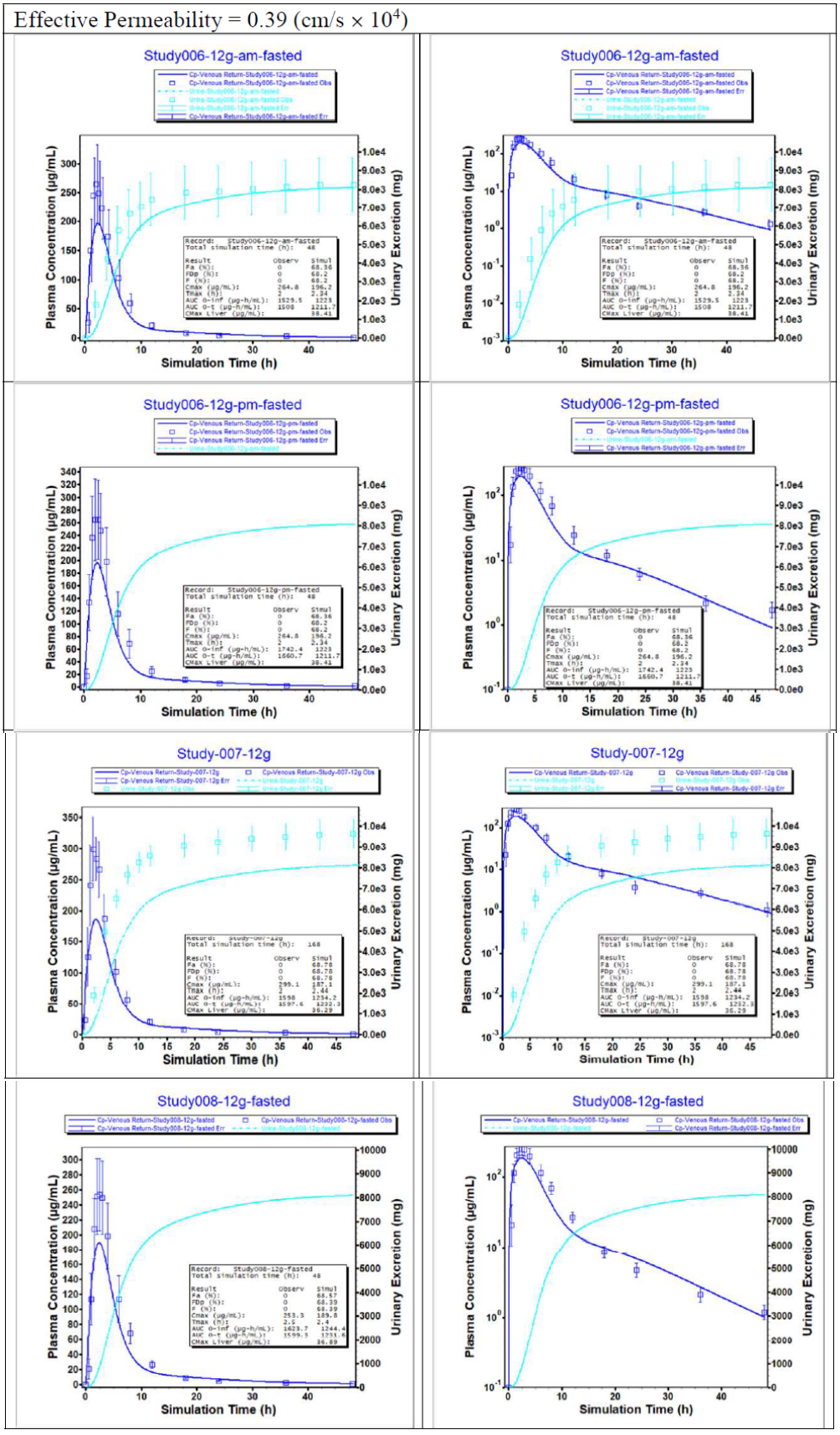
Property	Value	Reference
LogD (at pH = 4.0)	-2.93	ACADIA Pharmaceuticals, Inc.[a]
Aqueous Solubility (pH = 3.96)	800 mg/ml	ACADIA Pharmaceuticals, Inc.[b,c]
pKa Base Acid Acid	8.61 4.42 2.97	ACADIA Pharmaceuticals, Inc.[a]
Effective Permeability	Initial value: 0.29 (cm/s x 10^4) Final Value: 0.39 (cm/s x 10^4)	ADMET Predictor V 9.5 Model fit parameter
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 x 10^{-3} (ml/s/ml)	Model fit parameter

The following assumptions were used in the PBPK model development of trofinetide:

- Trofinetide is absorbed from the gut via a passive diffusion process.
- Trofinetide is eliminated in urine by passive renal filtration. No measurable amount of metabolism occurs for trofinetide.
- Trofinetide R_{bp} value of 0.525 was assumed for healthy adult subjects.

The final model refinement was based on the mean profiles for 12 g trofinetide oral dose administration in the fasted state. The simulated plasma concentration-time profiles and urinary excretion for a single 12 g trofinetide fasted dose using the final model settings provided a good prediction for the data from 3 clinical studies (Studies 006, 007, and 008) administering the clinically relevant 12 g oral trofinetide solution dose (Figure 12).

Figure 12: Trofinetide model development: fit of Study 006, 007 and 008 single-dose trofinetide oral administration: Original model setting, effective permeability of 0.39 cm/s 10^4 (PBPK Study MS-002)



Parameter sensitivity analyses indicated that effective permeability had the greatest overall impact on the C_{max}, $AUC_{0\text{-}inf}$ in plasma, and fraction of dose absorbed. The observed Rbp values ranging between 0.529 to 0.592 (mean of measured Rbp values from Study 007) resulted in less than 10% changes in trofinetide blood C_{max} values.

Similar to the results observed in the food effect study (Study 006), an exploratory evaluation of the impact of food predicted a small reduction in C_{max} and a slight delay in t_{max} for trofinetide when administered with a high fat meal as compared to the fasted state. The relative magnitude of the food effect on the rate of absorption differed to a small extent (approximately 10%) between the model predicted parameters and parameters estimated in Study 006. Further exploratory evaluation suggested that trofinetide doses of 18 and 24 g are absorbed more slowly and to a lesser extent than a 12 g dose, leading to a slightly less than dose proportional increase in systemic exposure over the range of 12 through 24 g. The less than dose proportional observation was also noted in the clinical dose proportionality evaluation (Study 008).

In PBPK Study MS-006, the main goal was to evaluate the expected trofinetide exposures in paediatrics, thus, providing a guide for trofinetide dosing in pediatric patients between 2 and 5 years of age, with a specific emphasis towards pediatric patients with Rett syndrome. To this end, virtual physiologies were created representing the age and size range of the intended patient pediatric patient population (2- to 5-year-old patients with RTT). Various dosing scenarios were simulated for trofinetide to evaluate the expected exposure of trofinetide and to define the doses required to achieve the previously determined target exposures in this pediatric patient population. For the purposes of these simulations, parameters, such as hematocrit, fraction of unbound drug in the plasma (fu_p), and GFR, in patients with RTT are assumed to be similar to and can be estimated from corresponding values in healthy pediatric subjects. In general, the predicted variability reasonably captured the observed trofinetide concentrations from the 5- to 16-year-old study patients, thus, providing additional confidence in use of this PBPK model for application to paediatric trofinetide exposure predictions.

In PBPK Study MS-003, the objective of the analysis was to estimate the trofinetide dose-exposure relationship for patients with mild, moderate, and severe renal impairment. To this end, various trofinetide dosing scenarios were simulated using validated healthy subject physiologies and physiologies representing the 4 stages of renal impairment, as defined in the corresponding FDA guidance document. Dosing adjustments for trofinetide were explored to achieve acceptable exposures for single-dose trofinetide administration for a renal impairment study 009.

In PBPK Study MS-011, the PBPK model as used in Study MS-003 related to renal impairment was validated using actual clinical data from paediatric Study 009. The goals were to use the PBPK model for trofinetide in humans and trofinetide concentration versus time profiles from renal impairment Study 010 to provide further validation of the PBPK model-predicted trofinetide concentrations in healthy subjects, and validate the PBPK model-predicted trofinetide concentrations in subjects with moderate renal impairment.

In PBPK Study MS-004, the impact of hepatic impairment on the predicted PK of trofinetide using the developed trofinetide PBPK Model MS-002 was assessed using deterministic and stochastic simulations for single-dose trofinetide administration in validated healthy subject physiologies and physiologies representing the stages of hepatic impairment (the Child-Pugh classification of hepatic impairment). Changes in the trofinetide R_{bp} due to reduced hematocrit values for each hepatic impairment level were taken into account in the predictions of blood concentrations and PK parameters. The standard hepatic impairment physiologies include a general renal impairment comorbidity. Therefore, to evaluate the impact due solely to hepatic impairment, the GFR for the hepatic impairment physiologies used in these simulations was set to be the same as for the healthy subjects.

In PBPK Study MS-005, the objective was to evaluate the DDI potential for trofinetide as the perpetrator drug and midazolam (oral or iv administered) as the victim drug following oral trofinetide administration. To this end, the DDI potential for trofinetide administration in humans to impact the PK of drugs eliminated primarily by CYP3A4 metabolism, such as midazolam (an index substrate for clinical DDI assessments) was evaluated using the trofinetide PBPK model MS-002.

In PBPK Study MS-012, the DDI potential for trofinetide administration in humans to impact the PK of drugs that are substrates of OATP1B1/B3 transporters and CYP3A4 enzyme, such as pravastatin was evaluated using the trofinetide PBPK model MS-002). Trofinetide was treated as the perpetrator drug and pravastatin as the victim drug following oral administration of both drugs.

The development of the PBPK models has been reported sufficiently. The final model refinement was based on the mean profiles for 12 g trofinetide oral dose administration in the fasted state. The simulated plasma concentration-time profiles and urinary excretion for a single 12 g trofinetide fasted dose using the final model settings provided a good prediction for the data from 3 clinical studies (Studies 006, 007, and 008) administering the clinically relevant 12 g oral trofinetide solution dose.

The PBPK model are considered fit for purpose when description of the trofinetide PK is concerned. However, in case of modification due to e.g. DDI or organ impairment, the models are not sufficiently qualified to be predictive. However, since PBPK modelling was mainly used for developing supportive data for certain situations of designing a clinical study the impact of the model is considered low, and the insufficient qualification will not be pursued.

Absorption

Following oral administration, trofinetide is rapidly absorbed into the circulation, with a Tmax of approximately 2 to 3 hours. Drug exposure was unaffected by the time of day of administration (morning versus evening dose), indicating the absence of circadian impact on trofinetide PK.

Similar trofinetide exposure was observed in subjects administered trofinetide orally or via G-tube.

Absolute bioavailability, based on the amount of trofinetide excreted renally following oral administration, is estimated to be at least 84%. The Applicant claims that trofinetide is considered a BCS class 1 product, based on high solubility and high absorption.

The proposed commercial oral solution has been used in the phase 3 studies and some of the Phase 1 studies. In the remainder of studies, a lyophilized product was used.

The commercial oral solution is considered suitable for the proposed posology, with 12.5 ml needed for the lowest 2.5g BID (in patients 9 kg to less than 12 kg with moderate renal impairment) and 60 ml for the highest 12g BID dose (in patients 50 kg or more).

The effect of food (high fat meal) was investigated with the final liquid formulation. This study showed a limited effect of food on C_{max} (20% decrease) and no effect on AUC. Based on this relatively small effect of food it is agreed that no dose adjustment is necessary when trofinetide is administered with or without food.

Distribution

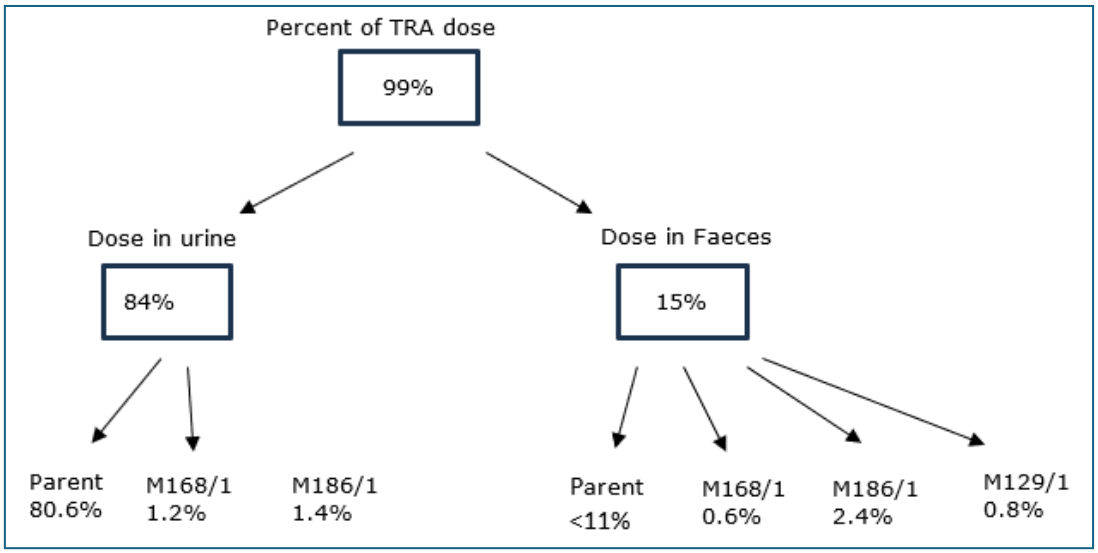
Distribution. Protein binding of trofinetide was low, with binding <6% in human plasma and <10% in HAS, independent of drug concentration. The blood-to-plasma-ratio (R_{bp}) of trofinetide remained constant over 12 hours after an oral dose (ranging from 0.529 to 0.592), indicating that trofinetide is not preferentially distributed into red blood cells.

Trofinetide V_d at steady state was approximately 70 L based on popPK analysis, and approximately 30 L based on non-compartmental analysis. In both cases, this indicates that trofinetide reaches beyond the extracellular fluid with moderate distribution into tissues (intracellularly).

Elimination and metabolism

Trofinetide was primarily (80.6%) excreted unchanged in urine. The recovery of trofinetide after an oral administration of [^{14}C]-trofinetide was complete (99%), with 15% and 84% of the TRA excreted in feces and urine, respectively. A large proportion (approximately 98%) of the administered dose has been identified.

Figure 13: Elimination of trofinetide



Elimination of orally administered trofinetide is characterized by an initial rapid elimination phase ($t_{½,α}$ 1.5 hours) followed by a relatively slow elimination phase ($t_{½,β}$ 30 hours). This is also reported in the proposed SmPC. The Applicant stated that 1.5 h is the effective $t_{1/2}$ and therefore no accumulation takes place.

Metabolism. Based on *in vitro* and *in vivo* studies, it is concluded that trofinetide is not significantly metabolised by CYP450 enzymes, and it is therefore primarily excreted as parent drug. Hepatic metabolism is not a significant route of trofinetide elimination. Based on these results, parent trofinetide is considered the active moiety, without relevant contribution from metabolites. For this reason pharmacokinetics of metabolites as well as pharmacogenetic polymorphisms of metabolising enzymes are considered not important, in light of the very low levels of metabolites formed.

Dose proportionality and time dependencies

Dose proportionality and time-dependence. No marked deviations from dose-proportionality of trofinetide PK were observed in the clinical dose range.

Variability. Interindividual variability for CL was modest, i.e., 13.6%.

PK in target population. No clinically relevant differences in PK were observed between healthy subjects and RTT patients. In the RTT population, the 90% CIs of the GMR fell within the bioequivalence boundaries (0.8 to 1.25). Thus, no dose adjustment is needed in the RTT population beyond applying the recommended body weight-based banded dosing.

Special populations

Impaired renal function. Results of renal impairment Study 010 confirmed previous PBPK simulations and support a 2-fold dose reduction in patients with moderate renal impairment as included in the proposed SmPC section 4.2. Based on the PBPK Study MS-003 and popPK Study MS-008, no dose adjustment is necessary in patients with mild renal impairment. The effect of severe renal impairment has only been investigated using PBPK analysis. Based on the PBPK results and in light of the results in subject with moderate renal impairment, administration of trofinetide to patients with severe renal impairment (eGFR 15 to 29 ml/min/1.73 m2) is not recommended. The advice is sufficiently worded in section 4.2 of the proposed SmPC.

Impaired hepatic function. Considering the limited importance of hepatic clearance for PK of trofinetide, no dose modification is considered necessary based on degree of hepatic impairment. This is supported by the results of PBPK and popPK studies. This information is sufficiently worded in section 4.2 of the proposed SmPC.

Gender. In the drug development plan, PK data was obtained both in male and female subjects, with 250 female subjects and 201 male subjects included. Male subjects were either healthy, non-penetrating traumatic brain injury or Fragile X syndrome patient. No male RTT patients were included in the dataset. Gender (sex) was not a significant covariate in the final popPK model.

Ethnicity. No information on the effect of ethnic factors on trofinetide PK was provided.

Body weight. The effect of body weight on PK parameters was investigated using popPK methods. The median (range) baseline weight in the analysis population was 62.5 kg (13.3 to 140 kg), with the larger weight being FXS and TBI patients. The heaviest RTT patients included was 98 kg.

In the RTT population, the 90% CIs of the trofinetide exposure GMR fell within the bioequivalence boundaries (0.8 to 1.25). Thus, no dose adjustment appears needed in the RTT population beyond applying the recommended body weight-based banded dosing over the studied body weight range.

Elderly.

The effect of age on PK parameters was investigated using popPK methods. The median (range) age in the analysis population was 21 years (5 to 64 years). No elderly subjects >65 years of age were included. Using the weight-based dose bands, the effect of age on trofinetide PK is considered negligible up to an age of 64 years when applying a 0.6-1.4 range for the 90% CI.

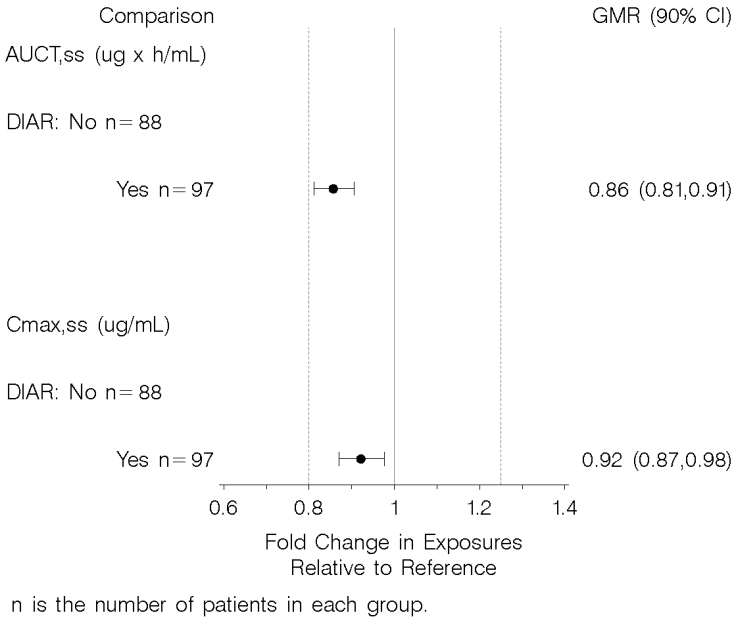
Table 15: Age ranges studied in the elderly population

	Age 65-74 (Older subjects number /total number)	Age 75-84 (Older subjects number /total number)	Age 85+ (Older subjects number /total number)
PK Trials	0	0	0

Paediatric population. Children aged 5 to 12 years were included in the pivotal Study RETT-003 (including patients aged 5 to 20 years). Younger subjects (n=13) with RTT aged 2 to 4 years were included in Study RETT-009. These studies were agreed in the PIP for trofinetide. Based on simulations in popPK Study MS-010, in both paediatric populations, i.e., 2 to 4 years and 5 to 20 years, the proposed weight-based banded dosing regimen achieved the target exposure. The median AUC_{0-12} values were within the target exposure range for all body weight-based bands.

Subjects with diarrhea. Diarrhea was the most commonly reported TEAE and TEAE leading to discontinuation in the RTT population (See Clinical Safety section of this AR). Based on popPK analysis, the occurrence of diarrhea resulted in an approximately 15% decrease in exposure (5). Although this should be confirmed applying the requested therapeutic margin (See LoQ), this decrease in exposure is unlikely to result in clinically relevant effects.

Figure 14: Forest plot of geometric mean ratios (90% confidence intervals) of diarrhea effects on trofinetide $AUC_{0\text{-}12,ss}$ and $C_{max,ss}$ in subjects with Rett Syndrome following the body weight-based banded dosing (popPK Study MS-008)



Source: Report MS-008 Figure 23

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; DIAR=diarrhea; GMR=geometric mean ratio.

Pharmacokinetic interaction studies

In vitro. The potential for trofinetide to be the victim or perpetrator of clinical DDIs was tested in various *in vitro* assays. The *in vitro* assays used and their positive and negative controls are considered adequate. The assays assessed the potential of trofinetide to serve as a substrate, inhibitor, or inducer of CYP enzymes or xenobiotic transporters, as well as UGT enzymes.

Effect other drugs on trofinetide PK. Overall, based on *in vitro* data, co-administered drugs that are CYP, UGT, or drug transporter inducers or inhibitors are not expected to affect trofinetide PK. Given the high anticipated intestinal concentration following oral doses, trofinetide has the potential for inhibitory interactions in the intestine when co-administered with drugs that are significant CYP3A4 substrates and/or are substrates of the P-gp and/or BCRP drug transporters.

Effect trofinetide on PK of other drugs. Trofinetide has a low potential for systemic interaction with CYP enzyme substrates or transporter substrates, with the possible exception of MATE1 and MATE2-K substrates in the kidney. This may in theory decrease the renal excretion of endogenous and exogenous organic cations, such as metformin. Further, *in vitro* CYP3A4 and CYP3A5 are investigated in a combined fashion. Although effects of/on CYP3A4 and 3A5 not always run in parallel, considering the limited effect on the combination of CYP3A4/3A5, this issue will not be further pursued.

The Applicant stated that trofinetide was a direct inhibitor of UGT1A9, UGT2B7, and UGT2B15, with IC_{50} values of 12, 10, and 23 mM, respectively, and the inhibitory action is also proposed to be indicated in the proposed SmPC. However, in light of the high IC_{50}, no relevant inhibition of UGTs is to be expected.

Overall drug-drug interaction potential based on in vitro data.

Trofinetide has a low risk of systemic interaction with CYP enzyme substrates or transporter substrates, with the potential exception of MATE1 and MATE2-K substrates in the kidney, such as metformin.

Further, given the high anticipated intestinal concentration, trofinetide has the potential for inhibitory interactions in the intestine when co-administered with drugs that have low bioavailability and/or are significant CYP3A4 substrates or substrates of the P-gp and/or BCRP drug transporters.

Co-administered drugs that are CYP, UGT, or drug transporter inducers or inhibitors are not expected to affect trofinetide PK.

In vivo. A PBPK model-predicted trofinetide to be a weak inhibitor of CYP3A4, and based on that PBPK study it was estimated that the impact is not likely to be clinically meaningful. In order to confirm this prediction, a DDI study was conducted to assess whether trofinetide would impact the PK of the CYP3A4/P-gp substrate loperamide. Combination of trofinetide with loperamide resulted in an up to 2-fold increased exposure to loperamide. This DDI is likely explained by inhibition of CYP3A4 and/or P-gp in the gastrointestinal tract. Although trofinetide is a relatively weak inhibitor of CYP3A4 and P-gp *in vitro*, the concentration obtained with the high 12 g dose are still potentially sufficient to result in inhibition in the gastrointestinal tract. The DDI is indicated in section 4.5 of the proposed SmPC.

In silico. PBPK modelling has been used to investigate the potential effect of inhibition of CYP3A4 and inhibition of transporters OATP1B1 and OATP1B3. In both cases the same basic PBPK model MS-002 was used, which was able to adequately describe the PK of trofinetide. However, the PBPK model has not been qualified for the effect of inhibition of CYP3A4, nor OATP1B1 and OATP1B3. However, with respect to CYP3A4, an *in vivo* DDI study (Study 012) was conducted, which more or less confirmed the outcome of the PBPK study, although a somewhat more pronounced effect of was found in the *in vivo* study.

With respect to the PBPK study related to OATP1B1 and OATP1B3, the model has not been qualified for describing the effect of inhibition of transporters. The outcome is therefore only supportive for no clinically relevant effect of inhibition of OATP1B1 and OATP1B43 by trofinetide. The outcome of this PBPK study is currently proposed to be mentioned in the SmPC, stating that the model predicted no clinically relevant effect on the AUC of pravastatin.

PK/PD relationships

The E-R for efficacy and safety analyses were based on data in pediatric and adult subjects with RTT collected in Studies Rett-001, RETT-002, and 003. Efficacy and safety adverse events models appear sufficiently validated. Routinely, pcVPC were conducted, illustrating that the final models corresponded well with the observed data. The efficacy and safety models are considered fit for purpose.

The E-R for efficacy included the Phase 3 Study 003 coprimary endpoints RSBQ and CGI-I and two secondary endpoints CSBS-DP-IT Social Composite Score and RTT-COMC. The suitability of the RSBQ, CSBS-DP-IT Social Composite Score and RTT-COMC as clinical endpoints in study 003 were questioned. It remained debatable whether the endpoints are validated, whether these are sensitive to change and what a minimal clinical important difference would be. Nevertheless, E-R relationships were established for RSBQ total scores, CSBS-DP-IT Social Composite Scores, and RTT-COMC scores, whereby higher trofinetide exposures were predictive of significant difference between trofinetide and placebo response

to each efficacy endpoint. No E-R relationship was found for CGI-I scores. At the target exposure associated with the proposed dosing regimen, E-R modeling of efficacy endpoints (RSBQ scores, CSBS-DP-IT Social Composite scores, and RTT-COMC scores) supports that trofinetide shows a response compared to placebo in Study 003. However, it remains unclear if this difference for the presumed efficacy endpoints is indicative of efficacy.

For safety, the most frequently reported TEAEs in the Phase 2 and Phase 3 studies were evaluated. These included diarrhea, vomiting, weight decreased, decreased appetite, seizure, and irritability. The E-R safety analyses of these TEAEs resulted in no E-R relationship for decreased appetite, irritability, or seizures. Significant E-R relationships were found for AEs of diarrhea, vomiting, and weight decreased, whereby higher trofinetide exposure was predictive of a higher probability of these TEAEs.

Dose justification

Support for the dosing regimen investigated in Phase 3

In popPK Study MS-007, only body weight was found to have a significant effect on systemic clearance (CL) and Vd, and consequently on the overall systemic exposure to trofinetide. This finding eventually led to a weight-based dosing regimen.

Based on exploratory analyses revealing that improvement in key efficacy endpoints including RSBQ and CGI-I were correlated with higher trofinetide exposure, the initial target range was developed based on the 10% highest exposures obtained by patients receiving 200 mg/kg BID trofinetide in Study RETT-002. In light of the limited safety concerns, higher doses than applied in Study RETT-002 were simulated in popPK Study MS-007 (up to 500 mg/kg BID). Overall, the MS-007 PK model-based simulations performed using the virtual paediatric population suggested that the weight-based dosing regimens are likely to result in exposure ranges that fall within the identified target exposure range of Day 14 AUC_{0-12} = 790 to 967 µg.h/ml. This led to the proposed weight banded dosing regimen that was further investigated in Phase 3. The E-R model-based simulations in popPK Study MS-007 in the virtual paediatric population suggest that the target exposure range is appropriate from an efficacy standpoint and likely to result in improvement in Rett syndrome symptoms measured by CGI-I and RSBQ scores.

The RSBQ model indicates that subjects treated with trofinetide, who obtain an AUC from 0 to 12 hours at steady state ($AUC_{0-12,ss}$) of 800 µg.h/ml, would be expected to experience a 5.1 times greater improvement from baseline in their RSBQ score after 42 days of treatment compared to placebo.

Results related to dosing in Phase 3

In popPK Study MS-008, including data from the pivotal Phase 3 Study 003, the previously identified target exposure range of $AUC_{0-12,ss}$=790 to 967 µg.h/ml, encompassing a median of 878.5 µg.h/ml, was rounded to a median $AUC_{0-12,ss}$=1000 µg.h/ml with a range expanding to ±20% around the median (800 to 1200 µg.h/ml). It is not clear what was the reason for this modified target range. Applying this new 800 to 1200 µg.h/ml target range, the median peak $AUC_{0-12,ss}$ values from Study 003 were contained within the target exposure range for all body weight ranges, and the distribution of $AUC_{0-12,ss}$ values overlapped with the target exposure range.

Likewise, after inclusion of data from the paediatric Study 009 (popPK Study MS-010), the distribution of $AUC_{0-12,ss}$ values was mostly contained within the target exposure range of 800-1200 µg.h/ml, with only subjects receiving 5 g BID (weighing 9 to <12 kg) falling slightly above the target exposure range.

2.6.2.2. Pharmacodynamics

Mechanism of action

The mechanism of action of trofinetide in RTT is unknown.

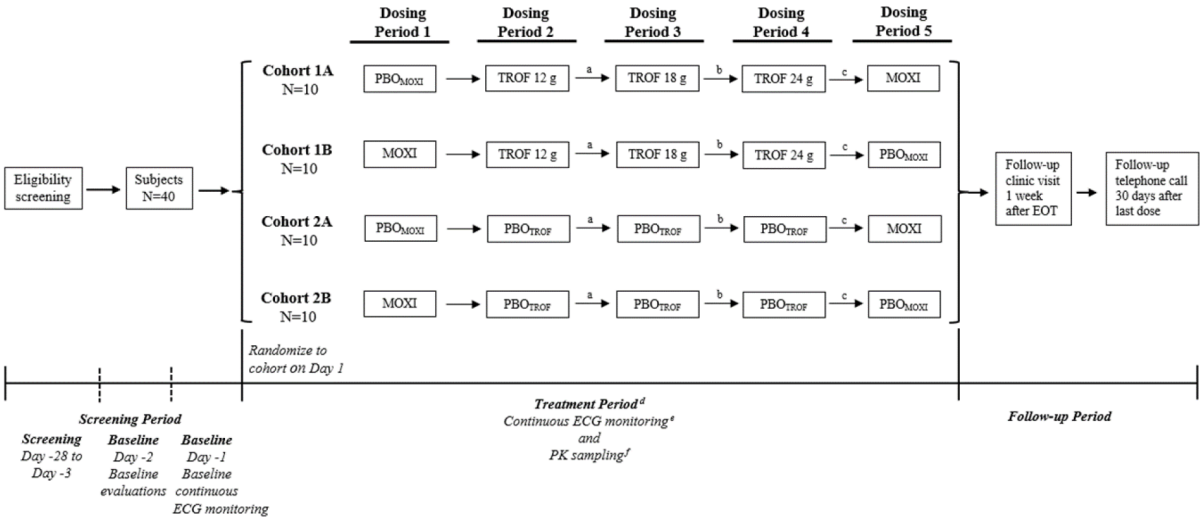
Primary and Secondary pharmacology

According to the Applicant, given the absence of identified mechanism of action, no dedicated PD study was conducted in humans and no PD-specific endpoints were included in the clinical studies.

Thorough QT Study

Study 008 was a Phase 1, randomized, DB, placebo-controlled, ascending dose, nested crossover study to evaluate the relationship between trofinetide and change in QT interval. Healthy subjects (n=40) were randomized into four cohorts, to receive a single dose of 12g (therapeutic dose), 18g, and 24 g (supratherapeutic dose) of trofinetide or matching placebo, as well as a single dose of 400 mg moxifloxacin and matching placebo. Moxifloxacin was included as a positive control. All subjects received in total five doses of study medication with ≥4 days between each administration. The detailed study design is shown in 6:

Figure 15: Schematic of Thorough QT Study Design



Abbreviations: ECG=electrocardiogram; EOT=end of treatment; MOXI=moxifloxacin; N=number of subjects; PBO_{MOXI}=placebo-matching moxifloxacin; PBO_{TROF}=placebo-matching trofinetide; PK=pharmacokinetics; TROF=trofinetide.

a A decision about Dosing Period 3 (whether to proceed and dose) will be made on the basis of blinded review by the Sponsor and Investigator of all available safety and tolerability data from Dosing Period 2.

b A decision about Dosing Period 4 (whether to proceed and dose) will be made on the basis of blinded review by the Sponsor and Investigator of all available safety, tolerability, and interim PK data from Dosing Periods 2 and 3.

c Dosing Period 5 will commence regardless of decisions made about Dosing Periods 3 and 4.

d A minimum of 4 days washout is required between all administrations of study drug.

e Continuous ECG monitoring will be conducted from predose to 24 hours postdose in Dosing Periods 2, 3, and 4; it will be conducted from predose to 6 hours postdose in Dosing Periods 1 and 5.

f PK blood sampling will be conducted predose and for 48 hours postdose in Dosing Periods 2, 3, and 4; PK sampling will not be conducted in Dosing Periods 1 and 5.

The relationship between ΔQTcF and trofinetide blood concentrations was investigated by linear mixed-effects modelling. The placebo adjusted ΔQTcF value was calculated at each dose level as the placebo-corrected ΔQTcF estimated from the model and its 1-sided 95% CI was then constructed. If the upper bounds of the one sided 95% CIs were less than 10 ms at all three doses, then it was concluded that trofinetide does not have a CS effect on QT prolongation, as per the ICH E14 guidance.

Overall, 15 (38%) subjects were White and 25 (63%) subjects were Black or African American, and the majority of subjects were not Hispanic or Latino (33 [83%]). The mean (SE) age at Screening was 34 (±0.9) years and ranged from 20 to 44 years. A total of 60% of subjects enrolled in the study were male (n=24). The mean (SE) weight at screening was 80 (±1.8) kg.

Following a single 12, 18, and 24 g dose of trofinetide, the PK profiles were qualitatively similar across dose groups. Trofinetide was rapidly absorbed into the circulation (median t_{max} approximately 2.5 to 3 hours), with an initial rapid decline followed by a relatively slow elimination phase ($t_{1/2}$ approximately

11 to 12 hours). Increase in C_{max} and AUC was less than dose proportional with an increase in dose. C_{max} following 12, 18, and 24 g trofinetide was 146, 183, and 206 µg/ml, and $AUC_{0-\infty}$ was 848, 1110, and 1342 µg.h/ml, respectively.

Table 16: Summary of concentration ΔQTcF Analyses – PK/PD Analysis Set

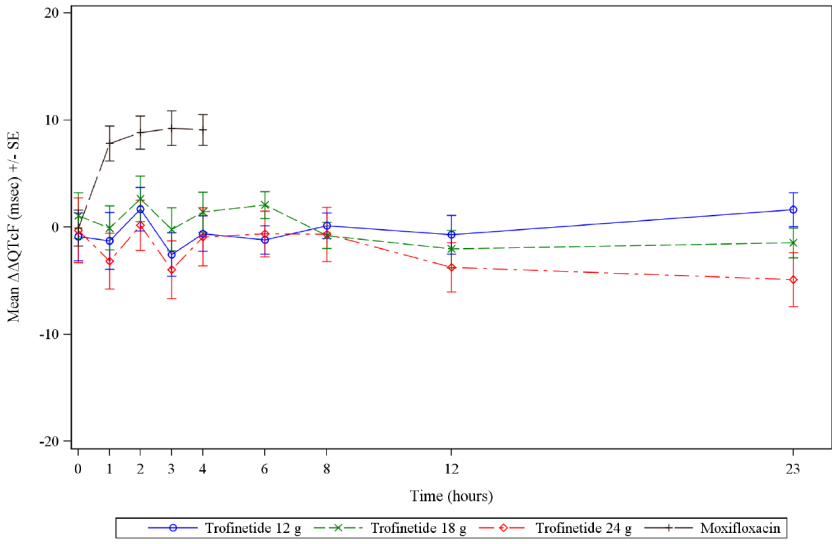
Model Parameter Predictions	N	Estimate (SE)	90% Confidence Interval
ΔQTcF-blood trofinetide concentration (slope)	40	0.0121	0.0003, 0.0239
ΔΔQTcF Trofinetide 12 g at Mean C_{max} (146 g/mL)	20	1.96 (2.109)	-1.555, 5.473
ΔΔQTcF Trofinetide 18 g at Mean C_{max} (183 g/mL)	20	1.87 (2.150)	-1.706, 5.455
ΔΔQTcF Trofinetide 24 g at Mean C_{max} (206 µg/mL)	18	-0.31 (2.213)	-3.989, 3.376
ΔΔQTcF Trofinetide 12 g at Maximum BC (179 g/mL)	20	2.36 (2.195)	-1.295, 6.010
ΔΔQTcF Trofinetide 18 g at Maximum BC (212 µg/mL)	20	2.22 (2.237)	-1.496, 5.945
ΔΔQTcF Trofinetide 24 g at Maximum BC (268 µg/mL)	18	0.44 (2.424)	-3.582, 4.466

Source: Table 14.2.2.1

Abbreviations: ΔQTcF=change from the time-matched baseline in QTcF; ΔΔQTcF=placebo-adjusted change from time-matched baseline in QTcF; BC=blood concentration; PD=pharmacodynamic; PK=pharmacokinetic; QTcF=QT interval using Fridericia's correction method; SE=standard error; UN=unstructured.

Estimates were obtained using a linear mixed effects model as ΔECG=Timepoint + Treatment + Concentration + Predose ECG with subject as a random effect and Subject*Period as a repeated effect using UN covariance structure.

Figure 16: ΔΔQTcF Interval vs. Time (Mean ±SE) (ECG Analysis Set)



Source: Figure 14.2.1.1.2.2

Abbreviations: ΔΔQTcF, placebo-adjusted change from time-matched baseline in QTcF; ECG, electrocardiogram; QTcF, QT interval using Fridericia's correction method; SE, standard error

The time-matched analysis for the QTcF interval change for 12, 18 and 24g doses showed that at all time points the point estimates were less than 2.4 ms and the upper bound of the 2-sided 90% CI did not exceed 10.0 ms (maximum of 6 ms for the 12 g dose). Therefore, there is no signal of cardiac repolarization for trofinetide at a single dose up to 24 g. For the moxifloxacin group, results of the time-matched analysis met the prespecified assay sensitivity criteria, whereby the Hochberg-adjusted 90% lower CI was >5 ms in at least 1 of 4 prespecified timepoints, and assay sensitivity was also confirmed.

2.6.3. Discussion on clinical pharmacology

2.6.3.1. Pharmacokinetics

Bioanalytical methods

Bioanalytical assays were developed and validated at three different sites. No formal cross-validation was conducted among the bioanalytical assays. However, the observed consistency in concentration levels and patterns observed in the popPK models suggests comparable analytical performance across bioanalytical laboratories. Moreover, the absence of systematic bias in the GOF plots stratified by assay site supports the conclusion that no significant assay-related discrepancies were present. Long-term stability results covering storage conditions for samples collected and analyzed in the clinical studies ACP-2566-006, ACP-2566-007, Neu-2566-RETT-001, ACP-2566-010, Neu-2566-RETT-002 were provided. Two assays were used for whole blood (SAP.1480, ATM-2546) and two for urine (SAP.2135, ATM-2554). The validated long-term stability periods (e.g., 1752 days at −80 °C; 267 days at −70 °C; 1030 days at −80 °C; 156 days at −20/−70 °C) cover the storage times shown for studies ACP-2566-006/-007/-010, Neu-2566-RETT-001/-002. Based on the data provided, the validated stability durations equal or exceed the maximum storage durations for the corresponding studies.

Evaluation and qualification of popPK models.

In general, the popPK models from Oosterholt *et al.* (2017) MS-007, MS-008 and MS-010 were validated sufficiently, and provide a reasonable description of the PK data from the actual clinical studies. The GOF plots and pcVPCs indicated that the model reasonably predicted both the central tendency of the concentration data over time, as well as the extent of variability in the observed data. However, higher exposures are underpredicted by all models.

The misfit at higher concentrations may in theory be caused by a miscoding of the exponential residual error or the absence of a 3rd compartment in the model.

In the second round the Applicant has re-evaluated the applied error model used in the popPK model. Overall, it was confirmed that indeed a proportional error model was applied instead of the initially assumed exponential error model.

Subsequently, the CHMP recommendations on model improvement were followed, although to a somewhat unsatisfying extent. Overall, no clear incentive to improve the exposure predictions at higher exposures (which was the overall reason for the questions raised and recommendations given) was noted in the efforts done by the Applicant. Instead, the above recommendations were followed in a somewhat restricted manner, sometimes introducing additional variations. First, the use of an exponential error model was tested and compared with the initial popPK model, although not in a fully satisfying manner, since the error model was stratified by population for the initial model and not stratified in the log-transformed both sides approach. Therefore, the comparison of the original, by mistake, proportional error model and the new model applying the exponential model was not optimal. Although it is agreed that from the limited GOF plots of the exponential error popPK model did not show improvement of fit as compared to the proportional error popPK model, it is uncertain if the stratification by population in the structure of the residual error in the two models affected this outcome. Again, at no occasion the Applicant expressed an interest in improving the fit of the data, instead focusing on demonstrating that the newly proposed models were no better than the original one (with suboptimal fit of the high exposure data).

Also the request to test the addition of a 3rd compartment to the model was given limited follow-up. Remarkably, the fit of the 3-compartmental model was indicated to be worse than the 2 compartmental model. This is an unexpected occurrence, since such additional compartment is by default expected to improve the fit (estimates close to 0 for model parameters associated with the 3rd compartment parameters should return to the same fit as the 2 compartment model) and therefore a local minimum in the objective function is likely reached. Output files were not provided, so the reported worse fit of the 3 compartmental model cannot be checked. Although not fully understood, based on the data provided, the fit of the 3 compartment model that was cited by the Applicant was worse than the original 2 compartment model, and no further incentive was shown to improve the model to obtain a better fit.

An additional comment on the initial model was made on the use of covariates related to body size. Covariates related to body weight are expected on all model parameters, and the lack of these covariates on some model parameters is questionable. The Applicant was requested to re-evaluate the covariate selection based on the optimized model, where all model parameters are scaled with a measure of body size (ideally bodyweight) and in line with allometric principles (ideally fixed exponents of 0.75 for clearance parameters and 1.0 for volume of distribution parameter when bodyweight is evaluated).

In the second round, the request to include allometric scaling in the model, to better align with theoretical principles and enhance extrapolation abilities of the model, was given suboptimal follow-up. Based on the output, it appears that not only allometric scaling was applied in the updated model, however at the same time also the effect of age on Vc and effect of RETT on CL was estimated or fixed (not clear from the provided output) to zero. No explanation for this was given by the Applicant. Although the consequences of these fixed parameters on the outcome of the model cannot be predicted, it is clear that next to the incorporation of allometric scaling, additional changes were implemented in the model. Overall, based on the provided output data, it cannot be concluded with certainty if inclusion of allometric scaling will improve the fit of the model.

Overall, the CHMP recommendations provided in order to improve the fit of the popPK model were given limited follow-up by the Applicant. The Applicant reconciles itself with the suboptimal fit of the high exposure values in the final popPK model. Although not optimal, and although it is expected that there is room for improvement of the popPK model, this was not further pursued. Reason for this is that both the 800-1200 µg.h/ml target range as well as the individual $AUC_{0\text{-}12}$ per patient were estimated based on the same, potentially suboptimal, popPK model. Therefore, the deviation in predicted exposures is considered to be a systematic error, affecting both target range as well as the individual AUC values to the same extent. In case an improved popPK model would be used, it is therefore expected that both the target range as well as the individual exposures will change in the same way, and the conclusion of fit or non-fit to the target range will not change.

The final model was used to check if patients 2-20 years achieved exposure within the predefined target exposure of 800-1200 µg.h/ml. In the updated analysis, it was observed that all medians by weight band fall within 800–1200 under the updated model, while the allometric model drifts at extremes. At a later round of assessment, simulations of steady-state $AUC_{0\text{-}12}$ values per age group were provided, with a summary of the number and percentage of subjects in each weight group and age group, respectively, with $AUC_{0\text{-}12,ss}$ values that fell below, within, and above the target exposure range of 800 – 1200 µg·h/mL. In all age subgroups (2-4, >4-12, >12-17, and ≥18 years of age), the majority (56.7-77.0%) of patients reached exposure between the 800-1200 µg.h/ml target range.

Absorption

Similar trofinetide exposure was observed in subjects administered trofinetide orally or via G-tube. Explicit information on the administration via a gastric feeding tube has been given in the proposed SmPC section 6.6. In section 4.2 of the SmPC, reference is made to this information.

Absolute bioavailability, based on the amount of trofinetide excreted renally following oral administration, is estimated to be at least 84%. Solubility of trofinetide is high (>500 mg/ml) at various pH within the range of pH 1-6.8. Findings are in line with solubility of the highest dose of 12 g in 25 ml as indicated in the posology for this product. According to the ICH M9 guideline, high permeability can be concluded when the absolute bioavailability is ≥85%, which has not been demonstrated in this case. Therefore, it is concluded at this stage that trofinetide is a BCS class 3 drug

The proposed commercial oral solution containing mannitol has been used in the phase 3 studies and some of the Phase 1 studies. In the remainder of studies, a lyophilized product without mannitol was used. In the revised popPK simulations with formulation applied as covariate, a statistically significant difference in relative bioavailability was observed between the formulations without and with mannitol (final formulation), resulting in a geometric mean ratio (GMR) and 90% confidence interval for $AUC_{ss,12}$ and $C_{max,ss}$ of 0.76 (0.73-0.79) and 0.65 (0.63-0.68), respectively. Exposure with the final formulation yielded higher exposures. Based on popPK simulations, subjects who received protocol specified dosing with the final formulation achieved $AUC_{0-12,ss}$ values within the 800-1200 µg.h/ml target exposure range, while subjects who received the old formulation had $AUC_{0-12,ss}$ values modestly below the target exposure range. It is agreed that key clinical studies ACP-2566-003, ACP-2566-004, and ACP-2566-009 utilized the final mannitol-containing formulation and as such pivotal efficacy and safety instrumental in establishing and confirming the recommended therapeutic doses.

2.6.3.2. Distribution

Trofinetide V_d at steady state was approximately 60 L based on popPK analysis. This V_d indicates that trofinetide reaches beyond the extracellular fluid with moderate distribution into tissues.

Elimination and metabolism

Elimination of orally administered trofinetide is characterized by an initial rapid elimination phase ($t_{½,α}$ 1.5 hours) followed by a relatively slow elimination phase ($t_{½,β}$ 30 hours). Plasma concentrations decline to negligible levels by 12 hours post-dose, falling to approximately 10% of C_{max}. This suggests that concentrations beyond 12 hours, corresponding to the beta phase, contribute minimally to the drug's therapeutic effect. Furthermore, the AUC of the alpha phase is substantially greater than that of the beta phase, confirming that the alpha phase is the primary contributor to overall exposure. Accordingly, the half-life of 1.5 h associated with the alpha phase represents the effective half-life and is indicated in the proposed SmPC.

2.6.3.3. Dose proportionality and time dependencies

Interconversion. Considering the presence of chiral centres in the trofinetide molecule, the possibility of inter conversion *in vivo* was discussed). No indication of interconversion was detected in the achiral bioanalysis. It is agreed that interconversion of one of the two chiral centers would result in a diastereoisomer, which would be expected to be detectable in the bioanalysis. Therefore, it is considered that no interconversion occurs after administration of trofinetide.

The post-hoc analysis indicated by the Applicant to yield accumulation of 6-10% over 5 days initially was not found in the study report nor dossier. Details on this post-hoc analysis were provided, and indicated that due to interday variation in apparent exposure, accumulation ratio's appeared to differ as well. Although reasons for this variation in exposure were not fully explained, bearing in mind the agreed effective half-life of 1.5 h and the results from the popPK analyses, it is agreed that accumulation can be considered low. The text in section 5.2 of the proposed SmPC indicating minimal to no accumulation was observed following multiple-dose administration is therefore considered acceptable.

Variability. Interindividual variability for CL was modest, i.e., 13.6%. No information is given on intra-individual variability. In light of the modest inter-individual variability, this topic will not be pursued.

Therapeutic window. Based on exposure–response analysis, in which trofinetide exposure and the primary efficacy endpoint, the Rett Syndrome Behaviour Questionnaire (RSBQ) total score, was used, an expanded therapeutic range, spanning $AUC_{0\text{-}12}$ values from approximately 650 to 1500 µg·h/mL, was concluded upon. This therapeutic window based on efficacy is acknowledged. No robust safety-based higher cut-off was proposed. Therefore, assuming safety at higher exposures than 1200 µg.h/ml is not limiting, the therapeutic window based on efficacy is assumed to be 650-1500 µg.h/ml. In a more conservative setting, assuming safety at higher exposures may be dose-limiting, a therapeutic window of 650-1200 µ.h/ml can be considered.

Special populations

Gender. In the drug development plan, PK data was obtained both in male and female subjects, with 250 female subjects and 201 male subjects included. Male subjects were either healthy, non-penetrating traumatic brain injury or Fragile X syndrome patient. No male RTT patients were included in the dataset. Gender (sex) was not a significant covariate in the final popPK model. Information on the trofinetide PK in male and female subjects has been adequately included in proposed SmPC section 5.2. Of note, PD in male and female RETT patients may be different, so comparable PK in male and female subjects cannot be considered evidence of comparable activity in male and female patients.

Ethnicity. Although the effect of ethnicity on Vc in the final population PK model was found to be statistically significant, no clinically meaningful differences in model-predicted $AUC_{0\ 12,ss}$ or $C_{max,ss}$ were observed between Hispanic/Latino and Non-Hispanic/Latino subjects. The geometric mean ratios (GMRs) and corresponding 90% confidence intervals (CIs) for for $AUC_{0\text{-}12,ss}$ and $C_{max,ss}$, were well within the predefined bioequivalence boundaries of 0.8 to 1.25. The information is adequately reported in the proposed SmPC section 5.2.

Body weight. In the provided sub-analysis comparing trofinetide exposure in patients weighing over 100 kg to those in the baseline 20–35 kg range revealed that the median $AUC_{0\text{-}12,ss}$ value for the >100 kg group was at the lower end of the target $AUC_{0\text{-}12,ss}$ exposure range (800-1200 µg·h/mL), with a large proportion under the lower exposure target. In the second round the Applicant presented simulations assuming all subjects are RTT and following protocol dosing. In the updated analysis, it was observed that median AUC in >100 kg is close to the lower bound of 800 µg·h/mL, while 20–35 kg is well within range. At a later stage, simulations of steady-state $AUC_{0\text{-}12h}$ values were presented per weight group, with a summary of the number and percentage of subjects in each weight group and age group, respectively, with AUC0-12,ss values that fell below, within, and above the target exposure range of 800 – 1200 µg·h/mL. It was shown that in patients weighing >9 - <100 kg, most patients obtained exposure within the 800-1200 µ.h/ml target range (54.2 – 85.4%). Information in the low-weight patients (>9-12 kg) is not interpretable, considering the small number of patients in that subgroup (n=3). With respect to patients >100 kg, a markedly smaller number of patients reached

exposure within the target range (28.6%), whereas 71.3% obtained a low exposure <800 ng/ml, so below the lower cut-off of the target range. Information on the lower exposure in patients >100 kg is reported in SmPC section 5.2.

Elderly. Using the weight-based dose bands, the effect of age on trofinetide PK is considered negligible up to an age of 64 years when applying a 80-125% range for the 90% CI. The lack of data in patients over 65 years of age has been indicated in proposed SmPC section 4.2 and 5.2, with an advice to monitor renal function in such patients. This is considered acceptable.

Paediatric population. Based on simulations in popPK Study MS-010, in both paediatric populations, i.e., 2 to 4 years and 5 to 20 years, the proposed weight-based banded dosing regimen achieved the target exposure. The median AUC_{0-12} values were within the target exposure range for all body weight-based bands. The Applicant provided age-stratified simulations (2–12, >12–17, ≥18) with both the original and updated models. In the updated analysis, it was observed that with the updated model the median AUC in all age groups is within 800–1200. The allometric model under-predicts in older groups. This addresses the specific request for children and shows no residual age bias with the final model. At a later round of assessment, simulations of steady-state AUC_{0-12} values per age group were provided, with a summary of the number and percentage of subjects in each weight group and age group, respectively, with AUC0-12,ss values that fell below, within, and above the target exposure range of 800 – 1200 µg·h/mL. In all age subgroups (2-4, >4-12, >12-17, and ≥18 years of age), the majority (56.7-77.0%) of patients reached exposure between the 800-1200 µg.h/ml target range.

Pharmacokinetic interaction studies

In vitro. Taking into account the *in vitro* data, the Applicant noted that trofinetide can inhibit MATE2-K *in vitro* (IC50 ≈ 0.951 mM) at therapeutic C_{max} and may reduce renal clearance of MATE2-K substrates. Typical substrates include metformin, acyclovir, ganciclovir, oxaliplatin, and fexofenadine. In Phase 3, fexofenadine was used in 1.7% of subjects, and its SmPC reports that 2–3-fold exposure increases did not raise safety concerns. The proposed SmPC (Section 5.2) now states that inhibition of MATE1/MATE2-K cannot be excluded and may increase substrate exposure (e.g., metformin). Since an interaction cannot be excluded, this information on MATE1 and MATE2-K was moved from section 5.2 to section 4.5 of the proposed SmPC.

In vivo. A PBPK model predicted trofinetide to be a weak inhibitor of CYP3A4, and based on that PBPK study it was estimated that the impact is not likely to be clinically meaningful. In order to confirm this prediction, a DDI study was conducted to assess whether trofinetide would impact the PK of the CYP3A4/P-gp substrate loperamide. Combination of trofinetide with loperamide resulted in an up to 2-fold increased exposure to loperamide. This DDI is likely explained by inhibition of CYP3A4 and/or P-gp in the gastrointestinal tract. Although trofinetide is a relatively weak inhibitor of CYP3A4 and P-gp *in vitro*, the concentration obtained with the high 12 g dose are still potentially sufficient to result in inhibition in the gastrointestinal tract. The DDI is indicated in section 4.5 of the proposed SmPC, in which it is indicated that the effect may be the result of inhibition of CYP3A4 and/or P-gp. Given the magnitude of interaction (1.8×) and modest increase in metabolite exposure, the DDI is not considered clinically significant for most P-gp substrates, but caution may be needed with narrow therapeutic index drugs transported by P-gp (e.g., cyclosporin, digoxin). Since concomitant use of an NTI drug cannot be excluded, as a general precautionary measure, the sentence '*Closely monitor when DAYBU is used in combination with orally administered CYP34A and/or P-gp sensitive substrates (e.g., cyclosporine, digoxin) for which a small change in substrate plasma concentration may lead to serious toxicities*', included in section 4.5 of the proposed SmPC, was included in section 4.4 of the proposed SmPC.

In silico. PBPK modelling has been used to investigate the potential effect of inhibition of CYP3A4 and inhibition of transporters OATP1B1 and OATP1B3. In both cases the same basic PBPK model MS-002 was used, which was able to adequately describe the PK of trofinetide. However, the PBPK model has not been qualified for the effect of inhibition of CYP3A4. However, with respect to CYP3A4, an *in vivo* DDI study (Study 012) was conducted, which more or less confirmed the outcome of the PBPK study, although a somewhat more pronounced effect of was found in the *in vivo* study. The information in the proposed SmPC section 4.5 based on the unqualified PBPK model was removed.

With respect to the PBPK study related to OATP1B1 and OATP1B3, the model has not been qualified for describing the effect of inhibition of transporters. The outcome is therefore only supportive for no clinically relevant effect of inhibition of OATP1B1 and OATP1B43 by trofinetide. The information in the proposed SmPC section 4.5 based on the unqualified PBPK model was removed.

Dose justification

Overall, the proposed dosing weight-banded regimen investigated in Phase 3 is supported by clinical pharmacological and popPK/PD simulations. Simulations indicate an expected improvement of RSBQ total scores and CGI-I scores at with the trofinetide exposures expected. The final target range for trofinetide is 800 to 1200 µg.h/ml however, it is not fully clear why this target range was changed from 790 to 967 µg.h/ml to 800 to 1200 µg.h/ml. In the response, the Applicant presented a model-based rationale. The higher AUC improves RSBQ with only a small increase in diarrhoea/vomiting risk. Therefore, the upper bound was extended to 1200 µg·h/mL and the range rounded for practicality. In the updated analysis, it was observed that this moves the range from a three-subject basis to an efficacy–tolerability basis.

The explanation is acceptable. However, to fully picture the B-R of the proposed exposure range the Applicant was asked to provide (a) a benefit–risk plot vs $AUC_{0-12,ss}$ showing the probability of achieving minimal clinically important difference on RSBQ and the probabilities of key AEs across the AUC range (including target exposure of 800–1200 µg·h/mL), with 95% CIs; and (b) a summary (updated model, final weight-band dosing) reporting, for each weight (including the heaviest patients >100kg) and age, the percent of subjects within 800–1200, percent <800, percent >1200, together with N, median AUC, and 5th/95th percentiles. Further, a pcVPC for the updated model, stratified by body-weight deciles, with explicit >100 kg panels was provided. It was concluded that the popPK model predicts the general tendency of exposure with reasonable precision at all weight categories.

Further, the QT study did not demonstrate a QT prolongation at doses up to 24 g. Considering that the achieved exposure did not reach the standard 2-fold margin, while the safety margins are considered suboptimal, in light of the lack of clinical evidence for an increased risk of QT prolongation this was not further pursued (see below).

Based on the provided exposure-efficacy (RSBQ total scores, CGI-I scores, CSBS-DP-IT Social Composite Scores and RTT-COMC scores), and exposure-safety (diarrhea, vomiting, weight decreased, seizures, irritability, and decreased appetite) evaluations, within the target AUC_{0-12} exposure range of 800 to 1200 µg.h/ml, the Applicant is of the opinion that trofinetide shows efficacy with an acceptable safety profile. Although safety may be acceptable, several issues regarding the efficacy of trofinetide remained.

Exposure response

Taking into consideration the E-R results for the efficacy parameters it is unclear what explains the lack of E–R relationship for CGI-I scores, despite its role as a co-primary endpoint. It was clarified that this

is attributed to the way the data was analysed, i.e. a dichotonmous outcome which can reduce the sensitivity of the analysis. It remains unclear why the CGI-I data were simplified from an ordinal to binary format, as this approach inevitable leads to loss of information and may limit the ability to detect E-R trends. Further, in the E-R analyses AUC_{0_12} and C_{max} were explored. The two parameters were highly correlated, and the differences in objective function values across models were minimal. The $AUC_{0\text{-}12,ss}$ was selected as a representative of exposure parameters for consistency and practicality, as it also was the base for the proposed dosing regimen. This is considered acceptable.

Pharmacodynamics

Mechanism of action

The mechanism of action in Rett syndrome is not clearly discussed or demonstrated. The Applicant was requested to elaborate more in detail on the (potential) mechanism of action and involved signaling pathways of trofinetide in Rett syndrome, to provide also a discussion in the clinical pharmacodynamics section and to update the discussion on non-clinical primary pharmacodynamics. Considering the role of synaptic function in brain development and the proposed indication from 2 years of age, such data was relevant for a discussion if there is a potential window of opportunity in which trofinetide should be administered to be effective. The only additional information provided by the applicant was on evidence from Mecp2 deficient mice suggesting that the window for GPE to impact synaptic plasticity extends from juvenile to young adult mice. Deficits in synaptic maturation or stabilization persisted into adulthood following MeCP2 deficiency, which was prevented upon GPE treatment. Additional discussions by the Applicant were not expected to provide more relevant information and the issue was not further pursued.

Primary and secondary pharmacology

No pharmacodynamic studies were performed by the Applicant.

The design and conduct of the TQT study were generally in line with the ICH E14 guidance. The study had a cross-over design, was placebo-controlled and included a positive control (moxifloxacin) to establish assay sensitivity. Data on drug concentrations around the time of ECG assessment were also evaluated.

Although selected doses (12, 18, 24g) of trofinetide appear to reach concentrations that are higher than those achieved following the recommended dose, the appropriateness of selected single doses cannot be confirmed at this time. Compared to the pivotal study (003) and the study in subjects aged 2-4 years (009), trofinetide blood concentrations were overall higher in the QT study (008) for doses of 18g and 24g. The mean Cmax in studies 003 and 009 was 156 µg/ml and the mean AUC0-12 was 939 µg.h/ml. In the QT study the Cmax was 183 µg/ml and AUC0-∞ 1097 µg.h/ml for the 18g dose, and 206 µg/ml and 1330 µg.h/ml for the 24g dose. For the pediatric population weighing 9-12kg (study 009) there is limited difference between blood concentrations reached with the therapeutic dose (5g BID) compared to the highest dose of 24g in the QT study. In these subjects the Cmax (203 µg/ml) was comparable to the Cmax with 24g, and the AUC (1108 µ.h/ml) was ~17% below the AUC for 24g in the QT study.

The mean ΔQT intervals corrected with Bazett's formula (QTcB) or individual subject corrected (QTcI) were not analysed, despite these being planned secondary endpoints and this has not been further pursued.

While the results of the QT study are overall negative, the dosing in the performed QT study was not optimal which hampers adequate evaluation of the results in this dedicated study. The achieved exposure did not reach the standard 2-fold margin. Higher doses were not evaluated due to safety

constraints (risk of GI AEs), however, a single higher dose in a population of healthy individuals is not expected to cause serious/severe safety issues. Non-clinical data indicate that trofinetide may cause cardiovascular effects, and an effect on QT prolongation cannot be excluded. Yet, based on the available data in the RTT population, both in the clinical program as well as post-marketing in the US, no risk of QT prolongation was identified. Further, RTT patients are routinely monitored by ECG, due to the risk of QT prolongation in this population. Therefore, it is considered that, despite non-clinical uncertainties and suboptimal QT Study design, the risk of serious consequences due to cardiac repolarization is minimal.

Studies in patients with RTT (003 and 009) included the collection of periodic ECGs.

The treatment of seizures in Rett syndrome is often done with antiseizure drugs. The clinical programme did not study pharmacodynamic interactions between trofinetide and ASDs. No specific PD assessment is provided, for example, on potential additive CNS effects (with benzodiazepines or cannabidiol), overlap of adverse events such as somnolence, decreased appetite, vomiting, or weight change, or any impact on seizure control. Post-hoc analyses do not identify a clinically meaningful signal for worsened seizure control with concomitant antiseizure medicines. However, the Applicant's approach is indirect (AE-based only) and post-hoc and therefore should be interpreted with caution. Nevertheless, at present there is no signal of a clinically relevant PD interaction between trofinetide and antiseizure medications.

2.6.4. Conclusions on clinical pharmacology

Overall, the PK of trofinetide has been investigated and described to a fair extend. Although the popPK model is considered suboptimal, this was not further pursued, since both the target range and individual $AUC_{0\text{-}12,ss}$ were simulated with the same model and conclusions are not expected to change if an optimised model is used.

2.6.5. Clinical efficacy

Table 17: Overview of clinical studies

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
003 (Phase 3)	Start: October 2019 Completed: October 2021 Enrolment Planned: 184 Randomized/Completed: 187/155 93 trofinetide / 94 placebo	R DB PC	Trofinetide 12-20 kg: 30 mL (6 g) >20-35 kg: 40 mL (8 g) >35-50 kg: 50 mL (10 g) >50 kg: 60 mL (12 g) Placebo Orally or via G-tube BID Duration: 12 weeks	Female aged 5-20 years Meets clinical criteria for RTT Documented disease-causing mutation in the MECP2 gene Post-regression* at Screening RTT-CSS score 10-36 CGI-S≥4
004	Start: January 2020	OL	Trofinetide	Completed 003

(Phase 3)	Completed: August 2022 <u>Enrolment</u> Entered/completed: 154 / 84		12-20 kg: 30 mL (6 g) >20-35 kg: 40 mL (8 g) >35-50 kg: 50 mL (10 g) >50 kg: 60 mL (12 g) Oral or G-tube BID Duration: 40 weeks	or Within the past 12 months, Completed 003, but did not directly rollover to 004 due to COVID-19 PHE or Within the past 12 months, discontinued from Study 003 due to COVID-19 PHE
005 (Phase 3)	Start: November 2020 Completed: June 2023 <u>Enrolment</u> Entered/completed: 77/0*	OL	32 months	Completed 004 May benefit from continued treatment with OL trofinetide Can still swallow study medication or take it by G-tube
RETT-002 (Phase 2)	Start: March 2016 Completed: January 2017 <u>Enrolment</u> Planned: 76 Randomized/completed: 82/81	R DB PC	<u>Trofinetide</u> 50 mg/kg 100 mg/kg 200 mg/kg <u>Placebo</u> Orally or via G-tube BID Duration: 42 days	Females aged 5-15 years Meets clinical criteria for RTT Proven mutation of the MECP2 gene Post-regression Hagberg Stage 3 or 4 RTT-CSS score 10-36 CGI-S≥4
009 (Phase 2/3)	Start: September 2021 Completed: May 2023 <u>Enrolment</u> Planned: 10-15 Randomized/completed: 15/0*	OL	<u>Trofinetide</u> Week 1: 10 mL (2 g) Week 2: 20 mL (4 g) Week 4: ≥9 to <12 kg: 25 mL (5 g) 12 to <20 kg: 30 mL (6 g) Oral or G-tube BID Duration: 12 weeks (period A), 21 months (Period B)	Females aged 2 – 4 years between ≥9 kg and <20 kg at Screening or 5 years old, ≥9 kg and <12 kg at Screening Meets clinical criteria for RTT Proven mutation of the MECP2 gene
RETT-001 (Phase 2)	Start: April 2013 Completed: September 2014 <u>Enrolment</u> Planned: 60 Randomized/completed: 56/53	R DB PC	<u>Trofinetide</u> 70 mg/kg 35 mg/kg <u>Placebo</u> Oral or G-tube BID	Females aged 16-45 years Meets clinical criteria for RTT Proven mutation of the MECP2 gene Not undergoing regression

			Duration: 2 weeks (cohort 0) or 4 weeks (cohorts 1/2)	RTT-CSS score 10-36 CGI-S≥4

BID= twice daily, CGI-S= Clinical Global Impression-Severity, COVID-19 PHE= covid-19 public health emergency, DB = double-blind, MECP2= methyl-CpG–binding protein 2, PC= placebo-controlled, R= randomized, RTT = Rett syndrome, , RTT-CSS= Rett Syndrome Clinical Severity Scale, *Study was terminated early due to FDA drug approval of trofinetide. *Post regression has been defined as "defined as having no loss or degradation within 6 months of Screening of ambulation (including gait, coordination, or independence of walking/standing), hand function, speech (including babbling, words, or previously developed communicative vocalizations), or nonverbal communicative or social skills (including eye gaze, using the body to indicate communicative intent, or social attentiveness)".

2.6.5.1. Dose response study

Study RETT-002

This was a Phase 2, randomized, double-blind, placebo-controlled, dose-ranging study of trofinetide in RTT. The total duration of treatment was 56 days. The primary objective was to investigate the safety, tolerability, and PK of treatment with trofinetide versus placebo BID. The secondary objective was to investigate measures of efficacy during treatment with trofinetide or placebo BID.

Methods

The study enrolled females between the ages of 5 and 15 years with a diagnosis of classical/typical RTT and a documented mutation of the MECP2 gene. Subjects were required to be post-regression Hagberg Stage 3 or 4, and weigh between 15 kg and 100 kg at Screening and Baseline. Subjects were required to have a severity rating of 10 to 36, inclusive, on the RTT-CSS at Screening, and a CGI-S score of 4 or greater. Each subject had to be able to swallow study drug provided as a liquid solution or be able to receive study drug via G-tube.

The initially planned sample size was 76 with subjects to be randomly assigned 1:1:1:1 to one of four treatment groups: trofinetide 50 mg/kg BID, trofinetide 100 mg/kg BID, trofinetide 200 mg/kg BID, or placebo BID. All randomized subjects received single-blind treatment with placebo for 14 days (Day 1 to Day 14). All subjects received a total of 42 days of DB treatment. Randomisation was stratified by age. The design and sample size were amended in Protocol version 3.0, where sample size was increased to 115, to have approximately 92 subjects randomized and dosed. The first 64 subjects (Cohort 1) received either 1 of 3 doses of trofinetide or placebo based on their randomized treatment assignment (1:1:1:1 in a DB fashion) as in the original protocol. The remaining subjects (Cohort 2) were randomized to receive 14 days of single-blind placebo followed by either 200 mg/kg BID or placebo (1:1 in a DB fashion).

Trofinetide was given orally or by G-tube. Subjects in all three trofinetide groups were up-titrated to the assigned doses and subjects in the 100 mg/kg BID and 200 mg/kg BID groups were down-titrated after 40 days of dosing according to the dose titration schedule (see Table 18).

Table 18: Dose Titration Schedule – Study RETT-002

Dose Level	Day of Study	Dose (mg/kg BID)	Total Dose/Day (mg/kg)
Placebo	15-56	Placebo	0
50 mg/kg BID	15	8.5	17
	16	35	70
	17-56	50	100
100 mg/kg BID	15	17.5	35

	16	35	70
	17	50	100
	18-55	100	200
	56	50	100
200 mg/kg BID	15	17.5	35
	16	35	70
	17	50	100
	18	100	200
	19	150	300
	20-54	200	400
	55	100	200
	56	50	100

Source: Study RETT-002 CSR Table 9-1
Abbreviation: BID=twice daily

Efficacy assessments were categorized into efficacy domains based on assessment type:

1. Clinician completed syndrome specific measures
    a. Rett Syndrome Natural History Motor Behavior Assessment (MBA)
    b. Clinician Rated Domain Specific Concerns VAS
2. Clinician completed syndrome specific global measures
    a. Clinical Global Impression of Severity (CGI-S)
    b. Clinical Global Impression of Improvement (CGI-I)
3. Caregiver completed measures
    a. Rett Syndrome Behavior Questionnaire (RSBQ)
    b. Caregiver Top 3 Concerns via a Visual Analogue Scale (VAS)
    c. Rett Syndrome Caregiver Burden Inventory
4. Physiological measures
    a. 3-lead ECG heart rate and respiratory assessment

Efficacy analyses were conducted for the modified Intent-to-treat (mITT) Population for all endpoints and for the Per-protocol (PP) population for core efficacy variables only. The mITT Population included all randomized subjects who received at least one dose of DB study drug. Primary analysis was performed in the mITT population according to the treatment actually received. Missing data at a scheduled visit was imputed with the median value for the subject's assigned dose group at that visit. The imputation will be performed for individual instrument items. Change from Treatment Baseline to Visit 7, (Day 54, designated End of Efficacy Assessment) was utilized as the primary analysis time point. No multiplicity adjustment was performed. The main efficacy analysis was conducted for the comparison of the 50 mg/kg BID group, 100 mg/kg BID group, and combined 200 mg/kg BID group, each compared with the combined placebo BID group for the core outcome variables.

Comparisons of mean change from Treatment Baseline (Day 14) to Visit 7 (Day 54, designated End of Efficacy Assessment) between each of the treatment groups and placebo were performed for each core efficacy endpoint using a generalized linear model. The core and secondary efficacy analyses included variable-specific (pre-treatment) placebo response and treatment baseline values in the general linear

model as covariates. The variables were retained in the model only if they were statistically significant at the p≤0.1 two-sided level. If not statistically significant at the 0.1 two-sided level for a given variable, the variable was dropped from the model. Pretreatment placebo response was defined as change from Pre-treatment Baseline (Visit 2) to Treatment Baseline (Visit 3, last day of the placebo run-in). As CGI-I is a global score, the score at Treatment Baseline (Visit 3) was used a (pretreatment) placebo response for this endpoint. The interaction of placebo response and treatment group was also explored.

Sensitivity analyses were performed in the PP population defined as all subjects in the mITT population who did not have a major protocol violation that would affect interpretation of the data and was defined prior to study unblinding. No imputation was performed in these analyses.

Results

A total of 82 subjects (24 subjects on placebo, 15 subjects on trofinetide 50 mg/kg BID, 16 subjects on trofinetide 100 mg/kg BID, and 27 subjects on trofinetide 200 mg/kg BID) were randomized, and all were included in the ITT and mITT Populations (in the group to which they were randomized). Prior to Protocol Amendment 3, 15 subjects were randomized into the placebo group and 16 subjects were randomized into the 200 mg/kg BID trofinetide group. After Protocol Amendment 3, 9 subjects were randomized into the placebo group and 11 subjects were randomized into the 200 mg/kg BID trofinetide group. No additional subjects were randomized into the 50 mg/kg BID or the 100 mg/kg BID groups.

One subject was prematurely discontinued from the 200 mg/kg BID treatment group due to an AE and was excluded from the Per-protocol (PP) population.

The mean age was 9.73 years (range: 5.1 to 15.9 years), and most of the subjects were White (94%). At baseline the mean RSBQ Total scores were 39.5, 44.7, 40.3 and 42.2 for placebo, 50 mg/kg, 100 mg/kg and 200 mg/kg trofinetide respectively.

Changes from Treatment Baseline (Day 14) to Day 54 are summarized for RSBQ total, RTT DSC total, Top 3 Caregiver Concerns, and MBA total in Table 19. For CGI-I, the score at Day 54 is summarized.

Table 19: Change From Treatment Baseline (Day 14) to Day 54 in Core Efficacy Outcomes– mITT Population – Study RETT-002

Core efficacy outcomes		Placebo BID (N=24)	Trofinetide 50 mg/kg BID (N=15)	Trofinetide 100 mg/kg BID (N=16)	Trofinetide 200 mg/kg BID (N=27)
RSBQ total					
D14 Treatment Baseline		39.5	44.7	40.3	42.2
Change D14-D54 (LS mean)[a]		-2.3	-3.0	-1.5	-6.7
p-value vs. placebo		--	0.768	0.749	0.042
CGI-I[b]					
Change D14-D54 (LS mean)		3.5	3.3	3.4	3.0
p-value vs. placebo			0.391	0.703	0.029
RTT-DSC total exact median test[c]					
D14 Treatment Baseline		473.3	450.0	445.3	516.6
Change D14-D54		-25.85	-32.50	-12.10	-76.00
p-value vs. placebo		--	0.999	0.748	0.025
Top 3 Caregiver Concerns Visual Analog Scale					
D14 Treatment Baseline		223.9	237.7	211.6	245.9
Change D14-D54 (LS mean)		-12.52	-16.56	-2.09	-18.54
p-value vs. placebo		--	0.776	0.455	0.619
MBA total					
D14 Treatment Baseline		48.8	46.6	48.6	46.6
Change D14-D54 (LS mean)[a]		-2.6	-2.8	-2.4	-2.9
p-value vs. placebo		--	0.872	0.925	0.840

Source: Study RETT-002 CSR Table 11-4
Abbreviations: BID=twice daily; CGI-I=Clinical Global Impression–Improvement; GLM=generalized linear model; MBA=Motor Behavior Assessment; n=number of subjects; mITT=modified Intent-to-treat; PR=placebo response; RSBQ=Rett Syndrome Behaviour Questionnaire; RTT-DSC=RTT Domain Specific Visual Analog Scale; VAS=Visual Analog Scale

[a] In the GLM, Treatment Baseline (TBL) and/or Placebo Response (PR) were included as covariates if $p \leq 0.1$ as specified in the statistical analysis plan. In the final analysis, PR and TBL were included in the analysis of the MBA and PR was included in the analysis of the RSBQ. The remaining three outcome measures were unadjusted.

[b] CGI-I has no Pretreatment Baseline value. The CGI-I values at Day 14 are the ratings of change from Day 0 (the Pretreatment Baseline) to Day 14 (End of Placebo Run-in). All CGI-I assessments done after Day 14 during the double-blind period are referenced to change from Day 14. CGI-I is, thus, not defined at Treatment Baseline (Day 14) by the mean value. A future score of 4 means no change from Day 14. Mean of actual scores were assessed at Day 54 (End of Efficacy Treatment).

[c] The SAP specified that if the distribution of data violated assumptions of normality for the GLM, nonparametric methods would be substituted. The distribution of data in the RTT-DSC was non-normal. Consequently, group medians were used in the analysis of this endpoint and statistical significance was determined by the exact median test

2.6.5.2. Main study

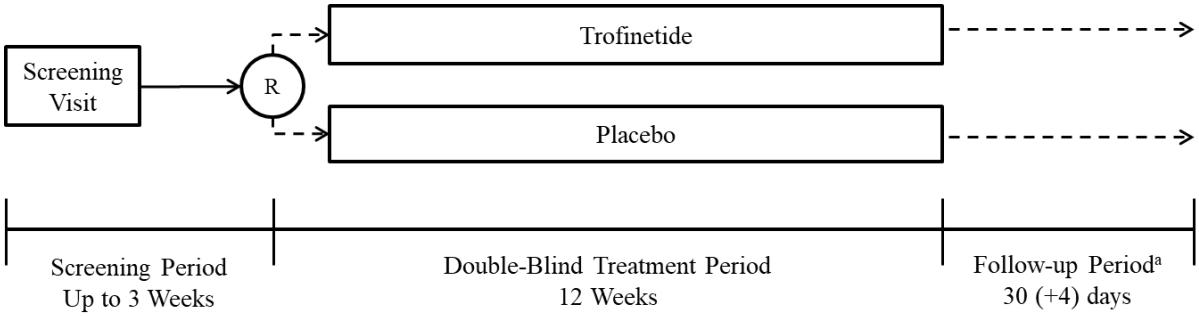
ACP-2566-003: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study of Trofinetide for the Treatment of Girls and Women With Rett Syndrome

Study design

Study 003 was a Phase 3, multicenter, randomized, double-blind (DB), placebo-controlled, parallel group study in girls and women aged 5 to 20 years with RTT. The duration of the DB treatment period

was 12 weeks, while overall participation for all periods in the study for individual subjects was up to 19 weeks (8).

Figure 17: Study schema



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

Study Participants

Subjects were girls and women aged 5 to 20 years weighing ≥12 kg at Screening with a diagnosis of classical or typical RTT according to the 2010 diagnostic criteria and nomenclature of RTT (Neul et al. 2010) with a severity rating of 10 to 36, inclusive, on the RTT-CSS at Screening, and a score of ≥4 on the CGI-S scale at Screening and Baseline. All subjects were required to have a documented disease-causing mutation in the MECP2 gene from a laboratory certified by the College of American Pathologists or under the Clinical Laboratory Improvement Act/Amendment or conduct genomic testing as part of Screening. Subjects were to be stable in their disease course, defined as having no loss or degradation within 6 months of Screening of ambulation (including gait, coordination, or independence of walking/standing), hand function, speech (including babbling, words, or previously developed communicative vocalizations), or nonverbal communicative or social skills (including eye gaze, using the body to indicate communicative intent, or social attentiveness). Since seizures are a common comorbidity of RTT, subjects were required to have a stable pattern of seizures or no seizures within 8 weeks of Screening, and that all concomitant anticonvulsant or other psychoactive medication to be either stable for at least 4 weeks or discontinued fewer than 2 weeks (or five half-lives, whichever was greater) before Baseline.

Subjects in this study were recruited from 21 sites scattered across the United States.

Treatments

In Study 003, each subject was dosed based on the weight-based band for the subject at baseline (Table 20). The dose was not to be changed if the subject's weight at a postbaseline visit put them in a new weight band. However, until the Week 6 visit, the dose could be decreased for poor tolerability (e.g., subject experienced diarrhea). The investigator could instruct the caregiver to reduce the study drug to a dose as low as half of the assigned dose. In addition, up to four total doses (consecutive or nonconsecutive) could be withheld within the first 6 weeks of treatment. The Investigator was to attempt to increase the dose as soon as it was possible based on the clinical situation, with an aim to return to the originally assigned dose or to the highest tolerable dose that is at least half of the originally assigned dose.

Table 20: Trofinetide Dosing Schedule Based on Weight at Baseline, Study 003

Weight	Dose	Total daily dose
12 to 20 kg	30 mL (6 g) BID	60 mL (12 g)
>20 to 35 kg	40 mL (8 g) BID	80 mL (16 g)
>35 to 50 kg	50 mL (10 g) BID	100 mL (20 g)
>50 kg	60 mL (12 g) BID	120 mL (24 g)

Source: Study 003 CSR Table 9-3
Abbreviation: BID=twice daily

Objectives

The primary objective of this pivotal study was to show efficacy of weight-banded doses of trofinitide BID relative to placebo on the mean change from baseline in RSBQ total score and the mean CGI-I score at 12 weeks, irrespective of treatment discontinuations.

A key secondary objective was to show efficacy of weight-banded doses of trofinitide BID relative to placebo on the mean change from baseline in CDBS-DP-IT Social Composite Score at 12 weeks, irrespective of treatment discontinuations.

Outcomes/endpoints

The coprimary efficacy endpoints in Study 003 were change from Baseline to Week 12 in the RSBQ total score and the CGI-I score at Week 12. The CSBS-DP-IT Social Composite Score was selected as the key secondary endpoint in this study due to the importance that caregivers of patients with RTT place on the patient's ability to communicate and interact in a meaningful way with their caregivers, family, and surroundings. All endpoints are described in Table 21 below.

Table 21: Efficacy Endpoints – Study 003

Endpoint	Domain	Endpoint assessment	
Primary efficacy	Rett syndrome globally	Clinical Global Impression–Improvement (CGI-I) at Week 12 (coprimary endpoint)	*Rett Syndrome Behaviour Questionnaire (RSBQ)* change from Baseline at Week 12 (coprimary endpoint)
Key secondary efficacy	Ability to communicate	*CSBS-DP-IT Social Composite Score* change from Baseline at Week 12	
Other secondary endpoints	Quality of life	*Overall Quality of Life Rating of the ICND*	
	Hand function	Rett Syndrome Clinician Rating of Hand Function (RTT-HF)	
	Walking	Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills (RTT-AMB)	
	Verbal communication	Rett Syndrome Clinician Rating of Verbal Communication (RTT-VCOM)	
	Nonverbal communication	Rett Syndrome Clinician Rating of Ability to Communicate Choices (RTT-COMC)	
	Rett syndrome globally	Clinical Global Impression–Severity (CGI-S)	
	Caregiver burden	*Rett Syndrome Caregiver Burden Inventory (RTT-CBI)*	

Endpoint	Domain	Endpoint assessment
	Impact on family and child	*ICND*

Source: Study 003 CSR Section 9.5.2.1
Abbreviations: CGI-S=Clinical Global Impression–Severity; CSBS-DP-IT=Communication and Symbolic Behavior Scales Developmental Profile™ Infant-Toddler Checklist; ICND=Impact of Childhood Neurologic Disability Scale
Note: Caregiver assessments are in italics.

Rett Syndrome Behaviour Questionnaire (RSBQ)

The RSBQ is a 45-item, caregiver-completed rating scale assessing core symptoms of RTT, including neurobehavioral, motor, and other symptoms known to be impaired in subjects with RTT (Mount et al., 2002). The RSBQ has been correlated with functioning and quality of life and has been characterized and validated across a range of ages (2 to 47 years) and genetic variations in RTT (Barnes et al., 2015; Cianfaglione et al., 2015; Robertson et al., 2006).

The caregiver rates each of the 45 items as "0" (not true), "1" (somewhat or sometimes true), or "2" (very true or often true). In general, the prompts for RSBQ represent symptoms of RTT, meaning that the score of "2" indicates greater severity or frequency of symptoms. Decreases in scores denote improvement. The exception is item 31 ("Uses eye gaze to convey feelings, needs, and wishes") for which the score of "2" (very true) indicates a better outcome. The score for item 31 ("Uses eye gaze to convey feelings, needs, and wishes") was reversed in the calculations of total score for the primary analysis and subscores because the score of "2" (very true) reflects a better outcome.

In order for all assessments to be administered in a standardized manner, caregiver-completed assessments were reviewed by study personnel and caregivers received standardized training and guidance on how to complete the measures. All efforts were made to maintain the same caregiver rater across visits for each subject. Before completing any ratings, caregivers watched a video with instructions on rating the RSBQ and were given short written instructions with each administration of the scale.

Clinical Global Impression–Improvement (CGI-I)

The Clinical Global Impression (CGI) scale, which includes both the Improvement (CGI-I) and the Severity (CGI-S) scales, is a well-established research rating tool used widely in clinical studies of CNS disorders (Guy, 1976), including neurodevelopmental disorders such as Angelman syndrome, FXS, and autism spectrum disorders (Arnold et al., 2000; Davenport et al., 2016; Kolevzon et al., 2021).

Neul et al. (2015) developed RTT-specific anchors for use with the CGI-I. The anchors were developed to provide a framework for considering the following factors related to symptom duration, onset, durability of change, and the context of sign/symptom change across the symptom domains. Information on factors to differentiate between the scores is provided (Neul et al., 2015).

To ensure ratings were consistently applied in the trofinetide clinical studies, training and standardization of the CGI-I ratings by all study raters and qualification of raters was required. To achieve this, an expert panel wrote a series of clinical case vignettes. All CGI raters were trained on anchors and case vignettes and discussed the panel's "gold standard" CGI-I ratings. During qualification, raters were asked to rate the CGI-I by independently rating case vignettes for fidelity as compared with a gold standard rating. All efforts were made to maintain the same clinician rater across visits for each subject.

Communication and Symbolic Behavior Scales (CSBS-DP-IT) Social Composite Score

The Infant-Toddler Checklist is one of three components of the CSBS-DP, which was originally developed to assess communication and pre-linguistic skills in young children (12 to 24 months of age)

(Wetherby et al., 2002), and has been used with older children with developmental delay (Anagnostou et al., 2015), including RTT (O'Leary et al., 2018).

The Social Composite score (CSBS-DP-IT Social Composite Score) in particular has been identified as containing items appropriate to the non-verbal modalities of people with RTT. Although it has not been validated in this pathology, the CSBS-DP-IT Social Composite Score was used in a previous placebo controlled study of girls (and women) with RTT (O'Leary et al., 2018) and was included as Study 003 key secondary endpoint as the ability to communicate is consistently found to be among the greatest concerns of parents and caregivers of people with RTT (Urbanowicz et al., 2016). Wetherby et al describes its validity and reliability as a screening instrument (Wetherby et al., 2002).

Because the communication ability and strategies in individuals with RTT of all ages are similar to the abilities of a toddler without a neurodevelopmental disorder, investigators have turned to assessments designed for toddlers as appropriate to use for all ages of RTT individuals

Rett Syndrome Clinician Rating of Hand Function (RTT HF)

The RTT-HF is a clinician-completed clinical assessment of the subject's ability to use her hands for functional purposes. The assessment is made on an 8-point Likert scale (0 to 7) with 0 denoting normal functioning and 7 the most severe impairment. Decreases in score denote improvement.

Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills (RTT-AMB)

The RTT-AMB is a clinician completed clinical assessment of the subject's ability to sit, stand, and ambulate. The assessment is made on an 8-point Likert scale (0 to 7) with 0 denoting normal functioning and 7 the most severe impairment. Decreases in score denote improvement.

Rett Syndrome Clinician Rating of Verbal Communication (RTT-VCOM)

The RTT-COMC is a clinician-completed clinical assessment of the subject's ability to communicate their choices or preferences, which can include the use of nonverbal means such as eye contact or gestures. The assessment is made on an 8-point Likert scale (0 to7) with 0 denoting normal functioning and 7 the most severe impairment. Decreases in score denote improvement.

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

The RTT-VCOM is a clinician completed clinical assessment of the subject's ability to communicate verbally. The assessment is made on an 8-point Likert scale (0 to 7) with 0 denoting normal functioning and 7 the most severe impairment. Decreases in score denote improvement.

Clinical Global Impression–Severity (CGI-S)

The CGI-S is a 7-point scale that requires the clinician to rate the severity of the subject's illness at the time of assessment relative to the clinician's experience with subjects who have the same diagnosis. The subject is assessed on severity of illness, i.e., Rett syndrome as a whole, at the time of rating: 1 (normal, not at all ill), 2 (borderline ill), 3 (mildly ill), 4 (moderately ill), 5 (markedly ill), 6 (severely ill), or 7 (extremely ill). Decreases in score denote improvement.

Rett Syndrome Caregiver Burden Inventory (RTT-CBI)

The RTT-CBI consists of 24 negatively worded items (Items 1 through 24). Frequency ratings are on a 5-point Likert scale including: 0 (never), 1 (rarely), 2 (sometimes), 3 (frequently), and 4 (nearly always). The RTT-CBI also includes two positively worded items (items 25 and 26) that comprise the Optimism Index that was not used for analysis. The total score, ranging from 0 to 96, was calculated as the sum of the scores for items 1 through 24.

Impact of Childhood Neurologic Disability (ICND) Quality of Life

Overall quality of life score rating of the ICND: The numeric scale of the child's overall quality of life ranges from 1 ("Poor") to 6 ("Excellent") with lower quality of life scores indicating lower quality of life.

Sample size

The sample size calculation was performed for the co-primary efficacy endpoints and took into account that both had to be significant at a two-sided significance level of 5%. The planned sample size of 184 subjects yields 95% power for detecting differences in mean change from baseline in RSBQ total score at week 12 when the true difference is means is 4.4 and assuming a standard deviation of 8 and accounting for a 5% discontinuation rate. The same sample size will yield >95% power for detecting differences in mean CGI-score when true difference in means is 0.5 and assuming a standard deviation of 0.7. The mean differences of 4.4 and 0.5 used in power analysis were derived from the phase II study.

Randomisation and blinding (masking)

Randomisation was stratified by RSBQ total score at baseline (2 strata, <35 and ≥ 35 total score) and age (5-10 years old, 11-15 years old, and 16-20 years old). The assignments were based on a pre-generated permuted-block randomization schedule. A minimum of 12 subjects were required to be randomized for each age stratum. A rationale for using the value of 35 as a cut-off for RSBQ total score was not well motivated and as well as the implications of scores above (or below) this cut-off discussed.

The sponsor, subjects, caregivers, and investigators were blinded to treatment assignment. Blinding was assured by restricting access of investigators and sponsor personnel and/or designee to the treatment codes, and providing identical packaging for the trofinetide and placebo treatments. The investigator was allowed to break the blind in case of a medical emergency if it was considered necessary for the care of the subject.

Statistical methods

The Full Analysis Set (FAS) consists of all randomized subjects, who received at least one dose of study drug, and have both a Baseline value and at least one post-baseline value for the RSBQ total score or at least one CGI-I score after taking study medication. Efficacy analyses were performed in the FAS. Excluding randomized subjects that do not have a post-baseline value from the primary analysis population is generally not considered acceptable. This is not further pursued as number of patients excluded for this reason is very low.

The Safety Analysis Set consists of all randomized subjects who received at least one dose of study drug and analyses in this set will be based on the actual treatment received.

Co-primary endpoints were both compared between treatment group using a mixed model for repeated measure (MRMM). The LS means in each treatment group, between-group difference in LS means with corresponding 95% confidence interval, p-value, and the effect size (Cohen's d) were presented for each post-baseline visit.

The model for comparing the coprimary endpoint of mean change from baseline in RSBQ total score at week 12 included fixed effects for treatment group, visit, treatment group-by-visit, baseline RSBQ total score, age group (5-10 years old, 11-15 years old, and 16-20 years old), baseline RSBQ total score stratum (<35 or ≥35 total score), baseline RSBQ total score-by-visit interaction and a random effect for

subject. It was clarified during the procedure why the score for item 31 ("Uses eye gaze to convey feelings, needs, and wishes") was reversed in the calculations of total score for the primary analysis.

The model for comparing the coprimary endpoint of mean CGI-I at week 12 included fixed effects for treatment group, visit, treatment group-by-visit, baseline CGI-S score, age group (5-10 years old, 11-15 years old, and 16-20 years old), baseline RSBQ total score stratum (<35 or ≥35 total score), baseline CGI-S score-by-visit interaction and a random effect for subject.

The key secondary endpoint CDBS-DP-IT Social Composite Score at week 12 was also compared between treatment groups using a MMRM. The model included fixed effects for treatment group, visit, treatment group-by-visit, baseline CSBS-DP-IT Social Composite Score, age group (5-10 years old, 11-15 years old, and 16-20 years old), baseline RSBQ total score stratum (<35 or ≥35 total score), baseline CSBS-DP-IT Social Composite Score-by-visit interaction and a random effect for subject.

Type I error was controlled over co-primary and key secondary endpoints by means of a gatekeeping procedure where the key secondary endpoint was only tested when both co-primary endpoints were significant. All tests used a two-sided significance level of 5%.

The estimands associated with the primary and key secondary objectives handled all intercurrent events with a treatment policy strategy. In the estimators, a missingness at random assumption for the missing outcomes was made. Several predefined sensitivity analyses for comparing the coprimary endpoints were performed, including analyses using reference-based imputation for missing outcomes in the trofinetide arm (after early withdrawal in general and early withdrawal not due to COVID-19), an analysis to investigate the impact of remote assessments, and analysis to assess the impact of using the incorrectly calculated RSBQ total score for stratification. Additional estimands were defined that handled all IEs with a hypothetical strategy.

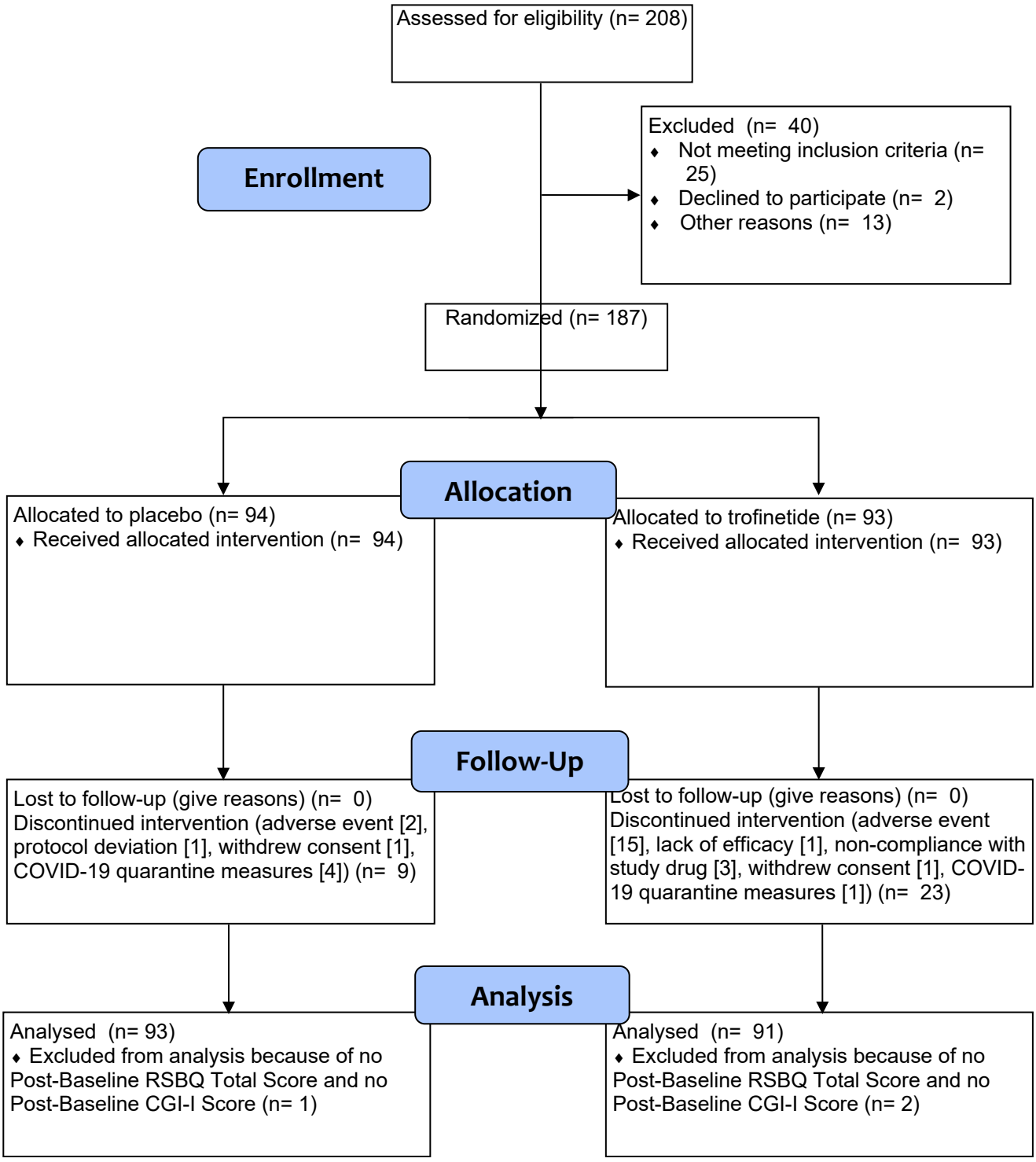
Predefined subgroup analyses included analyses stratified by age group, RSBQ score at baseline and (pooled) MECP2 mutation group.

MMRM analyses similar to those used for coprimary and key secondary endpoint were used for secondary endpoints not included in the testing strategy. An exploratory analysis compared proportion of responders, defined as subjects with CGI-I scores at week 12 of 1 or 2 (much or very much improved), between the study arms using logistic regression adjusted for baseline CGI-S, age group, and baseline RSBQ total score stratum. Responder analysis will be performed in two ways:1) Missing CGI-I outcomes will be imputed as non-responder and 2) using observed cases only (subjects with missing CGI-I will be excluded).

Results

Participant flow

Figure 18: Participant flow



Recruitment

First patient randomized/enrolled: 29 October 2019

Screening/enrollment paused: 18 March 2020

Screening/enrollment restarted: 12 June 2020

Study completion date: 28 October 2021

Conduct of the study

As a result of the COVID-19 public health emergency (PHE), screening and enrolment for this study were paused on 18 March 2020. Screening and enrolment resumed on 12 June 2020, provided various risk mitigation measures were maintained.

Study protocol was amended twice (27 April 2020 and 7 Augustus 2020). As a result of the COVID-19 public health emergency, protocol Amendment 2 incorporated revisions to study methodology in accordance with the FDA Guidance on Conduct of Clinical Trials of Medical Products during the COVID-19 Public Health Emergency. Specifically, alternative methods of performing safety and efficacy assessments include provisions for remote (off-site) assessments.

Baseline data

The overall mean age was 10.9 years and ranged from 5 to 20 years (27). Of the 184 subjects, 58.2% of subjects were 5 to <12 years, 24.5% of subjects were 12 to <17 years, and 17.4% of subjects were ≥17 years. Mean body weight was 29.7 kg and ranged from 13 to 78 kg. Subjects were predominantly White (91.8%) and not Hispanic or Latino (91.3%), and all subjects were female.

Table 22: Demographics – Full Analysis Set

Parameter	**Placebo** (N=93)	**Trofinetide** (N=91)	**Total** (N=184)
Sex, n (%)			
Female	93 (100.0)	91 (100.0)	184 (100.0)
Age (years)			
n	93	91	184
Mean (SE)	10.8 (0.47)	11.0 (0.50)	10.9 (0.34)
SD	4.57	4.73	4.64
Median	10.0	10.0	10.0
Min, max	5, 20	5, 20	5, 20
Age categories, n (%)			
5 to <12 years	55 (59.1)	52 (57.1)	107 (58.2)
12 to <17 years	23 (24.7)	22 (24.2)	45 (24.5)
≥17 years	15 (16.1)	17 (18.7)	32 (17.4)
Randomization age categories, n (%)			

Parameter	Placebo (N=93)	Trofinetide (N=91)	Total (N=184)
5 to 10 years	52 (55.9)	48 (52.7)	100 (54.3)
11 to 15 years	23 (24.7)	24 (26.4)	47 (25.5)
16 to 20 years	18 (19.4)	19 (20.9)	37 (20.1)
Primary race, n (%)			
White	89 (95.7)	80 (87.9)	169 (91.8)
Black or African American	1 (1.1)	1 (1.1)	2 (1.1)
Asian	1 (1.1)	5 (5.5)	6 (3.3)
American Indian or Alaska native	- -	- -	- -
Native Hawaiian or other Pacific islander	- -	1 (1.1)	1 (0.5)
Other	2 (2.2)	4 (4.4)	6 (3.3)
Non-white	4 (4.3)	11 (12.1)	15 (8.2)
White	89 (95.7)	80 (87.9)	169 (91.8)
Ethnicity, n (%)			
Hispanic or Latino	10 (10.8)	6 (6.6)	16 (8.7)
Not Hispanic or Latino	83 (89.2)	85 (93.4)	168 (91.3)
Height (cm)			
n	91	90	181
Mean (SE)	127.5 (1.68)	128.4 (1.73)	128.0 (1.20)
SD	16.04	16.43	16.20
Median	127.5	128.1	127.6
Min, max	92, 171	94, 170	92, 171
Weight (kg)			
n	93	91	184
Mean (SE)	29.1 (1.08)	30.2 (1.30)	29.7 (0.84)
SD	10.43	12.42	11.44
Median	27.2	29.3	28.0
Min, max	13, 57	13, 78	13, 78
BMI (kg/m²)			

Parameter	Placebo (N=93)	Trofinetide (N=91)	Total (N=184)
n	90	90	180
Mean (SE)	17.2 (0.37)	17.5 (0.45)	17.4 (0.29)
SD	3.48	4.25	3.88
Median	16.6	16.8	16.7
Min, max	12, 28	10, 34	10, 34
Is the subject of childbearing potential?, n (%)			
Yes	34 (36.6)	34 (37.4)	68 (37.0)
No	59 (63.4)	57 (62.6)	116 (63.0)
Has the subject reached menarche?, n (%)			
Yes	34 (36.6)	33 (36.3)	67 (36.4)
No	59 (63.4)	58 (63.7)	117 (63.6)

Source: Study 003 CSR Table AH14.1.5.2
Abbreviations: BMI=body mass index; max=maximum; min=minimum; SD=standard deviation; SE=standard error
Note: Number of subjects in each treatment group was used as the denominator for calculating percentages.

Mean Baseline scores on the RSBQ, CGI S, and RTT-CSS assessments were similar for both treatment groups (Table 23). Mean Baseline CGI-S score was 4.9, and most of the subjects were moderately ill (34.2%) or markedly ill (42.9%).

Table 23: Baseline Efficacy Variables – Full Analysis Set

Parameter	Placebo (N=93)	Trofinetide (N=91)	Total (N=184)
IRT Baseline RSBQ total score			
n	93	91	184
Mean (SE)	45.9 (1.29)	44.8 (1.19)	45.4 (0.88)
SD	12.46	11.35	11.90
Median	48.0	43.0	43.5
Min, max	16, 76	21, 74	16, 76
Derived Baseline RSBQ total score[a]			
n	93	91	184

Parameter	Placebo (N=93)	Trofinetide (N=91)	Total (N=184)
Mean (SE)	44.5 (1.26)	43.7 (1.21)	44.1 (0.87)
SD	12.20	11.52	11.84
Median	43.0	42.0	43.0
Min, max	14, 69	21, 74	14, 74
Baseline CGI-S score			
n	93	91	184
Mean (SE)	4.9 (0.08)	4.9 (0.08)	4.9 (0.06)
SD	0.76	0.77	0.76
Median	5.0	5.0	5.0
Min, max	4, 7	4, 6	4, 7
Baseline CGI-S category, n (%)			
1=Normal, not at all ill	- -	- -	- -
2=Borderline ill	- -	- -	- -
3=Mildly ill	- -	- -	- -
4=Moderately ill	32 (34.4)	31 (34.1)	63 (34.2)
5=Markedly ill	42 (45.2)	37 (40.7)	79 (42.9)
6=Severely ill	18 (19.4)	23 (25.3)	41 (22.3)
7=Among the most extremely ill patients	1 (1.1)	- -	1 (0.5)
Baseline CSBS-DP-IT Social Composite Score			
n	93	91	184
Mean (SE)	8.8 (0.34)	8.7 (0.35)	8.8 (0.24)
SD	3.24	3.31	3.27
Median	9.0	9.0	9.0
Min, max	2, 16	2, 16	2, 16
RTT Clinical Severity Scale Score at Screening			
n	93	91	184

Parameter	Placebo (N=93)	Trofinetide (N=91)	Total (N=184)
Mean (SE)	24.1 (0.69)	24.2 (0.66)	24.1 (0.48)
SD	6.70	6.33	6.50
Median	23.0	24.0	24.0
Min, max[a]	12, 40	11, 36	11, 40

Sources: Study 003 CSR Table AH14.1.6.2 and Table 14.2.3.1
Abbreviations: CGI-S=Clinical Global Impressions–Severity; CSBS-DP-IT=Communication and Symbolic Behavior Scales Development Profile™ Infant-Toddler Checklist; IRT=interactive response technology system; max=maximum; min=minimum; RSBQ=Rett Syndrome Behaviour Questionnaire; RTT=Rett syndrome; SD=standard deviation; SE=standard error
Note: IRT and derived Baseline RSBQ total score differ in that scoring of item 31 is reversed for the derived Baseline RSBQ total score. The IRT RSBQ score was used only to determine a subject's randomization stratum.
Note: Number of subjects in each treatment group was used as the denominator for calculating percentages.
[a] One subject enrolled with an RTT Clinical Severity Scale score of >36. This subject was excluded from the Per-protocol analysis.

The mean (SE) age at RTT diagnosis was 2.4 years, with mean age at first symptoms noticed of 0.8 years (Table 2.7.3 11) The distribution of mutation types was similar between the placebo and trofinetide groups.

Applicability of a US study population to an EU patient setting as provided by the Applicant

Similarity of the populations

The age at RTT diagnosis was not reported in RETT-002 study. In Study 003, the mean age at RTT diagnosis was 2.2 ± 1.04 years in the trofinetide group and 2.6 ± 0.21 years in the placebo group (Study ACP-2566-003 CSR Table 11-4). This was in the range of what has been reported in the literature in the EU (ranging from 2 years in Denmark to 7.8 years in the Netherlands; (Zade et al., 2024)).

In the RTTDB Pool, the mean weight of subjects was approximately 31 kg and the mean age was approximately 13 years (placebo and all trofinetide groups) (Table 2.7.4-15). Weight and height of RTT patients have been reported very infrequently in the literature, limiting the ability to compare weight and height between the EU target and trofinetide studies populations. A literature review was conducted by Zade et al., 2024 to enable the comparison of their Irish cohort to other RTT cohorts worldwide. In a Danish cohort with a mean age of 40.7 years, the mean weight was 42 kg; in an Irish cohort with a mean age of 20.9 years, the mean weight was 38 kg (Zade et al., 2024).

Baseline RTT symptoms were not recorded as such in the trofinetide studies, but medical history can still inform the disease presentation at the time of inclusion. In the RTTDB Pool, the most common (>10% incidence) medical histories in the all trofinetide and placebo groups were constipation (78.4% vs. 80.5%, respectively), gastroesophageal reflux disease (50.6% vs. 45.1%), seizure (45.5% vs. 51.1%), gastrostomy (30.7% vs. 31.6%), insomnia (18.2% vs. 24.1%), anxiety (23.3% vs. 20.3%), physiotherapy (16.5% vs. 21.1%), epilepsy (19.9% vs. 15.8%), occupational therapy (16.5% vs. 19.5%), speech rehabilitation (15.3% vs. 21.1%), dystonia (13.6% vs. 15.8%), drooling (11.9% vs. 12.0%), and scoliosis surgery (11.4% vs. 10.5%) (Section 2.7.4.1.3.2.1). The array of RTT symptoms is consistent with what has been observed in EU (either or several of the following cohorts: Italy, Denmark, the Netherlands, the United Kingdom, Poland, Sweden, and Ireland): regression (~96% of RTT patients of the cohort), behavioral regression (77% to 68%), speech regression (23% to 84%), regression of hand use (41% to 88%), stereotypies (70% to 99%), gastrointestinal problems (82% to

89%), seizures (43% to 82%), trouble night sleep (16% to 77%), anxiety (71% to 74%), and involuntary movements (40% to 84%), among other manifestations (Zade et al., 2024).

Disease severity at inclusion was rated using the RSBQ in Studies RETT-002 and 003. The mean baseline RSBQ total score was 39.5 ± 11.83 and 42.2 ± 10.99 in the placebo and trofinetide 200 mg/kg BID in Study RETT-002 (Study RETT-002 CSR Table 11-3), and 45.9 ± 12.4 and 44.9 ± 11.25 in the placebo and trofinetide groups of Study 003 (Study ACP-2566-003 CSR Table 11-3). In a national survey of UK RTT patients (n=91), the mean RSBQ was 42.31 ± 14.85 (Cianfaglione 2015).

The demographic characteristics of RTT patients in the US and EU are comparable, with similar age ranges, symptom severity, and comorbid conditions.

Similar medical practice

In both the US and the EU, the clinical management of RTT focuses on a symptomatic approach with a treatment plan tailored to the individual's needs. Both regions predominantly use symptomatic prescription medications and over-the-counter treatments to manage these symptoms. Key aspects of clinical management include (Fu et al., 2020; May et al., 2023; Peron et al., 2022; RETT UK, 2013):

- Seizure management: anti-epileptic drugs are commonly used in both regions to control seizures, which are a frequent symptom of RTT.
- Respiratory support: US and EU healthcare systems provide similar interventions for respiratory issues, including supplemental oxygen and respiratory therapies.
- Gastrointestinal management: treatments for constipation and other gastrointestinal issues are similar, with the use of laxatives, antacids, dietary adjustments, and other supportive measures.
- Musculoskeletal issue management: both regions commonly use physical and occupational therapy to maintain mobility and flexibility.
- Sleep disturbance management: treatment for night terrors and other sleep issues is similar. It involves improving sleep hygiene practices and supporting them with melatonin supplements and medications to regulate sleep patterns.

The primary differences in the management of RTT between the US and EU are due to each region's unique medical care systems and cultural contexts, rather than differences in the disease itself. These differences include:

- Healthcare accessibility: while the US healthcare system is primarily insurance-based, many EU countries have universal healthcare systems, which may affect accessibility and timeliness of care.
- Cultural approaches to care: cultural attitudes towards healthcare and patient management can vary, influencing how treatments are administered and the patient support systems in place.

While differences in healthcare systems and cultural contexts exist, these should not significantly impact the pharmacological response to trofinetide. Any variations in patient management due to healthcare accessibility or cultural approaches can be mitigated through tailored implementation strategies and healthcare provider education.

In conclusion, the clinical management of RTT in the EU is sufficiently similar to that in the US to support the applicability of US clinical trial data for trofinetide to the EU population.

The core principles of symptom management and treatment approaches are consistent across both regions, and the differences in medical care systems do not significantly affect the expected outcomes of trofinetide treatment. Therefore, the efficacy and safety profile of trofinetide observed in US trials is likely to be replicated in the EU.

Similar clinical studies conduct

Trofinetide studies were conducted in accordance with ICH guidelines and GCP, which apply universally.

Numbers analysed

The Randomized Analysis Set consisted of 187 subjects. Of these 187 subjects, 184 subjects (98.4%) comprised the full analysis set (FAS). In the FAS, 93 subjects were randomised to placebo and 91 were randomised to trofinetide.

Outcomes and estimation

Co-primary endpoints

RSBQ

The least squares mean (LSM) [SE] change from Baseline to Week 12 in the RSBQ total score was 1.7(0.90) in the placebo group compared to 4.9 (0.94) in the trofinetide group (LSM difference [95% confidence interval (CI)]: 3.1[5.7, 0.6]; p=0.0175, Table 24). A forest plot with the RSBQ subscales is provided in Figure 19.

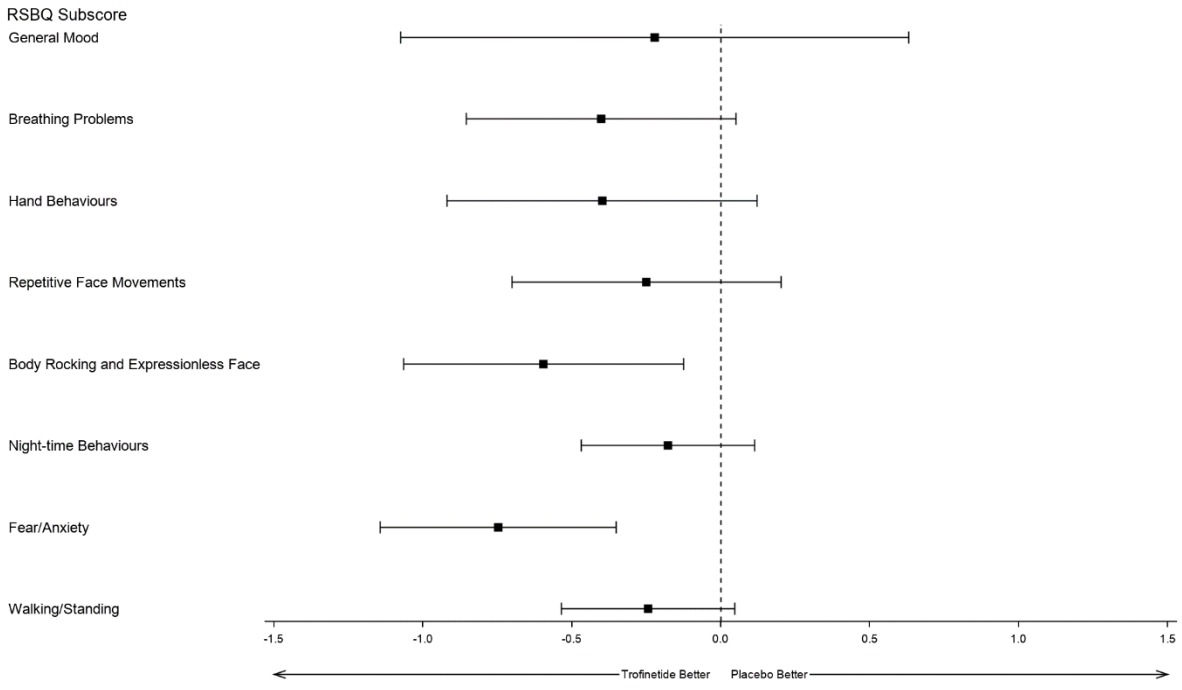
Table 24: Primary Analysis of RSBQ Total Score and Change From Baseline by Visit (MMRM) – Full Analysis Set

Visit	Placebo (N=93)	Trofinetide (N=91)
Baseline		
N	93	91
Mean (SE)	44.5 (1.26)	43.7 (1.21)
SD	12.20	11.52
Median	43.0	42.0
Min, max	14, 69	21, 74
Week 12		
N	85	76
Mean (SE)	42.8 (1.42)	39.9 (1.38)
SD	13.05	12.02
Median	41.0	40.5
Min, max	16, 69	9, 69
Change from Baseline to Week 12		
N	85	76
Mean (SE)	-1.7 (0.98)	-5.1 (0.99)
SD	9.05	8.67
Median	-2.0	-3.5
Min, max	-31, 40	-34, 10
MMRM Analysis[a]		
LS mean (SE)	-1.7 (0.90)	-4.9 (0.94)

95% CI	(-3.5, 0.0)	(-6.7, -3.0)
Difference from placebo		
LS mean difference (SE)		-3.1 (1.30)
95% CI		(-5.7, -0.6)
2-sided p-value		0.0175
Effect size (Cohen's *d*)		0.37

Source: Study 003 CSR Table 14.2.1.1
Abbreviations: CI=confidence interval; LS=least squares; max=maximum; min=minimum; MMRM=mixed effects model for repeated measures; RSBQ=Rett Syndrome Behaviour Questionnaire; SD=standard deviation; SE=standard error
Notes: Baseline was the latest nonmissing value prior to the first dose of study drug.
[a] The MMRM includes age group, baseline RSBQ severity, planned treatment, study visit, treatment-by-visit interaction, Baseline-by-visit interaction, and Baseline total score as fixed effects. An unstructured matrix was used to model within-subject errors. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

Figure 19: Forest Plot of Treatment Difference in LSM Change From Baseline to Week 12 in RSBQ Subscales and Corresponding 95% CI (MMRM) – Full Analysis Set



Source: Study 003 CSR Figure AH1.3.4
Abbreviations: CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behaviour Questionnaire

CGI-I

The MMRM LSM (SE) in CGI-I score at Week 12 was 3.8 (0.07) in the placebo group and 3.5 (0.07) in the trofinetide group, yielding a difference in favor of trofinetide group of 0.3, which was statistically significant (95% CI: -0.5, -0.1; p=0.0030, Table 25).

Table 25: Primary Analysis of CGI-I Score by Visit (MMRM) – Full Analysis Set

Visit	Placebo (N=93)	Trofinetide (N=91)
Week 12		
N	86	77

Visit	Placebo (N=93)	Trofinetide (N=91)
Mean (SE)	3.8 (0.06)	3.5 (0.08)
SD	0.55	0.74
Median	4.0	4.0
Min, max	2, 5	2, 5
MMRM analysis[a]		
LS mean (SE)	3.8 (0.07)	3.5 (0.07)
95% CI	(3.7, 4.0)	(3.4, 3.7)
Difference from placebo		
LS mean difference (SE)		-0.3 (0.10)
95% CI		(-0.5, -0.1)
Two-sided p-value		0.0030
Effect size (Cohen's *d*)		0.47

Source: Study 003 CSR Table 14.2.2.1
Abbreviations: CGI-I=Clinical Global Impression–Improvement; CGI-S=Clinical Global Impression–Severity; CI=confidence interval; LS=least squares; MMRM=mixed-effects model for repeated measures; max=maximum; min=minimum; RSBQ=Rett Syndrome Behaviour Questionnaire; SD=standard deviation; SE=standard error
[a] The MMRM included age group, baseline RSBQ severity, planned treatment, study visit, treatment-by-visit interaction, Baseline CGI-S-by-visit interaction, and Baseline CGI-S as fixed effects. An unstructured covariance matrix was used to model within-subject errors. Kenward-Roger method was used for calculating the denominator degrees of freedom for tests of fixed effects.

Key secondary endpoint CSBS-DP-IT Social Composite Score

The MMRM LSM change from Baseline to Week 12 in the CSBS DP IT Social Composite Score demonstrated a statistically significant separation in favor of the trofinetide group compared with the placebo group (LSM difference: 1.0; 95% CI: 0.3, 1.7; p=0.0064, Table 26).

Table 26: Key Secondary Analysis of CSBS-DP-IT Social Composite Score and Change From Baseline at Week 12 (MMRM) – Full Analysis Set

Visit	Placebo (N=93)	Trofinetide (N=91)
Baseline		
N	93	91
Mean (SE)	8.8 (0.34)	8.7 (0.35)
SD	3.24	3.31
Median	9.0	9.0
Min, max	2, 16	2, 16
Week 12		
N	81	73
Mean (SE)	7.5 (0.33)	8.9 (0.44)
SD	2.99	3.74
Median	7.0	8.0
Min, max	2, 18	3, 19
Change from Baseline to Week 12		
N	81	73
Mean (SE)	-1.1 (0.28)	-0.1 (0.28)
SD	2.55	2.38
Median	-1.0	0.0
Min, max	-9, 4	-5, 7

Visit	Placebo (N=93)	Trofinetide (N=91)
MMRM Analysis[a]		
LS mean (SE)	-1.1 (0.25)	-0.1 (0.26)
95% CI	(-1.6, -0.6)	(-0.6, 0.5)
Difference from placebo		
LS mean difference (SE)		1.0 (0.37)
95% CI		(0.3, 1.7)
Two-sided p-value		0.0064
Effect size (Cohen's *d*)		0.43

Source: Study 003 CSR Table 14.2.3.1

Secondary/exploratory endpoints

All other secondary endpoints are displayed in Table 27.

Table 27: Analyses of the Other Secondary Endpoints – Full Analysis Set

Other secondary endpoints	Trofinetide group comparison (Trofinetide-Placebo)		
	LSM difference (SE)	Two-sided p-value	Effect size (Cohen's *d*)
QoL rating of ICND	0.1 (0.13)	0.2507	0.19
RTT-HF	-0.1 (0.11)	0.3649	0.14
RTT-AMB	-0.1 (0.10)	0.2114	0.19
RTT-COMC	-0.3 (0.15)	0.0257	0.36
RTT-VCOM	0.0 (0.08)	0.9799	0.00
CGI-S	0.0 (0.05)	0.5304	0.10
RTT-CBI	-0.8 (1.40)	0.5855	0.09
ICND	-4.5 (4.67)	0.3376	0.22

Sources: Study 003 CSR Tables 14.2.4.1, 14.2.5.1, 14.2.6.1, 14.2.7.1, 14.2.8.1, 14.2.9.1, 14.2.10.1, and 14.2.11.1

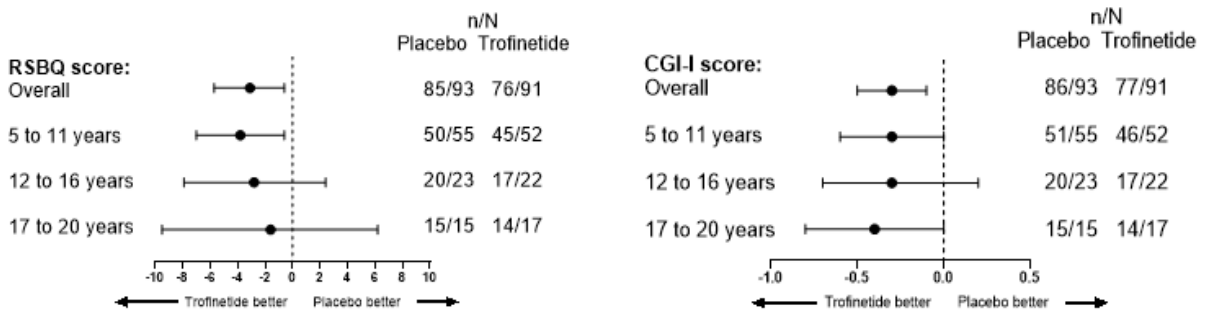
Abbreviations: CGI-S=Clinical Global Impression–Severity; ICND=Impact of Childhood Neurologic Disability Scare; LSM=least squares mean; QoL=quality of life; RTT-AMB=Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills; RTT-CBI=Rett Syndrome Caregiver Burden Inventory; RTT-COMC=Rett Syndrome Clinician Rating of Ability to Communicate Choices; RTT-HF=Rett Syndrome Clinician Rating of Hand Function; RTT-VCOM=Rett Syndrome Clinician Rating of Verbal Communication; SE=standard error

Ancillary analyses

Pre-defined subgroup analyses

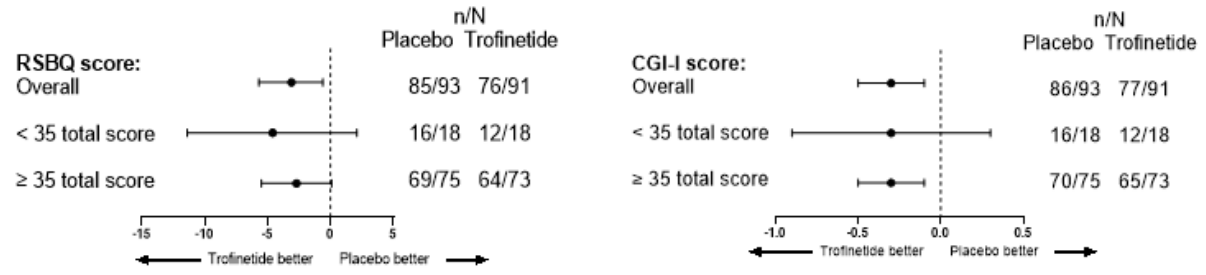
Forest plots of LSM difference (95% CI) for the coprimary endpoints (RSBQ change from Baseline to Week 12 and CGI-I at Week 12) by age group, Baseline RSBQ score, and mutation severity category are provided in Figure 20, Figure 21 and Figure 22.

Figure 20: Forest Plot of LSM Treatment Difference With 95% CI (MMRM) for Co-primary Endpoints by Age Group - Full Analysis Set



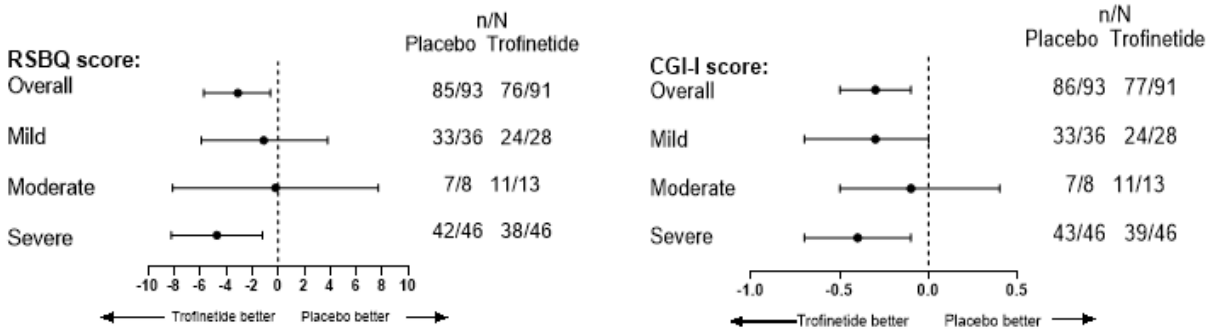
Sources: Study 003 CSR Figures AH1.2 and AH2.2 and Study 003 CSR Tables AH14.2.1.1.3 and AH14.2.2.1.3
Abbreviations: CGI I=Clinical Global Impression–Improvement; CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behaviour Questionnaire

Figure 21: Forest Plot of LSM Treatment Difference With 95% CI (MMRM) by Baseline RSBQ Severity - Full Analysis Set



Sources: Study 003 CSR Figures 1.2 and 2.2
Abbreviations: CGI I=Clinical Global Impression–Improvement; CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behaviour Questionnaire.

Figure 22: Forest Plot of LSM Treatment Difference With 95% CI (MMRM) by MECP2 Mutation Severity - Full Analysis Set



Sources: Study 003 CSR Figures 1.2 and 2.2
Abbreviations: CGI I=Clinical Global Impression–Improvement; CI=confidence interval; LSM=least squares mean; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behaviour Questionnaire

Pre-defined post hoc analyses

CGI-I responder analysis

A prespecified analysis of response on CGI-I was conducted in which a responder was defined as a subject with a CGI-I score of 1 (very much improved) or 2 (much improved) using missing imputed nonresponders. At Week 12, 4.3% of subjects in the placebo group and 11.0% of subjects in the trofinetide group were considered CGI-I responders by these criteria (odds ratio: 2.8 [95% CI: 0.8, 9.3; nominal p=0.0955] Table 28).

Table 28: Analyses of CGI-I Response (CGI-I⩽2) Week 12 (Logistic) - Full Analysis Set

	Placebo Week 12	**Trofinetide Week 12**	**Treatment group comparison (Placebo - Trofinetide)**		
CGI-I: Response of 1 or 2	**n/N (%)**	**n/N (%)**	**Odds ratio**	**(95% CI)**	**2-sided p-value**
Missing as nonresponders	4/93 (4.3)	10/91 (11.0)	2.8	(0.8, 9.3)	0.0955
Observed cases	4/86 (4.7)	10/77 (13.0)	3.1	(0.9, 10.6)	0.0637

Sources: Study 003 CSR Tables 14.2.12.1 and 14.2.12.2
Abbreviations: CGI-I=Clinical Global Impression–Improvement; CI=confidence interval

Post hoc analyses

CGI-I responder analysis (any improvement)

An additional responder analysis was performed where a response is defined by having any improvement on CGI-I (i.e., score 1, 2, or 3) at Week 12, while subject with missing data at Week 12 is considered as non-responder. Based on this definition, the percentage of responders is 14% (13/93) in the placebo group compared to 32% (29/91) in the trofinetide group, yielding a nominal p-value=0.0042 using a logistic regression adjusted for age group, baseline RSBQ severity category and baseline CGI-S as a covariate.

Table 29: CGI-I Responder Analysis by Visit (MN, Logistic) (Study ACP-2566-003) - Full Analysis Set

Visit	**Response Criteria**	**Placebo (N=93)**	**Trofinetide (N=91)**
Week 2, n (%)	CGI-I scores of 1, 2, or 3	16/93 (17.2)	23/91 (25.3)
Logistic regression[a]			
Odds Ratio			1.6
95% CI			(0.8, 3.4)
Two-sided p-value			0.1794
Week 6, n (%)	CGI-I scores of 1, 2, or 3	19/93 (20.4)	25/91 (27.5)
Logistic regression[a]			
Odds Ratio			1.5
95% CI			(0.7, 2.9)
Two-sided p-value			0.2623
Week 12, n (%)	CGI-I scores of 1, 2, or 3	13/93 (14.0)	29/91 (31.9)
Logistic regression[a]			
Odds Ratio			2.9
95% CI			(1.4, 6.1)
Two-sided p-value			(0.0042)

Source: Table 5.2a-responder-cgii-mn
Abbreviations: CGI-I=clinical global impression-improvement; MN=missing imputed as non-responders; SE=standard error; CI=confidence interval
Missing values imputed as non-responders.
A: Logistic regression on the outcome of response adjusted for age group, Baselined RSBQ severity, and planned treatment factors and Baseline CGI-S as a covariate.

Summary of main efficacy results

The following table summarise the efficacy results from the main study supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 30: Summary of efficacy for trial 003

Title: Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study of Trofinetide for the Treatment of Girls and Women With Rett Syndrome				
Study identifier	ACP-2566-003 NCT04181723			
Design	Phase 3, multicentre, randomised, double-blind, placebo-controlled, parallel-group study			
	Duration of main phase:		12 weeks	
	Duration of Run-in phase:		Not applicable	
Hypothesis	Superiority			
Treatments groups	Trofinetide		Weight-based dosing for 12 weeks	
			Weight	**Dose** **Total daily dose**
			12 to 20 kg	30 mL (6 g) BID 60 mL (12 g)
			>20 to 35 kg	40 mL (8 g) BID 80 mL (16 g)
			>35 to 50 kg	50 mL (10 g) BID 100 mL (20 g)
	Placebo		Same weight-based dosing for 12 weeks 94 subjects randomised	
Endpoints and definitions	Co-Primary endpoint	RSBQ	Change from baseline to Week 12 in RSBQ total score	
	Co-Primary endpoint	CGI-I	CGI-I score at Week 12	
	Key Secondary endpoint	CSBS-DP-IT Social Composite Score	Change from Baseline to Week 12 in the CSBS-DP-IT Social Composite Score	
Database lock	11 November 2021			
Results and Analysis				
Analysis description	**Primary Analysis**			

Analysis population and time point description	Full Analysis Set (FAS): all subjects who were randomised, received at least one dose of study drug, and had both a baseline value and at least one postbaseline value for the RSBQ total score of have at least one CGI-I score after taking the study medication.		
Descriptive statistics and estimate variability	Treatment group	Placebo	Trofinetide
	Number of subjects	93	91
	RSBQ Change from baseline to Week 12	-1.7	-4.9
	95% CI	(-3.5, 0.0)	(-6.7, -3.0)
	CGI-I	3.8	3.5
	95% CI	(3.7, 4.0)	(3.4, 3.7)
Effect estimate per comparison	Co-Primary endpoint: RSBQ	Comparison groups	Trofinetide and placebo
		LS mean difference	-3.1
		95% CI	(-5.7, -0.6)
		P-value (MMRM)	0.0175
	Co-Primary endpoint: CGI-I	Comparison groups	Trofinetide and placebo
		LS mean difference	-0.3
		95% CI	(-0.5, -0.1)
		P-value (MMRM)	0.0030

Notes	For RSBQ, the mixed-effects model for repeated measures included effects for treatment group, age group (5 to 10 years old, 11 to 15 years old, and 16 to 20 years old), RSBQ severity, visit, treatment-by-visit interaction as fixed effects, Baseline RSBQ total score, and the Baseline RSBQ total score-by-visit interaction as covariates and subjects as random effect. The MMRM for the CGI-I analysis included effects for treatment group, age group (5 to 10 years old, 11 to 15 years old, and 16 to 20 years old), Baseline RSBQ severity (<35 total score and ≥35 total score), visit, treatment-by-visit interaction as fixed effects, subjects as random effect, and baseline CGI-S score, and the baseline CGI-S score-by-visit interaction as covariates. Drop-outs: 9 discontinuations in placebo group (AE: 2; protocol deviation: 1; consent withdrawal: 1; other: 5) and 23 discontinuations in trofinetide group (AE=16; lack of efficacy=1; non-compliance=4; consent withdrawal=1; other=1).		
Analysis description	**Secondary analysis** Key secondary endpoint (pre-specified analysis)		
Analysis population and time point description	FAS Week 12		
Descriptive statistics and estimate variability	Treatment group	Placebo	Trofinetide
	Number of subjects	93	91
	CSBS-DP-IT Social Composite Score Change from baseline to Week 12	-1.1	-0.1
	95% CI	(-1.6, -0.6)	(-0.6, 0.5)
Effect estimate per comparison	Key secondary endpoint: CSBS-DP-IT Social Composite Score	Comparison groups	Trofinetide and placebo
		LS mean difference	1.0
		95% CI	(0.3, 1.7)
		P-value	0.0064
Notes	Analysis done using a mixed-effects model for repeated measures.		

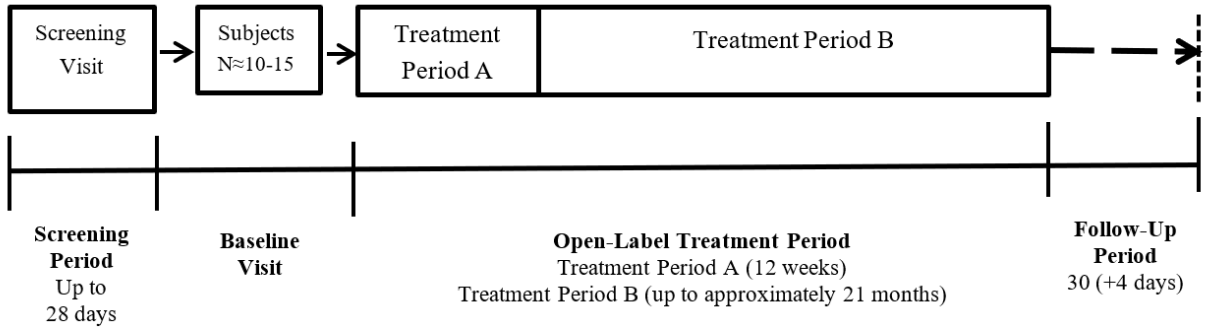
2.6.5.3. Clinical studies in special populations

Study 009 in subjects with Rett Syndrome aged 2 to 3 years

Methods

Study 009 was a multicenter, OL study consisting of 3 periods: Screening (up to 4 weeks), Treatment Periods A (12 weeks) and B (up to approximately 21 months), and Safety follow up period (30 [+4] days), see Figure 23.

Figure 23: Schematic of Study Design for ACP-2566-009



Source: Study 009 CSR Figure 9-1

All subjects began treatment with trofinetide 10 mL (2 g) BID. The dose was increased to 20 mL (4 g) BID at the Week 2 visit. At the Week 4 visit, the dose was increased to 25 mL (5 g) BID for subjects who weighed ≥9 to <12 kg (based on weight at Baseline visit) or 30 mL (6 g) BID for subjects who weighed 12 to <20 kg. In each case, the dose was only increased if the Investigator judged that the subject was showing acceptable tolerability of the treatment. The final assigned dose (i.e., highest tolerated dose) was given BID, wherein the morning and afternoon or evening doses were identical.

The study enrolled females between the ages of 2 and 4 years with a body weight ≥9 kg and <20 kg. They had a diagnosis of classical/typical RTT or possible RTT according to the Rett Syndrome Diagnostic Criteria. The diagnosis of "possible" RTT is given to those individuals under 3 years old who have not lost any skills but otherwise have clinical features suggestive of RTT. Subjects were required to have a documented mutation of the MECP2 gene. Subjects were required to have a CGI-S score of 4 or greater at Screening and Baseline. Each subject had to be able to swallow study drug provided as a liquid solution or be able to receive study drug via G-tube.

The efficacy objectives in this study were exploratory as the focus of the study was on safety, tolerability, and PK assessments. Efficacy assessments consisted of two clinician rated scales, CGI-I and CGI-S, and two caregiver rated scales, CaGI-I and ICND QoL.

Results

A total of 15 subjects were enrolled. Two subjects (13.3%) were discontinued from the study due to an AE; one subject (6.7%) was discontinued from the study due to non-compliance with the study drug, and 12 subjects (80.0%) were discontinued after termination of the study by the sponsor when commercial trofinetide became available post-FDA approval. The overall mean (SE) age was 3.1 (0.21) years and ranged from 2 to 4 years. The overall mean (SE) age at RTT diagnosis for all subjects was 1.98 (0.106) years and ranged from 1.1 to 3.0 years.

No hypothesis testing was planned. Descriptive summaries of all efficacy endpoints at week 12 (end of period A) are presented below:

- The mean (SE) CGI-I score was 3.1 (0.22)
- The mean (SE) CaGI-I score was 2.3 (0.12)
- The change from baseline in mean (SE) of the Overall Quality of Life Rating of the ICND was 0.3 (0.19)
- The mean (SE) CGI-S score was 4.7 (0.19)

2.6.5.4. In vitro biomarker test for patient selection for efficacy

Not applicable

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable

2.6.5.6. Supportive studies

Open-label extension studies 004 & 005

Studies 004 and 005 were open-label extension studies that lasted up to 40 weeks (study 004) and up to an additional 32 months of treatment (study 005). Subjects who completed preceding Study 003 were eligible to enroll in study 004; in addition, subjects who discontinued Study 003 due to the COVID-19 PHE or those who completed Study 003 but could not immediately continue to Study 004 due to the COVID-19 PHE were allowed to be re-evaluated for enrolment eligibility. Subjects who completed Study 004 were eligible to enrol in Study 005.

Subjects received oral trofinetide BID dosed by weight, according to the same banding as in Study 003. The assigned dose in Study 004 was based on the subject's weight at Baseline in Study 004, and the dose in Study 005 was the final dose received in Study 004. The same endpoints were evaluated as in study 003 and only descriptive summary statistics were provided.

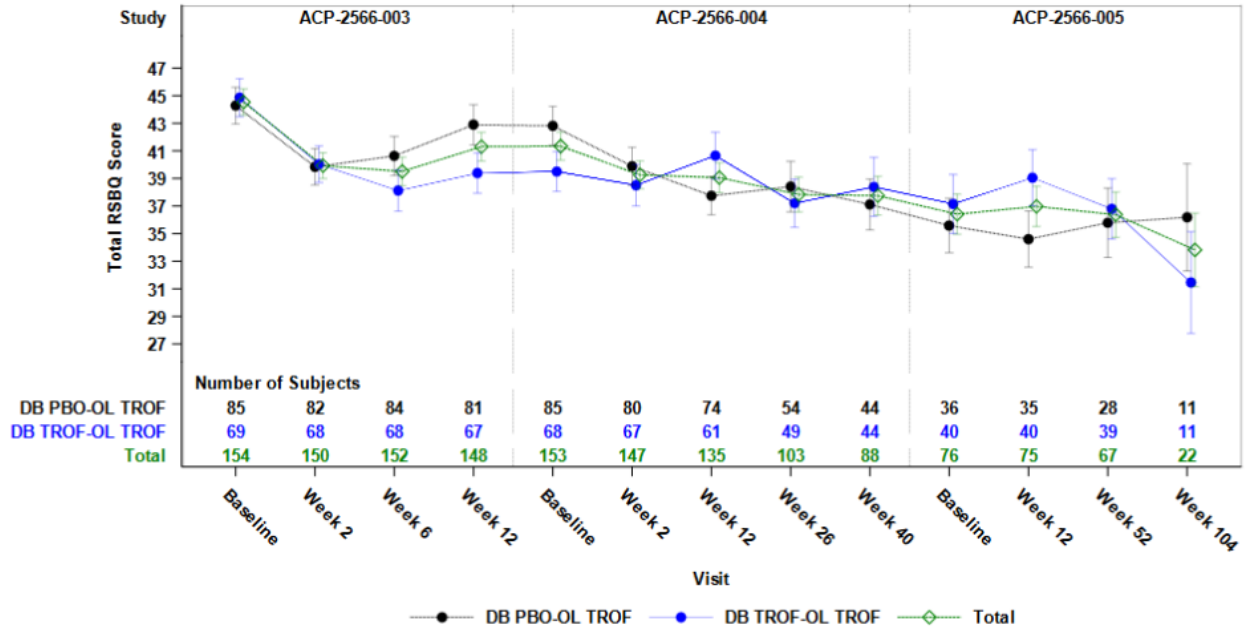
Results

In study 004, 154 subjects enrolled into the extension study and 84 subjects (54.5%) completed the study. The most common reason for ET was an AE (35.7%); of the 55 subjects who discontinued due to an AE, 'diarrhoea' was reported in 33 subjects. Other reasons for ET included lack of efficacy and non-COVID-19-related consent withdrawal (3.2% each), non-compliance with study drug (1.9%), and Other not related to COVID 19 (1.3%).

In study 005, 77 subjects enrolled into study 005. Most subjects (79.2%) terminated before the 130-week visit due to study termination by the Sponsor (i.e., subjects who switched to the commercially available product were not required to complete the follow-up visit). Five subjects (6.5%) terminated early due to an AE, and 4 subjects (5.2%) terminated early due to death. Other reasons for ET were lack of efficacy (3 subjects, 3.9%), noncompliance with study drug (2 subjects, 2.6%), and other not related to COVID-19 (2 subjects, 2.6%).

The RSBQ per visit in the open-label studies is displayed in Figure 24 below.

Figure 24: RSBQ Total Score from DB Baseline by Visit (Mean ± SE) - Safety Analysis Set (Subjects who Continued from ACP-2566-003 to Open-label Extension Study(ies))



Study RETT-001

Methods

Study RETT-001 was an exploratory Phase 2, randomized, DB, placebo-controlled, dose escalation study of the safety and tolerability of oral treatment (or via G-tube) with trofinetide (35 or 70 mg/kg BID) in 56 adolescent and adult females with RTT, aged 16 to 45 years. Trofinetide was administered for 4 weeks.

The analysis of results focused on a composite of clinical response. The efficacy variables in this study were categorized into 4 domains: clinician-completed syndrome-specific measures, clinician-completed syndrome-specific global measures, caregiver-completed syndrome-specific and general measures, and physiological measures, with each domain including core (primary), secondary and/or exploratory variables. The 6 core efficacy variables were as follows:

- Clinician-completed syndrome-specific variables (efficacy domain 1)
    - Mean change from baseline to end of treatment at the protocol-specified dose level in MBA change index
    - Mean change from baseline to end of treatment at the protocol-specified dose level in CSS change index
- Clinician-completed syndrome-specific global variables (efficacy domain 2)
    - Actual values at end of treatment at the protocol-specified dose level for CGI-I score
- Caregiver-completed syndrome-specific and general variables (efficacy domain 3)
    - Mean change from baseline to end of treatment at the protocol-specified dose level in the total VAS score for the Caregiver Top 3 Concerns (syndrome-specific)
    - Mean change from baseline to end of treatment at the protocol-specified dose level (in ABC total score (general)
- Physiologic variables (efficacy domain 4)
    - Mean change from baseline to end of treatment at the protocol-specified dose level in modified apnea index, obtained from video EEG sessions

The study outcome was considered positive (i.e., indicative of overall biological activity of trofinetide) if efficacy was detected at the group-level and/or subject-level, and if a corresponding trend was evident in the other (i.e., either group- or subject-level) analysis. A corresponding trend was defined as numerical superiority for point estimates that may or may not have been statistically significant (at $p < 0.2$). Overall conclusions regarding biological activity/efficacy were based on the totality of all available data.

Results

A total of 56 female subjects from 3 study sites, were enrolled in this study: 20 subjects received placebo; 18 subjects in Cohorts 0 and 1 received 35 mg/kg b.i.d. of trofinetide; and 17 subjects in Cohort 2 received 70 mg/kg b.i.d trofinetide. 53 subjects completed the study. The mean age of all subjects was 25.3 years (range 15.9 to 44.2 years), the majority of subjects were white (91%), and the mean BMI of all subjects was 21.84 kg/m2.

The results of the efficacy outcomes are summarised in Table 31. Trofinetide 70 mg/kg BID was associated with benefit over placebo ($p<0.2$) in three core variables from three different efficacy domains.

Table 31: Group-Level Analysis of Core Efficacy Variables by Individual Cohort – mITT Population

Variable Timepoint	Minimal clinically significant difference	Cohort 0		Cohort 1		Cohort 2	
		Placebo (N=4)	35 mg/kg BID (N=5)	Placebo (N=5)	35 mg/kg BID (N=13)	Placebo (N=11)	70 mg/kg BID (N=17)
Clinician-completed syndrome-specific measures (Efficacy domain 1) - MBA change index							
Change to end of treatment[a]	2.3						
LS mean		-3.00	-2.20	-2.00	-1.92	-0.62	-2.01
SE		1.916	1.714	1.250	0.775	0.721	0.580
p-value vs. placebo[b]			0.765		0.959		0.146*
Clinician-completed syndrome-specific measures (Efficacy domain 1) - RTT-CSS change index							
Change to end of treatment[a]	0.8						
LS mean		-0.54	-0.97	-1.60	-1.15	-0.62	-0.83
SE		0.530	0.473	0.401	0.249	0.449	0.360
p-value vs. placebo[b]			0.564		0.359		0.713
Clinician-completed syndrome-specific global measures (Efficacy domain 2) – CGI-I							
End of treatment[a]							
LS mean		3.50	3.40	3.80	3.38	3.64	3.24
SE		0.387	0.346	0.351	0.218	0.218	0.175
p-value vs. placebo[b]			0.853		0.330		0.164*
Caregiver-completed syndrome-specific and general measures (Efficacy domain 3) - Caregiver Top 3 Concerns VAS total score							
Change to end of treatment[a]	21						
LS mean		-60.75	-97.20	-33.84	-53.18	-23.71	-62.59
SE		26.238	23.468	35.435	21.976	16.388	13.183
p-value vs. placebo[b]			0.335		0.649		0.076*
Caregiver-completed syndrome-specific and general measures (Efficacy domain 3) - ABC total score							
Change to end of treatment[a]	3.2						
LS mean		-15.79	-21.97	-7.07	-9.51	-8.23	-7.20
SE		2.577	2.291	8.277	5.112	3.731	2.993
p-value vs. placebo[b]			0.132		0.806		0.832
Physiological measures (Efficacy domain 4) – Modified apnea index							
Change to end of treatment[a]	1.9						
LS mean		5.36	3.30	1.96	-2.66	-6.32	-4.36
SE		5.857	5.222	2.941	1.824	1.514	1.214
p-value vs. placebo[b]			0.805		0.202		0.324

Source: Study RETT-001 CSR Table 11-5

Abbreviations: ABC=Aberrant Behavior Checklist; ANCOVA=analysis of covariance; BID=twice daily; CGI-I=Clinical Global Impression–Improvement; MBA=Motor Behavior Assessment; mITT=modified Intent-to-treat; LS=least squares; RTT-CSS=Rett Syndrome Clinical Severity Scale; SE=standard error of the mean; VAS=Visual Analog Scale

* Met the prespecified threshold for significance: if a difference in favor of the active treatment group over placebo (p-value<0.2) was demonstrated for at least two core efficacy variables from two different efficacy domains, provided there was no clinically significant worsening in any other core efficacy variable.

[a] End of treatment refers to Day 14 for Cohort 0 and Day 26 for Cohorts 1 and 2.

[b] p-values are based on an ANCOVA with dose group as a main effect and baseline value as a covariate. If baseline values were not significant at the 0.1 two-sided significance level, it was dropped from the model.

2.6.6. Discussion on clinical efficacy

The claimed indication for trofinetide oral solution is: "*for the treatment of Rett syndrome in adults and paediatric patients 2 years of age and older.*"

The clinical development plan of trofinetide consisted of 6 studies: pivotal study 003 and supportive studies RETT-002, 004, 005, 009 and RETT-01.

Design and conduct of pivotal study 003

The pivotal study 003 was a phase 3, randomized, double-blind, placebo-controlled study in girls and women with RTT aged 5 to 20 years. The duration of the double-blind treatment period was 12 weeks and subjects were randomized 1:1 to trofinetide and matching placebo. Treatments were administered based on weight-banded dosing (12 – 20 kg, >20 – 35 kg, >35 – 50 kg and >50 kg). The general design of the study is sufficient to allow for an evaluation of short term efficacy of trofinetide in RTT. The duration is too short to establish long term efficacy of trofinetide.

Subjects eligible for inclusion were girls and women aged 5 to 20 years weighing ≥12 kg with a diagnosis of classical or typical RTT, a RTT-CSS severity rating of 10 to 36 and a score of ≥4 on the CGI-S scale, which corresponds to a population markedly to moderately ill. In addition, all subjects were required to have a documented mutation in MECP2 gene and had to be stable in their disease course (i.e. post regression stage). Subjects who have been treated with growth hormone or IGF-1 or with insulin within 12 weeks of Baseline were not eligible to the Study.

- The co-primary endpoints for the trial were the RSBQ and the CGI-I and the choice to combine these specific endpoints was based on previous interactions with the FDA. As part of the national scientific advices provided by France (July 2020), Germany (July 2020), Sweden (September 2020) and the Netherlands (September 2020) overall, the agencies considered the proposal as being acceptable with the proper justification on validity and clinical relevance included in the MAA.

The RSBQ is a caregiver completed questionnaire that covers behavioural symptoms related to mood, hand behaviours, repetitive face movements etc. It was initially developed as a tool to differentiate females with RTT from those with other severe intellectual disability. It remains unclear whether the RSBQ has been validated as an endpoint for clinical trials as scientific literature is inconclusive. The RSBQ may not be sensitive enough to detect a change due to the scoring system used (0- not true / 1- somewhat or sometimes true / 2- very or often true), especially as when an overall score is used on a group level. Although it remains unclear whether the RSBQ is an adequate primary endpoint for evaluation of clinical efficacy in RTT, it was the endpoint that was formally evaluated as co-primary endpoint in the study and the applicant was not able to provide any further conclusive evidence of its validity.

The CGI-I is considered a validated endpoint which is widely used in clinical trials, including for rare diseases. RTT- specific anchors were used for scoring.

The key secondary endpoint was the CSBS-DP-IT Social Composite Score. It is agreed that communication with the RTT patient is an important aspect to the parent/caregiver, and the Social Composite Score (emotion, eye gaze, communication and gestures) focuses more on nonverbal aspects, which could be more suitable in the setting of RTT as most patients do not speak. This questionnaire is developed for use in infants and toddlers, but the rationale can be followed that there

is no reason to assume communication would be substantially different in older RTT patients as speech regression occurs early in life.

Additional secondary and exploratory endpoints measure the quality of life, hand function (RTT-HF), ambulation and motor skills (RTT-AMB), verbal communication (RTT-VCOM), ability to communicate choices (RTT-COMC), severity (CGI-S), caregiver burden (RTT-CBI) and impact of disability (ICND). Most of the endpoints measure specific aspects which are also covered by the RSBQ, e.g. hand movement. The RTT-HF, RTT-AMB, RTT-COMC and RTT-VCOM have been derived from the Rett Syndrome Domain Specific Visual Analog Scale (RTT-DSC), which is a scale that assesses the severity across 8 domains. It remains unclear if any of these endpoints have been validated for use as a clinical outcome measure, and how the natural disease fluctuations impact the outcome measures.

No functional measurements or outcomes have been included in the study specifically related to other core symptoms of RTT such as apnea/breathing, intellectually disability, sleep disturbance and seizures, although some of these aspects are to a minor extent covered by the RSBQ.

As a result of the COVID-19 pandemic, screening and enrolment of the study were temporarily paused on 18 March 2020 and resumed on 12 June 2020 with various risk mitigation measures. COVID-19 related events and treatment discontinuations have been addressed in the estimand and sensitivity analyses.

The issues related to the duration of the double-blind treatment period of the study and the co-primary endpoints RSBQ and CGI-I were highlighted previously in various national Scientific Advices.

Efficacy data and additional analyses of study 003

Study population

187 subjects were randomized in the study, of which 94 were allocated to placebo and 93 to trofinetide. 155 subjects (≈ 84%) completed the study, and the most common reason for premature study discontinuation in the trofinetide group was adverse event (n = 15). According to the Study protocol, in total, 208 unique subjects were screened, but some were rescreened, for a total of 227 screenings.

It is noted that during the double-blind treatment period, study visits 3 (Week 2) and 4 (Week 6) could have been performed off-site rather than in the clinic with the prior approval of the Sponsor or Medical Monitor.

There were 39 subjects included in study 003 that have previously participated in the Phase 2 trofinetide study 002. Of these 39, 10 received trofinetide in both studies. The Applicant's notion that this is 'common practice' for rare diseases is not agreed. Although the gap of 34 months between study periods would be sufficient to washout prior trofinetide exposure, this does not account for any potential bias introduced due to familiarity with effects/adverse events of the active treatment or in case of placebo lack thereof. Potential unblinding due to knowledge of the adverse event profile can also not be excluded. In contrast to the Applicant's notion that this is "common practice for rare diseases", even in rare disease trials usually efforts are made to not include patients previously included in another trial (unless it is an open label extension study), as this bias is difficult to mitigate. A sensitivity analysis was provided excluding the 39 subjects that participated in the phase II study. The effect estimates in this sensitivity analysis were somewhat larger, with conclusions being similar to the original analysis.

The study population was on average 11 years old and included 32 adult patients with RTT who were between 17 – 20 years of age. Baseline RSBQ scores were comparable between study arms and most

subjects were considered moderately or markedly ill according to baseline CGI-S scores. The study was conducted exclusively in the US but adequate justification was provided on the applicability of this study to an EU RTT patient population.

A substantially higher number of subjects from placebo group was treated with antidepressants compared to trofintide group (20.2% vs 8.6%), in particular for trazodone. Based on the doses of trazodone used (up to 62.5mg in all but one subject) it is expected this was mostly used to treat insomnia.

Dose reductions of up to 50% of the target dose were permitted up until week 6 of the study in the event of tolerance issues (e.g. gastrointestinal issues) as it was assumed that events related to diarrhoea would occur early in treatment and that dose adjustments were likely not necessary after 6 weeks. Temporary dose discontinuations also occurred at various timepoints during the study. The impact of dose reductions on efficacy outcomes remains uncertain. A modest change from baseline in RSBQ and CGI-I was observed, irrespective of dose modifications. Patients treated with trofinetide requiring a dose reduction, however, seem to have a smaller change from baseline in RSBQ versus placebo (-3.3±1.8 vs 0.4±4.3), compared to those who received their planned dose (-6.0±1.2 vs -1.8±1.0). Since impact of dose reductions on efficacy of trofinetide cannot be excluded, upon request from CHMP the Applicant has agreed to place a statement on the impact of dose reduction on the efficacy at the beginning of the dose modification section in the proposed SmPC (section 4.2), together with the recommendation to reinstate the planned dose as clinically appropriate (see safety section, of proposed SmPC). In the statistical analysis, dose adjustments were considered part of treatment policy, and that primary analysis was based on intention-to-treat using outcomes irrespective of dose-reductions.

It is noted that medication to treat AEs were prescribed by the investigator. The most commonly used medication to treat AEs were antidiarrhoeal medications.

Efficacy

After 12 weeks of treatment, trofinetide resulted in a statistically significant reduction in RSBQ score of 3.1 (p= 0.0175) and a statistically significant difference on the CGI-I of -0.3 (p = 0.0030) when compared to placebo. The clinical relevance of the observed difference cannot be evaluated. It is unclear whether a reduction in RSBQ scores indicate an improvement of symptoms, stabilization of the disease or whether this for example reflects disease fluctuation over time. The Applicant uses Cohen's d, a distribution-based calculation of MCID in the current population to interpret clinical relevance. A Cohen's d of 0.37 has been observed, which could correspond to a small effect. However, clinical relevance is usually determined based on a predefined MCID (based on clinical rather than statistical considerations) and/or supportive responder rates and secondary endpoints reflecting the relevance of the effect. Such an MCID has not been established for this endpoint in this condition.

The CGI-I has been evaluated as a population level mean difference rather than a category shift. The observed mean difference of -0.3 over placebo is not very informative. Adequacy of the primary analysis method for CGI-I score is of concern, because the CGI-I is a 7-point categorical endpoint, with observed range 2 to 5 at Week 12 in both placebo and trofinetide group, and identical medians in both groups. The pre-specified MMRM analysis resulted in a statistically significant but rather small mean treatment difference (-0.3). Considering the distribution of the CGI-I scale, and imbalance in dropout rates, it is likely that the MMRM, although pre-specified, is less suitable for analysis of CGI-I than non-parametrical methods. Sensitivity analysis was performed for CGI-I at Week 12 by using a non-parametric Cochran-Mantel-Haenszel (CMH) test with modified ridit scores stratified by the same covariates as originally included in the primary MMRM model. The conclusion was in line with the primary analysis for CGI-I, showing a significant difference between trofinetide and placebo (p = 0.0035).

An additional responder analysis was performed (as requested) where a responder was defined as having any improvement on the CGI-I (i.e. a score of 1, 2 or 3) at week 12. The percentage of responders is 14% (13/93) in the placebo group compared to 32% (29/91) in the trofinetide group. Thus, trofinetide treated patients had a higher chance of having any improvement on the CGI-I compared to placebo. This is considered a clinically relevant finding, although this is a post hoc analysis. In order to help further contextualize the findings on the RSBQ, the relationship between improvement on the RSBQ and CGI-I responder analysis was further evaluated and showed minimal concordance. Additional analyses with respect to responders on both RSBQ and CGI-I further complicate the interpretation of clinical relevance. It is argued by the Applicant that caregivers and HCPs consider any minimal improvement clinically relevant. Insights from the SAG-N, which included both RTT experts and patient representatives do not concur with this statement. They concluded that no difference was seen in the domains considered most important by caregivers based on the data presented. Therefor it is unclear how the findings on the co-primary endpoints translate into a benefit for a RTT patients daily functioning.

Considering the large number of subjects that experienced diarrhoea under trofinetide treatment, the impact of a possible functional unblinding due to diarrhoea cannot be excluded. Several analyses have been provided to evaluate to what extent the results on the co-primary endpoints could have been impacted by differential occurrence of diarrhoea. The provided analyses do shed some light on the complex relationship between treatment with trofinetide, reporting of diarrhoea and the co-primary endpoints, but they do not directly answer the question regarding the potential impact of functional unblinding. Differences in outcomes under trofinetide and differences compared to placebo seem to be present between those reporting diarrhoea and not reporting diarrhoea. Such differences could occur because of trofenetide treatment being discontinued after experiencing diarrhoea or when stronger clinical improvement is associated with higher risk of diarrhoea because of another mechanism (as a result of a confounder or underlying biological mechanism). It cannot be ruled out that functional unblinding occurred and potentially impacted the outcomes. Additional analyses have been provided to further evaluate the potential impact of functional unblinding. Although in the sensitivity analyses only small indirect effects of treatment through diarrhoea were observed, it remains uncertain if and how functional unblinding impacted the results.

Estimates for the co-primary endpoints were largely robust across the different sensitivity analyses. However, sensitivity analysis on subjects accounting for impact of remote assessments on the coprimary endpoints demonstrated a nominally significant interaction for subjects who had at least one remote RSBQ assessment (p=0.0323) but not for subjects who had at least one remote CGI-I assessment (p=0.1630).

For the key secondary endpoint CSBS-IT-DP Social composite score, a statistically significant difference was observed in favour of trofinetide of 1.0 (p= 0.0064). It is unknown how to interpret this treatment difference and what change on this endpoint would be anticipated in the natural course of RTT.

None of the secondary or exploratory endpoints have been formally evaluated as the Type-I error correction ended at the key secondary endpoint. With the exception of the RTT-COMC, none of the endpoints reach nominal statistical significance. More importantly, there appears to be no difference in motor function (RTT-AMB), hand function (RTT-HF) or verbal communication (RTT-VCOMB) as reflected in small numerical differences. In addition, no change in severity has been observed on the CGI-S, nor any improvement in quality of life (QoL rating of ICND) or caregiver burden (RTT-CBI).

It has been highlighted by the patient organization engagement that there is a need for treatments that target multiple core aspects of the syndrome, i.e. overall health, respiratory and sleep, neurological/seizures, motor function and communication and daily functioning. As discussed previously, not all RTT core features/symptoms have been covered by the endpoints selected for this

study. This was also noted by the SAG-N. A consistent (relevant) effect of trofinetide across the endpoints that do evaluate RTT core aspects has not been shown. In other words, there is no benefit across the whole disease spectrum. In light of this, the nonspecific mechanism of action, absence of evaluation of specific core RTT features and the findings that small numerical differences are observed for specific RTT symptoms, the sought indication "*treatment of Rett Syndrome*" is not supported.

Subgroup analyses for the co-primary endpoints have been performed for age, RSBQ severity and MECP2 mutation severity. For all subgroups, the point estimates favor trofinetide but have large confidence intervals. Some of the individual subgroups are too small to allow for any conclusions. Nevertheless in the analysis by MECP2 Mutation Severity Category, mild and moderate subjects appear to have a lower response based on RSBQ score analysis compared to severe subjects. Subgroup analysis based on MECP2 mutation severity category was based on categories in which specific mutations are placed, not the clinical severity of the illness of the patient. Therefore, it is not possible to draw any conclusions regarding benefits of trofinetide in subjects across mutations.

Thus, although the study met its objective i.e., showed a statistically significant effect in favour of trofinetide for the co-primary and key secondary endpoints, efficacy, clinical meaningfulness and consistency remain unsubstantiated.

Supportive studies

Dose ranging study 002

Study 002 was a randomized, double-blind placebo controlled study that evaluated 50 mg/kg, 100 mg/kg and 200 mg/kg BID trofinetide in girls with RTT aged 5 – 15 years. The total duration of treatment was 56 days. The primary analysis performed for the study are not considered standard practice (e.g. analysis based on dose actually received, retaining variables in the model at $p < 0.1$ etc.). In addition, there was no correction for multiple testing.

At the end of the double-blind treatment period, 200 mg/kg trofinetide was nominally statistically significant vs. placebo on three of the five prespecified "core variables": RSBQ Total, CGI-I and RTT-DSC Total. Large numerical improvements compared to baseline are observed for this group for particularly the RSBQ total and RTT-DSC total (-6.7 and -76.00, respectively), but it must be noted that large baseline differences were seen on these scales (especially RTT-DSC). In contrast, the 100 mg/kg trofinetide group performs worse than the 50 mg/kg group and for some endpoints worse than placebo (e.g. RSBQ total). Pharmacokinetic modelling has suggested a linear relationship between trofinetide dose and efficacy outcomes, which is not supported by the lack of a dose-response relationship in study RETT-002. The paradoxal effects were not further explained.

Open-label extension studies 004 & 005

Subjects who completed study 003 were eligible to enroll into the open-label extension study 004 (40 weeks) and subsequent extension study 005 (up until FDA drug approval). The primary objective of both studies was to evaluate the long-term safety and tolerability of trofinetide.

In study 004 for subjects for whom it was not possible to use the Week 12/EOT visit of the antecedent study as the Baseline visit (Visit 1) due to COVID-19, were re-evaluated for study eligibility at the Baseline visit of the present study, with approval by the Sponsor or Medical Monitor. There were a total of 15 subjects who were not able to use the Study 003 Week 12/ET/EoT visit as the Baseline visit in Study 004.

Trofinetide dosing was the same as in study 003. If the subject could not tolerate the full assigned dose (e.g., because of diarrhea), the dose could be reduced to a dose as low as half the assigned dose; additionally, up to four doses could be withheld for this reason. 79 (51.3%) subjects had dose reduced at least once during the Study. However, 18 of these subjects resumed back to initial dose level and 3

subjects eventually increased the dose level. No conclusions on influence of dose reduction can be made since there were large differences in the time spent with dose reduced (the mean duration was 59.1 days (SD 82.85 days), and a median of 21.0 days with minimum of 1 day and maximum of 291 days).

In study 004, 154 subjects enrolled into the extension study and 84 subjects (54.5%) completed the study. The most common reason for ET was an AE (35.7%); of the 55 subjects who discontinued due to an AE, 'diarrhea' was reported in 33 subjects. 77 subjects enrolled into study 005. Most subjects (79.2%) terminated before the 130-week visit due to study termination by the Sponsor (i.e., subjects who switched to the commercially available product were not required to complete the follow-up visit). Five subjects (6.5%) terminated early due to an AE, and 4 subjects (5.2%) terminated early due to death. Neither one of the studies can be used to substantiate long term efficacy/maintenance of effect of trofinetide. First, both studies lack a control arm for comparison. Second, selection bias cannot be excluded, as subjects for whom a treatment benefit is perceived are more likely to continue treatment. Third, almost half of the subjects prematurely discontinued study 004, and the high dropout rate hampers interpretation of the effects. In study 004, around 55% in the placebo – trofinetide group and around 35% in the trofinetide – trofinetide group prematurely discontinued the study. In both groups the most common reason given is adverse event, and the incidence is higher in subjects who switched to active treatment. It was concluded that long term studies are insufficient to support a claim of maintenance of effect and the proposed SmPC included a recommendation of regular re-assessment of treatment benefit.

The longest placebo-controlled data available is 12 weeks in study 003, despite an overall recommendation from the national competent authorities providing scientific advice that a 24-week duration would be expected. The 12 weeks duration of study 003 was considered insufficient to establish trofinetide efficacy. No conclusions on long term efficacy can be drawn from studies 004 and 005 due to the open-label design, selection bias and high dropout due to adverse events. In absence of a controlled study, maintenance of effect of trofinetide for the treatment of Rett syndrome cannot be claimed.

Use in children < 5 years of age

Study 009 in children aged 2 – 4 years of age

Study 009 was an open-label exploratory study in female RTT patients between the ages of 2 and 4. The main objective of the study was to evaluate the PK and safety of trofinetide in this particular age group. All efficacy related endpoints were evaluated as exploratory.

A total of 15 subjects were enrolled. Two subjects (13.3%) were discontinued from the study due to an AE. The study was terminated due to FDA approval of trofinetide and subjects were switched to commercial formulation.

The study included no endpoints that measured specific RTT features, only global evaluations of improvement (e.g. CGI-I) and severity (CGI-S). Considering that in this age group developmental regression is expected, it would have been interesting to evaluate the effects of trofinetide on such specific endpoints, to examine if it would prevent or slow regression. In absence of a control arm, it is difficult to evaluate efficacy of trofinetide in this age group. Moreover, several of the endpoints do not include a baseline measurement, so a comparison cannot be made with pre- and post treatment.

Thus, study 009 is insufficient to support efficacy of trofinetide in patients younger than 5 years of age.

Paediatric extrapolation

In absence of clinical efficacy data, paediatric extrapolation of efficacy from older children, adolescents and adult patients with RTT could be considered. Additional information to support paediatric

extrapolation has been provided by the Applicant on similarity in disease progression and response to intervention, similarity in exposure (see discussion of clinical pharmacology) and safety (see discussion of safety).

One of the key assumptions in paediatric extrapolation is that efficacy has been established in an (older) age group. The efficacy of trofinetide in older children > 5 years, adolescents and adults (i.e. plateau phase) has not been demonstrated. By default this would mean that paediatric extrapolation would not be feasible. In the event efficacy of trofinetide is established in the plateau phase, this cannot be extrapolated to the younger patients in the regression phase. Although the underlying MECP2 mutation remains the same and symptoms may be similar across the two phases, the frequency and severity may not. Similarity in these disease stages remains unclear. Moreover, it is also unknown whether a similar treatment response can be anticipated. This has also been highlighted by the SAG-N, indicating that different instruments might be needed to examine efficacy in the regression phase.

Use in older adults (> 20 years of age)

Exploratory study 001

Study RETT-001 can be considered an exploratory proof of concept study of trofinetide in patients with RTT aged 16 to 45 years. This is the only study in the clinical development program which includes older adult patients with RTT (study 003 included 32 subjects aged 17 – 20 years). Efficacy on a group level was considered shown if a difference in favor of trofinetide over placebo was demonstrated for at least 2 core efficacy variables from 2 different efficacy domains at a p-value of < 0.2. The rationale for this high p-value instead of the conventional p-value of 0.05 is not supported, as a high p-value increases the risk for chance findings.

The findings of the study are suitable for evaluating whether to continue with the clinical development program of trofinetide in RTT. But the results do not provide support for the sought indication, as the doses of trofinetide evaluated are substantially lower than the proposed posology in the proposed SmPC and the duration of treatment was 4 weeks. Next to the statistical issues highlighted above, small numerical differences were observed between the placebo and trofinetide groups over the cohorts, but it cannot be evaluated if these are clinically meaningful (e.g. compared to the MCID defined by the Applicant in the study). It is questioned whether 4 weeks of treatment would have been sufficient to show a clinically meaningful effect. Finally, the study did not include any subgroup analyses. An analysis per age group, to evaluate consistency of effect in older adults with RTT could have been interesting, but in light of the issues described above would not allow for any conclusions with respect to efficacy in older adults.

The claimed indication for trofinetide does not have an upper age limit and this is the only study that enrolled patients older than 20 years of age. A discussion was requested on the available data to support an indication in older adults. The degree of similarity between RTT disease presentation in adulthood and adolescence/childhood is inconclusive. There is literature that highlights differences but also literature that describes a type of stabilization phase in adulthood. High level interim results from the RWE Lotus study were provided for adult patients but the limited description of the data hampers the interpretation of results and selective reporting cannot be excluded. Subgroup analysis for this age group has been provided from the ongoing RWE Lotus study. Although it is somewhat reassuring to see that patients who have initiated trofinetide remain in the study, the results cannot be adequately interpreted due to the formulation of the endpoint (i.e. Caregiver perception of behavioral improvements). The issue remains that the available data in this age subgroup is limited. It was discussed that in principle there is no reason to discontinue trofinetide treatment in patients who become > 20 years if they benefit from the treatment. However, considering that efficacy has not been

demonstrated in study 003 (for any age group), the issue of extrapolation to older age groups was not further pursued.

Use in male patients with RTT

The sought indication makes no distinction between male and female patients with RTT. The pivotal study 003, as well as all other clinical efficacy studies of trofinetide, only enrolled females with RTT. According to the Applicant, there is no reason to assume difference in efficacy as there is no indication that the PK or PD of trofinetide would be different (see also section on clinical pharmacology). However, it is known that male patients with RTT, those that survive infancy, generally have a more severe phenotype compared to female patients. Therefore, CHMP requested additional justification to support the efficacy of trofinetide in male patients with RTT. The response provided by the Applicant was considered as unsatisfactory, as most of the discussion provided focused on the rarity of RTT in male patients and the inability to conduct a clinical trial and generate high quality evidence in this population. Additional discussion has been provided which indicate that the same diagnostic criteria are applied to male patients as to female patients with RTT. Although a direct comparison between phenotypes in males and females was not provided, it can be followed that overlap in symptomatology can be expected in particular as the same diagnostic criteria are applied regardless of sex. Limited data from males (n = 7) have been provided from the ongoing RWE Lotus study, although a conclusion with respect to trofinetide efficacy cannot be drawn. From a pragmatic perspective, given the rarity of RTT in males, that trofinetide is symptomatic treatment and there appears to be overlap in symptomatology, there is no need to restrict the indication to female patients with RTT. However, considering that efficacy has not been demonstrated in study 003 (for any age group or sex), the issue of extrapolation to male subjects was not further pursued.

2.6.7. Conclusions on the clinical efficacy

Support for the sought indication is primarily based on the (single) pivotal study (study 003), which showed a statistically significant difference in favour of trofinetide on the co-primary endpoints RSBQ total score, CGI-I and key secondary endpoint CSBS-DP-IT Social Composite score. However, the findings of pivotal study 003 preclude conclusions with respect to efficacy of trofinetide for the treatment of RTT. The clinical meaningfulness of the observed effects on the co-primary endpoints RSBQ and CGI-I and consistency across endpoints has not been demonstrated. The double-blind treatment phase of 12 weeks is too short to establish efficacy and the impact of functional unblinding due to the high incidence of diarrhoea remains uncertain. The provided efficacy data is insufficient to support use of trofinetide in paediatric patients in the regression phase, because efficacy cannot be extrapolated from the plateau phase to the regression phase of the condition and moreover efficacy has not been demonstrated in the plateau phase. The oral explanation and subsequent discussion did not lead to relevant new insights with respect to resolving these outstanding major objections.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

Evaluation of safety is mainly based on the double-blind placebo-controlled phase 3 Study ACP-2566-003 (n=187) and open-label extension studies ACP-2566-004 (n= 154) and ACP-2566-005 (n=77), in subjects with RTT. Data on the following additional studies, are also included:

- Phase 2 dose-ranging study Neu-2566-RETT-002 (n=82) to evaluate safety and tolerability in girls (5-16 years) with RTT, treated up to 6 weeks with placebo or trofinetide up to 200 mg/kg.

- Phase 2 dose escalation study Neu-2566-RETT-001 (n=56) to evaluate safety and tolerability of trofinetide up to 70 mg/kg, in females (16- 44 years) with RTT, up to 4-weeks.
- Open-label study ACP-2566-009 (Study 009) to evaluate safety and PK of trofinetide in subjects with RTT aged 2 to 4 years (n=15), up to 104 weeks of treatment.

None of the studies solicited the collection of AEs of special interest.

In total 10 phase I studies in healthy subjects were completed with intravenous or oral trofinetide, including studies assessing specific aspects: ACP-2566-008 was a study to evaluate the effects of single doses of trofinetide on QTc interval (n=58), ACP-2566-010 was a study to evaluate safety and tolerability in subjects with moderate renal impairment versus healthy subjects (n=20), and ACP-2566-012 was a study to evaluate the interaction of trofinetide with loperamide (n=13).

Pooling strategy

For safety evaluation, data were integrated and analysed across four pools, representing the randomized double-blind studies in RTT, fragile X syndrome (FXS), and traumatic brain injury (TBI), and open-label studies of long-term safety in RTT.

RTTDB Pool: Safety data from placebo-controlled studies in RTT (003, Rett-002, Rett-001).

ALLDB Pool: Safety data from the placebo-controlled studies in RTT (003, Rett-002, Rett-001) with the addition of two placebo-controlled studies in FXS and TBI.

RTTLT Pool: Safety data from Study 003 and subsequent open-label extension Studies 004 and 005.

RTTOL Pool: Safety data from the open-label extension Studies 004 and 005.

Safety data from study 009 (subjects with RTT aged 2 to 4 years) were not included in the safety pools due to the small sample size, and since it was the only study with subjects <5 years of age.

The detailed description of the pools is summarised in Table 32.

Table 32: Pooling Datasets for Integrated Safety Analyses

Pooled population	Description (study numbers)	Groups presented
RTTDB	Placebo-controlled double-blind data in RTT: • ACP-2566-003 • Neu-2566-RETT-001 • Neu-2566-RETT-002	Placebo <200 mg/kg BID TROF ≥200 to 500 mg/kg BID TROF All TROF ACP-2566-003 placebo ACP-2566-003 TROF
ALLDB	Placebo-controlled, double-blind data in all indications: • ACP-2566-003 • Neu-2566-RETT-001 • Neu-2566-RETT-002 • Neu-2566-TBI-003 • Neu-2566-FXS-001	Placebo <200 mg/kg BID TROF ≥200 to 500 mg/kg BID TROF All TROF ACP-2566-003 placebo ACP-2566-003 TROF
RTTLT	RTT Phase 3 long-term data: • ACP-2566-003 • ACP-2566-004 • ACP-2566-005	All TROF
RTTOL	RTT Phase 3 long-term, open-label data: • ACP-2566-004 • ACP-2566-005	All TROF

Abbreviations: ALLDB=all subjects in double-blind oral-treatment studies; BID=twice daily; RTTDB=Rett syndrome in double-blind oral-treatment studies; RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; RTTOL=trofinetide-treated subjects with Rett syndrome in open-label long-term extension studies; TROF=trofinetide

Exposure in RTT population

The mean duration of exposure in Study 003 was 74 days in the trofinetide group and 82 days in the placebo group. In total 72% in the trofinetide group and 86% in the placebo group were exposed for ≥12 weeks.

The RTTDB Pool combines Phase 2 studies that utilized a mg/kg dosing regimen at multiple dose levels. Whereas Study 003 utilized a single dose level based on weight bands to achieve target exposure. The lower dosing groups from Phase 2 studies are shown in the <200 mg/kg column, the highest dose group from Study 002 (200 mg/kg) overlaps with the dosing in Phase 3, and is shown in the ≥200 to 500 mg/kg column.

In the RTTDB Pool, the mean duration of exposure to trofinetide was 58 days, 72% were exposed for ≥ 6 weeks, and 40% were exposed for ≥12 weeks.

Drug exposures for the RTTDB Pool and Study 003 are shown in Table 33.

Table 33: Study Drug Exposure - RTTDB Pool and Study 003

	Placebo (N=133)	Trofinetide			Study ACP-2566-003[a]	
		<200 mg/kg BID (N=67)	≥200 to 500 mg/kg BID (N=115)	All trofinetide (N=176)	Placebo (N=94)	Trofinetide (N=93)
Duration of double-blind exposure (days)[b]						
n	133	67	115	176	94	93
Mean (SE)	69.0 (2.47)	33.0 (1.19)	68.8 (2.39)	57.5 (2.21)	81.5 (1.66)	73.5 (2.50)
SD	28.46	9.72	25.67	29.27	16.07	24.14
Median	85.0	28.0	84.0	42.0	85.0	85.0
Minimum, maximum	10, 128	1, 43	8, 124	1, 130	10, 100	8, 93
Duration of double-blind exposure - categorical, n (%)						
<1 week	- -	1 (1.5)	- -	1 (0.6)	- -	- -
≥1 week to <2 weeks	1 (0.8)	- -	6 (5.2)	5 (2.8)	1 (1.1)	6 (6.5)
≥2 weeks to <6 weeks	23 (17.3)	36 (53.7)	7 (6.1)	43 (24.4)	3 (3.2)	8 (8.6)
≥6 weeks to <12 weeks	28 (21.1)	30 (44.8)	33 (28.7)	57 (32.4)	9 (9.5)	12 (12.9)
≥12 weeks	81 (60.9)	- -	69 (60.0)	70 (39.8)	81 (86.2)	67 (72.0)

Source: Table RTTDB.MAA.4 and Study 003 CSR Table 14.1.12.1

Abbreviations: BID=twice daily; RTTDB=Rett syndrome in double-blind oral-treatment studies; SD=standard deviation; SE=standard error of the mean

Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study ACP-2566-003. Subjects can be counted in multiple dose/drug columns if a different dose/drug was administered in these studies. However, these subjects are only counted once in the placebo or all trofinetide column.

N is used as the denominator for calculating percentages within each column.

a Subjects who received trofinetide in Study ACP-2566-003 are also included in the ≥200 to 500 mg/kg BID and the all trofinetide columns. Subjects who received placebo in Study ACP-2566-003 are also included in the all placebo group.

b Duration of exposure to double-blind study drug within each study is calculated as last double-blind study dose date - first double-blind study dose date +1.

Long-term exposure in RTT

For subjects in the studies comprising the RTTLT pool, a single trofinetide dose level was dosed based on weight bands to achieve target systemic exposure. In this report these dose levels are considered one active treatment arm ("all trofinetide group").

In the RTTLT Pool, the mean duration of exposure to trofinetide was 396 days, and 134 subjects were exposed for ≥12 weeks. Subjects with exposure of ≥6, ≥9, and ≥12 months were 56% (100), 49% (87), and 45% (80), respectively (Table 34).

Table 34: Study Drug Exposure in the RTTLT Pool

	All trofinetide (N=178)
Duration of open-label exposure (days)[a]	
n	178
Mean (SE)	396.6 (25.83)
SD	344.60
Median	260.0
Minimum, maximum	8, 1190
Duration of open-label exposure - categorical, n (%)	
<6 weeks	23 (12.9)
≥6 weeks to <12 weeks	21 (11.8)
≥12 weeks to <6 months	34 (19.1)
≥6 months to <9 months	13 (7.3)
≥9 months to <12 months	7 (3.9)
≥12 months to <18 months	12 (6.7)
≥18 months to <24 months	24 (13.5)
≥24 months	44 (24.7)

Source: Table RTTLT.MAA.4

Abbreviations: RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; SD=standard deviation; SE=standard error of the mean

Note: N is used as the denominator for calculating percentages within each column.

[a] Duration of exposure to trofinetide within each study is calculated as last trofinetide dose date - first trofinetide dose date +1. If a subject experienced continuous study drug interruption for more than 30 days, the duration of exposure was calculated as last trofinetide dose date – first trofinetide dose date + 1 - duration of interruption. For a given subject, the total duration of trofinetide exposure is calculated as the sum of trofinetide exposure durations from all studies in which the subject received trofinetide.

Total exposure in RTT studies

Cumulative exposure to trofinetide across **all clinical studies in RTT** (Rett-001, Rett-002, 003, 004, 005, and 009) included 92 subjects exposed for at least 12 months, and 44 subjects exposed for 24 months (Table 35).

Table 35: Duration of Exposure to Trofinetide in RTT Studies (Rett-001, Rett-002, 003, 004, 005, 009)

Duration of Trofinetide Exposure[1], n(%)	Total (N=260)
<=2 weeks	17 (6.5)
>2 to 6 weeks	38 (14.6)
6 weeks to <12 weeks	55 (21.2)
12 weeks to <6 months	38 (14.6)
6 months to <9 months	11 (4.2)
9 months to <12 months	9 (3.5)
12 months to <18 months	22 (8.5)
18 months to <24 months	26 (10.0)
24 months	44 (16.9)
Total	260 (100.0)

Exposure in other RTT studies

Fifteen subjects 2 to 4 years of age were enrolled and were exposed to trofinetide treatment in **study 009**. The mean (SE) duration of exposure to trofinetide was 434 (46) days. In total 13/15 subjects were exposed for ≥12 weeks, 12 subjects were exposed for ≥12 months, and 2 subjects were exposed for ≥18 months.

Exposure in phase I studies

In the 10 Phase 1 studies, a total of 187 healthy adult subjects received trofinetide, including 77 subjects (46 males and 31 females) who received intravenous trofinetide and 110 subjects (73 males and 37 females) who received oral trofinetide.

2.6.8.2. Adverse events

Overview of adverse events

In Study 003 (n=187), the overall incidence of TEAEs was 93% with trofinetide and 54% with placebo, of which the majority was considered related to trofinetide (84% vs 21% in the placebo group). Overall, 41% and 38% of subjects experienced mild AEs, and 45% and 11% experienced moderate AEs, with trofinetide and placebo respectively. Severe AEs were reported in 7% with trofinetide and 3% with placebo. In total, 3% of subjects in both groups, experienced an SAE, of which 2% (trofinetide) and 1% (placebo) were considered related. In total 17% of subjects with trofinetide and 2% of subjects with placebo, experienced TEAEs leading to discontinuation (Table 36).

In Study RETT-002 (n=82), the incidence of TEAEs was 67% in placebo, 60% in the 50 mg/kg, 81% in the 100 mg/kg and 78% in the 200 mg/kg group. Overall, most TEAEs were of mild or moderate severity. Three subjects reported SAEs (1 per group in the placebo, 100 mg/kg, 200 mg/kg). One subject in the 200 mg/kg group discontinued due to TEAEs (reflux, diarrhoea, vomiting).

The overall incidence of TEAEs in the RTTDB Pool (n=309) was 83% in the all trofinetide group and 59% in the placebo group. Severe TEAEs were experienced by 6% and 3%, serious TEAEs by 5% and 3%, and TEAEs leading to discontinuation by 10% and 2%, in the trofinetide and placebo group, respectively. Related serious TEAEs were similar between the groups (1% for both) (Table 36).

Table 36: Overview of AEs – RTTDB Pool and Study 003

	Subjects, n (%)					
		Trofinetide			ACP-2566-003[a]	
	Placebo (N=133)	<200 mg/kg BID (N=67)	≥200 to 500 mg/kg BID (N=115)	All trofinetide (N=176)	Placebo (N=94)	Trofinetide (N=93)
Any TEAE	79 (59.4)	47 (70.1)	102 (88.7)	146 (83.0)	51 (54.3)	86 (92.5)
Any severe TEAE	4 (3.0)	3 (4.5)	8 (7.0)	11 (6.3)	3 (3.2)	6 (6.5)
Any serious TEAE	4 (3.0)	4 (6.0)	4 (3.5)	8 (4.5)	3 (3.2)	3 (3.2)
Any TEAE leading to discontinuation of study drug or study termination	2 (1.5)	2 (3.0)	16 (13.9)	18 (10.2)	2 (2.1)	16 (17.2)
Any related TEAE	26 (19.5)	12 (17.9)	87 (75.7)	98 (55.7)	20 (21.3)	78 (83.9)
Any related serious TEAE	1 (0.8)	- -	2 (1.7)	2 (1.1)	1 (1.1)	2 (2.2)
Any fatal TEAE	- -	- -	- -	- -	- -	- -

Source: Table RTTDB.MAA.6.1 and Study 003 CSR Table 14.3.1.2.1

Abbreviations: BID=twice daily; RTTDB=Rett syndrome in double-blind oral-treatment studies; TEAE=treatment-emergent adverse event

Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study 003. Subjects can be counted in multiple dose/drug columns if a different dose/drug was administered in these studies. However, these subjects are only counted once in the placebo or all trofinetide column.

The definition of TEAE can be found in each individual study's statistical analysis plan except for Study RETT-002, where adverse events with onset date/time on or after the first double-blinded study dose date/time are considered as TEAEs for the integrated analyses. A subject may have more than one TEAE per category. A subject is counted at most once per category.

N is used as the denominator for calculating percentages for subject incidences.

[a] Subjects who received trofinetide in Study 003 are also included in the ≥200 to 500 mg/kg BID and all trofinetide columns. Subjects who received placebo in Study 003 are also included in the all placebo group.

In the RTTLT Pool (n=173), 97% experienced at least one TEAE. In total 19% experienced AEs of mild intensity and 62% experienced AEs of moderate intensity. The percentage of subjects with TEAEs at <6 and ≥6 weeks after the first trofinetide dose were 89% and 76%, respectively (proportions based on subjects remaining on treatment). A total of 39 subjects (22%) experienced at least one SAE. The percentage of subjects with SAEs was 3% at <6 weeks and 20% at ≥6 weeks. Related serious TEAEs were reported in a total of 3 subjects (2%). A total of 77 subjects (43%) experienced TEAEs leading to discontinuation. The percentage of subjects with TEAEs leading to discontinuation was 26% at <6 weeks from the first trofinetide dose and 18% at ≥6 weeks. Fatal TEAEs were reported in 3 subjects (2%), all occurred ≥6 weeks from the first trofinetide dose (Table 37).

Table 37: Overview of AEs – RTTLT Pool

	Number (%) of subjects		
	<6 weeks (N=178)	≥6 weeks (N=169)	Overall trofinetide (N=178)
Any TEAE	159 (89.3)	128 (75.7)	173 (97.2)
Any severe TEAE	12 (6.7)	17 (10.1)	29 (16.3)
Any serious TEAE	6 (3.4)	34 (20.1)	39 (21.9)
Any TEAE leading to discontinuation of study drug or study termination	46 (25.8)	31 (18.3)	77 (43.3)
Any related TEAE	148 (83.1)	67 (39.6)	158 (88.8)
Any related serious TEAE	2 (1.1)	1 (0.6)	3 (1.7)
Any Related TEAE Leading to Discontinuation of Study Drug or Study Termination	45 (25.3)	22 (13.0)	67 (37.6)
Any fatal TEAE	- -	3 (1.8)	3 (1.7)

Source: Table RTTLT.MAA.6.1
Abbreviations: RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; TEAE=treatment-emergent adverse event
Notes: A subject may have more than one TEAE per category in the given time period. A subject is counted at most once per category in the given time period.
N is the number of subjects who are eligible for adverse event reporting for the given dose group during the given time period, where subjects are eligible to be counted up to the minimum of (last dose date+30, death date).
N is used as the denominator for calculating percentages for subject incidences.

Common adverse events

In Study 003, the most common (≥5% of subjects in either treatment group) TEAEs by PT were diarrhoea, vomiting, seizure, pyrexia, decreased appetite, and irritability. The most common TEAE was diarrhoea (placebo: 19%; trofinetide: 81%). Vomiting was the second most frequent TEAE (placebo: 10%; trofinetide: 27%). Seizure was reported in 5 subjects (5%) in the placebo group and 8 subjects (9%) in the trofinetide group. A TEAE of partial seizures occurred in the placebo group. Irritability was observed in 6 subjects (7%) in the trofinetide group.

The most frequently reported TEAEs (≥5% of subjects) considered to be related by the investigator were diarrhoea (placebo: 13%; trofinetide: 80%), vomiting (placebo: 1%; trofinetide: 17%), and irritability (placebo: 0%; trofinetide: 5%).

Most of the reported TEAEs were of mild or moderate severity. All TEAEs of seizure or partial seizures were mild or moderate in severity. Treatment-emergent adverse events rated as severe were reported for 3% in the placebo group and 7% in the trofinetide group. Severe diarrhoea, experienced by 2 subjects in the trofinetide group, was the only severe TEAE reported in more than 1 subject.

In Study RETT-002, overall, the most common AEs (>10% of) were diarrhoea (35%), vomiting (20%), pyrexia (16%) and upper respiratory tract infection (15%).

In terms of AEs from the start of the double-blind treatment period, for the 50mg/kg BID treatment group (n=15), diarrhoea (27%) was the most commonly reported AE, compared to 4% for the placebo group. Other AEs that were commonly reported with a higher incidence than the placebo group for the 50mg/kg BID treatment group included pharyngitis streptococcal, decreased appetite, somnolence, rhinorrhea and dermatitis diaper (7% for all events).

In the 100mg/kg BID treatment group (n=16), pyrexia (19%), diarrhoea and vomiting (13% for both events) were the most commonly reported AEs from the start of the double-blind period, compared to 17%, 4%, and 13% for the placebo group. Other AEs that were commonly reported with a higher

incidence for the 100mg/kg BID group than for the placebo group included gastroenteritis, tonic convulsion, irritability, sinus congestion (6% for each event).

In the 200mg/kg BID treatment group (n=27), diarrhoea (56%), vomiting (22%), and upper respiratory tract infection (19%) were the most commonly reported AEs, compared to 4%, 13%, and 17% for the placebo group. Other AEs with a higher incidence for the 200mg/kg BID group than for the placebo group included constipation, sinus congestion (7% for both events), gastroenteritis, irritability and dermatitis diaper (each 4%).

Overall in the RTTDB Pool, the most frequent TEAEs (≥5% incidence) in the all trofinetide group with a higher incidence than the placebo group were diarrhoea (57% vs. 17%), vomiting (20% vs. 9%), and irritability (7% vs. 2%).

The most common TEAEs (≥2%) in the RTTDB Pool and Study 003 are shown in Table 38.

Table 38: AEs by PT experienced by >2% of subjects in the trofinetide group – RTTDB and Study 003

	Subjects, n (%)					
		Trofinetide			ACP-2566-003[a]	
	Placebo (N=133)	<200 mg/kg BID (N=67)	≥200 to 500 mg/kg BID (N=115)	All trofinetide (N=176)	Placebo (N=94)	Trofinetide (N=93)
Any TEAE	**79 (59.4)**	**47 (70.1)**	**102 (88.7)**	**146 (83.0)**	**51 (54.3)**	**86 (92.5)**
Diarrhoea	22 (16.5)	14 (20.9)	88 (76.5)	100 (56.8)	18 (19.1)	75 (80.6)
Vomiting	12 (9.0)	4 (6.0)	31 (27.0)	35 (19.9)	9 (9.6)	25 (26.9)
Pyrexia	12 (9.0)	5 (7.5)	12 (10.4)	17 (9.7)	4 (4.3)	8 (8.6)
Irritability	3 (2.3)	5 (7.5)	7 (6.1)	12 (6.8)	- -	6 (6.5)
Upper respiratory tract infection	8 (6.0)	3 (4.5)	7 (6.1)	10 (5.7)	4 (4.3)	2 (2.2)
Seizure	8 (6.0)	2 (3.0)	8 (7.0)	10 (5.7)	5 (5.3)	8 (8.6)
Decreased appetite	3 (2.3)	2 (3.0)	5 (4.3)	7 (4.0)	2 (2.1)	5 (5.4)
Somnolence	2 (1.5)	5 (7.5)	2 (1.7)	7 (4.0)	1 (1.1)	3 (3.2)
Dermatitis diaper	1 (0.8)	1 (1.5)	5 (4.3)	6 (3.4)	1 (1.1)	4 (4.3)
Gastrooesophageal reflux disease	1 (0.8)	1 (1.5)	4 (3.5)	5 (2.8)	1 (1.1)	3 (3.2)
Insomnia	- -	4 (6.0)	1 (0.9)	5 (2.8)	- -	- -
Nasopharyngitis	3 (2.3)	2 (3.0)	3 (2.6)	5 (2.8)	1 (1.1)	2 (2.2)
Pharyngitis streptococcal	1 (0.8)	1 (1.5)	4 (3.5)	5 (2.8)	- -	3 (3.2)
Bruxism	- -	1 (1.5)	3 (2.6)	4 (2.3)	- -	3 (3.2)
Fall	4 (3.0)	1 (1.5)	3 (2.6)	4 (2.3)	1 (1.1)	2 (2.2)
Nasal congestion	2 (1.5)	2 (3.0)	2 (1.7)	4 (2.3)	1 (1.1)	- -
Retching	1 (0.8)	- -	4 (3.5)	4 (2.3)	1 (1.1)	4 (4.3)

Source: Table RTTDB.MAA.6.2, Table RTTDB.MAA.6.3, and Study 003 CSR Table 14.3.1.3.1 and Table 14.3.1.5.1
Abbreviations: BID=twice daily; MedDRA=Medical Dictionary for Regulatory Activities; RTTDB=Rett syndrome in double-blind oral-treatment studies; TEAE=treatment-emergent adverse event
Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study 003. Subjects can be counted in multiple dose/drug columns if a different dose/drug was administered in these studies. However, these subjects are only counted once in the placebo or all trofinetide column.
Adverse events are coded using MedDRA version 24.0.
The definition of TEAE can be found in each individual study's statistical analysis plan except for Study RETT-002, where adverse events with onset date/time on or after the first double-blinded study dose date/time are considered as TEAEs for the integrated analyses.
A subject may have more than one TEAE for each level of summary. A subject is counted at most once for each level of summary.
N is used as the denominator for calculating percentages for subject incidences.
[a] Subjects who received trofinetide in Study 003 are also included in the ≥200 to 500 mg/kg BID and all trofinetide columns. Subjects who received placebo in Study 003 are also included in the all placebo group.

The overall most frequent TEAEs (≥5% incidence) in the RTTLT Pool were diarrhoea (85%), vomiting (37%), COVID-19 (20%), pyrexia (16%), seizure (15%), upper respiratory tract infection, (12%) urinary tract infection (11%), decreased appetite (10%), weight decreased (8%), constipation (8%), irritability (8%), pneumonia, cough, and lethargy (6% each). Incidence of diarrhoea was higher at <6 weeks from the first dose of trofinetide than at ≥6 weeks (80% vs. 30%). Incidence of seizure was higher at ≥6 than at <6 weeks from the first dose of trofinetide (12% vs. 4%).

The maximum severity of TEAEs was mild or moderate for the majority of subjects in the trofinetide group (mild: 19%; moderate: 62%) and 16% of subjects had severe TEAEs. Incidences for all severe TEAE PTs were <2.0%, except for diarrhoea, which had an overall incidence of 3%.

The TEAEs in the RTTLT Pool, with a subject incidence ≥2% are presented by PT in Table 39.

Table 39: AEs by PT experienced by ≥2% of subjects in the Overall group – RTTLT Pool

	Number (%) of subjects		
	<6 weeks (N=178)	**≥6 weeks (N=169)**	**Overall (N=178)**
Any TEAE	**159 (89.3)**	**128 (75.7)**	**173 (97.2)**
Diarrhoea	143 (80.3)	51 (30.2)	152 (85.4)
Vomiting	40 (22.5)	37 (21.9)	66 (37.1)
COVID-19	2 (1.1)	34 (20.1)	36 (20.2)
Pyrexia	8 (4.5)	21 (12.4)	28 (15.7)
Seizure	7 (3.9)	20 (11.8)	26 (14.6)
Upper respiratory tract infection	4 (2.2)	19 (11.2)	21 (11.8)
Urinary tract infection	6 (3.4)	16 (9.5)	19 (10.7)
Decreased appetite	9 (5.1)	10 (5.9)	18 (10.1)
Weight decreased	6 (3.4)	9 (5.3)	14 (7.9)
Constipation	- -	14 (8.3)	14 (7.9)
Irritability	6 (3.4)	9 (5.3)	14 (7.9)
Pneumonia	1 (0.6)	10 (5.9)	11 (6.2)
Cough	- -	11 (6.5)	11 (6.2)
Lethargy	3 (1.7)	7 (4.1)	10 (5.6)
Dehydration	1 (0.6)	7 (4.1)	8 (4.5)
Dermatitis diaper	5 (2.8)	3 (1.8)	8 (4.5)
Gastroenteritis	2 (1.1)	7 (4.1)	9 (5.1)
Pharyngitis streptococcal	1 (0.6)	8 (4.7)	9 (5.1)
Influenza	- -	8 (4.7)	8 (4.5)
Alanine aminotransferase increased	3 (1.7)	6 (3.6)	8 (4.5)
Gastroenteritis viral	- -	7 (4.1)	7 (3.9)
Nasopharyngitis	2 (1.1)	5 (3.0)	7 (3.9)
Flatulence	2 (1.1)	5 (3.0)	7 (3.9)
Nasal congestion	- -	6 (3.6)	6 (3.4)
Somnolence	6 (3.4)	1 (0.6)	6 (3.4)
Rash	1 (0.6)	5 (3.0)	6 (3.4)
Nausea	4 (2.2)	1 (0.6)	5 (2.8)
Ear infection	2 (1.1)	4 (2.4)	5 (2.8)
Acute respiratory failure	1 (0.6)	4 (2.4)	5 (2.8)
Pneumonia aspiration	- -	5 (3.0)	5 (2.8)
Viral upper respiratory tract infection	- -	5 (3.0)	5 (2.8)
Viral infection	- -	5 (3.0)	5 (2.8)
Sinusitis	- -	5 (3.0)	5 (2.8)
Nephrolithiasis	- -	4 (2.4)	4 (2.2)

	Number (%) of subjects		
	<6 weeks (N=178)	≥6 weeks (N=169)	Overall (N=178)
Bruxism	3 (1.7)	1 (0.6)	4 (2.2)
Anxiety	1 (0.6)	3 (1.8)	4 (2.2)
Sinus congestion	1 (0.6)	3 (1.8)	4 (2.2)
Status epilepticus	2 (1.1)	2 (1.2)	4 (2.2)
Rhinovirus infection	1 (0.6)	3 (1.8)	4 (2.2)
Retching	4 (2.2)	1 (0.6)	4 (2.2)
Respiratory syncytial virus infection	- -	4 (2.4)	4 (2.2)
Fatigue	2 (1.1)	2 (1.2)	4 (2.2)
Fall	2 (1.1)	2 (1.2)	4 (2.2)
Enterovirus infection	1 (0.6)	3 (1.8)	4 (2.2)
Electrocardiogram QT prolonged	- -	4 (2.4)	4 (2.2)
Gamma-glutamyltransferase increased	1 (0.6)	3 (1.8)	4 (2.2)
Fungal infection	- -	4 (2.4)	4 (2.2)
Gastrooesophageal reflux disease	4 (2.2)	- -	4 (2.2)

Source: Table RTTLT.MAA.6.2

Abbreviations: MedDRA=Medical Dictionary for Regulatory Activities; RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; TEAE=treatment-emergent adverse event

Notes: Adverse events are coded using MedDRA version 24.0.

A subject may have more than one TEAE for each level of summary in the given time period. A subject is counted at most once for each level of summary in the given time period.

N is the number of subjects who are eligible for adverse event reporting for the given dose group during the given time period, where subjects are eligible to be counted up to the minimum of (last dose date+30, death date).

N is used as the denominator for calculating percentages for subject incidences.

Gastrointestinal AEs

Diarrhoea was the most common TEAE (trofinetide: 81%; placebo: 19%) in Study 003. There were no SAEs of diarrhoea. The mean onset for diarrhoea was 7 days with a mean duration of 51 days. Diarrhoea severity was mild in 52%, moderate in 45%, and severe in 3% of cases. Diarrhoea was persistent in 33%, and recurred in 23% of patients (Table 42). In total 12 subjects in the trofinetide treatment group had a TEAE of diarrhoea leading to withdrawal. Among patients with diarrhoea in the trofinetide group (n=75), 1 subject had concurrent dehydration, and 3 subjects had concurrent weight loss.

During Study 003, a diarrhoea management plan was recommended, which included the recommendation to stop laxatives or anti-constipation medication, and start loperamide and increased fibre (i.e., Metamucil®). A cereal or BRAT diet was also suggested. Additionally, investigators were allowed to hold up to four doses of study drug, and to decrease the assigned dose by 50% (before the Week 6 visit). The dose should be attempted to be increased as soon as possible, with the aim to return to the originally assigned dose.

Antidiarrhoeal treatment was used at some point in the study by 51% and 3%, in the trofinetide and placebo group, respectively. Among those with diarrhoea, 74% of patients with trofinetide took concomitant antidiarrhoeal treatment.

The dose of study medication was reduced in 36% of patients with trofinetide compared to 5% with placebo. Although this was typically due to the management of diarrhoea, reasons for dose reduction were not specified.

In the RTTDB Pool, the incidence of TEAEs of diarrhoea was higher among subjects treated with the recommended dose 200 to 500 mg/kg BID trofinetide (77%) compared with subjects treated with <200 mg/kg BID trofinetide (21%).

In the RTTLT Pool, the overall incidence of TEAEs of diarrhoea was 85%. The incidence was higher among subjects with <6 weeks of treatment compared with subjects treated for ≥6 weeks (80% versus 30%). The mean onset for diarrhoea was 19 days with a mean duration of 232 days (Table 41). No subject had a SAEs of diarrhoea. Diarrhoea severity was mild in 45%, moderate in 51%, and severe in 4% of cases. Diarrhoea was persistent in 13%, and recurred in 40% of subjects (Table 42). In total 19% of patients with TEAEs of diarrhoea discontinued the study. Among patients with diarrhoea (n=152), 34 had weight loss, 14 had urinary tract infection, 8 had dermatitis diaper, 6 had dehydration and 2 had abdominal pain/discomfort.

A total of 81% of the subjects with diarrhoea in the RTTLT Pool used antidiarrhoeal treatment as concomitant medications.

The dose of study medication was reduced in 62% of patients, although this was typically due to the management of diarrhoea, reasons for dose reduction were not specified.

Table 40: Concomitant antidiarrhoeal treatment

	Subjects Received Trofinetide	
	ACP-2566-003 (N=93)	**ACP-2566-003, -004, -005 (RTTLT) (N=178)**
Any diarrhoea TEAE, n (%)	75 (80.6)	152 (85.4)
Had diarrhoea and took concomitant antidiarrhoeal[a] treatment, n (%[b])	55 (73.3)	123 (80.9)

Source: Table CHMPxQ46

a: Concomitant antidiarrhoeal treatment includes 'LOPERAMIDE', 'LOPERAMIDE HYDROCHLORIDE', 'LOPERAMIDE HYDROCHLORIDE;SIMETICONE', 'PLANTAGO OVATA', or "FIBRE, DIETARY'.

b: Percentage calculated using number of subjects with diarrhoea in given cohort as the denominator.

Vomiting was the second most common TEAE (trofinetide: 27%; placebo: 10%) in Study 003. There were no SAEs of vomiting. The mean onset for vomiting was 20 days with a mean duration of 26 days. Cases of vomiting were mild in 72%, moderate in 24%, and severe in 4%. Vomiting did not resolve while on trofinetide in 5 patients and recurred in 2 (Table 42). One subject in the trofinetide treatment group had a TEAE of vomiting leading to discontinuation.

In the RTTDB Pool, the incidence of TEAEs of vomiting was higher among subjects treated with the recommended dose trofinetide (200 to 500 mg/kg BID) compared with subjects treated with a lower dose trofinetide (<200 mg/kg BID), the incidence of vomiting was 27% versus 6%, respectively.

The overall incidence of TEAEs of vomiting was 37% (66 of 178 subjects) in trofinetide-treated subjects of the RTTLT Pool. Two (1%) subjects had a serious TEAE of vomiting. The mean onset for vomiting was 88 days with a mean duration of 60 days (Table 41). Vomiting resolved while on treatment for 48%, recurred in 36% and was not persistent (Table 42). In total 6% of patients with TEAEs of vomiting discontinued the study. Among patients with TEAE vomiting (n=66), 15 had weight loss, 4 had aspiration/aspiration pneumonia and 2 had abdominal pain/discomfort.

Table 41: Onset and duration of AEs diarrhoea and vomiting - RTTLT Pool

TEAE	Subjects Overall N=178 n (%)	Onset Day Mean, Median (Min, Max)	Event Duration (days) Mean, Median (Min, Max)
Diarrhoea	152 (85.4)	18.5, 3.0 (1, 773)	232.0, 109.0 (1, 1039)
Vomiting	66 (37.1)	87.5, 12.0 (1, 642)	57.9, 10.0 (1, 801)

Table 42: Diarrhoea and vomiting resolution status

	Subjects Received Trofinetide	
	ACP-2566-003 (N=93) n (%)	ACP-2566-003, -004, -005 (RTTLT) (N=178) n (%)
Any Diarrhoea TEAE, n (%)	75 (80.6)	152 (85.4)
Diarrhoea completely resolved while on trofinetide and did not recur, n (%)	14 (18.6)	28 (18.4)
Diarrhoea resolved after last dose of trofinetide and did not recur, n (%)	9 (12.0)	14 (9.2)
Diarrhoea resulted with drug withdrawn and did not recur, n (%)	10 (13.3)	29 (19.1)
Diarrhoea did not resolve while on trofinetide, n (%)	25 (33.3)	20 (13.2)
Diarrhoea recurred after resolution, n (%)	17 (22.7)	61 (40.1)
Any Vomiting TEAE, n (%)	25 (26.9)	66 (37.1)
Vomiting completely resolved while on trofinetide and did not recur, n (%)	13 (52.0)	32 (48.4)
Vomiting resolved after last dose of trofinetide and did not recur, n (%)	4 (16.0)	6 (9.1)
Vomiting resulted with drug withdrawn and did not recur, n (%)	1 (4.0)	4 (6.1)
Vomiting did not resolve while on trofinetide, n (%)	5 (20.0)	- -
Vomiting recurred after resolution, n (%)	2 (8.0)	24 (36.4)

This table was adapted by the clinical assessor to provide proportions with the correct denominator (those with respective TEAE)

Seizures

In Study 003, 67% of subjects on trofinetide had medical history of seizures or epilepsy versus 70% of subjects on placebo. In total, 8 (9%) subjects on trofinetide reported 11 TEAEs of seizure (of which 7 with history of seizure/epilepsy), and 6 subjects (6%) on placebo reported 6 TEAEs of seizure or partial seizures (of which 5 had history of seizure/epilepsy). Three TEAEs of seizure on trofinetide were mild and 5 were moderate, and study drug was withdrawn in 2 subjects. Two TEAEs of seizure in the trofinetide group were considered related to study drug, one of which was a serious TEAE. Four TEAEs of seizure on placebo were mild and two were moderate, the majority of which were not related (5/6), and none led to study drug discontinuation.

In the RTTDB Pool the percentage of subjects with a medical history of seizure or epilepsy was respectively: 46% and 20% for the trofinetide group, and 51% and 16% in the placebo group. In total 7% in the trofinetide group and 7% in the placebo group reported a TEAE of seizure. Seizure TEAEs

considered by the Investigator to be related to study drug occurred in 2 (1%) subjects in the all trofinetide group and 1 (1%) subject in the placebo group. Most events of seizure were mild or moderate in severity. One subject in the all trofinetide group had a severe TEAE of seizure cluster and 1 subject in the placebo group had a severe TEAE of seizure. Two subjects in the trofinetide group had TEAEs of seizure that led to study discontinuation.

The TEAEs experienced by subjects related to seizures are presented by PT in Table 43.

Table 43: AEs under the convulsion SMQ by SOC and PT – RTTDB Pool and Study 003

	Number (%) of subjects					
		Trofinetide			ACP-2566-003[a]	
	Placebo (N=133)	<200 mg/kg BID (N=67)	≥200 to 500 mg/kg BID (N=115)	All trofinetide (N=176)	Placebo (N=94)	Trofinetide (N=93)
Any TEAE under the Convulsions SMQ	**9 (6.8)**	**3 (4.5)**	**9 (7.8)**	**12 (6.8)**	**6 (6.4)**	**8 (8.6)**
Nervous system disorders	**9 (6.8)**	**3 (4.5)**	**9 (7.8)**	**12 (6.8)**	**6 (6.4)**	**8 (8.6)**
Seizure	8 (6.0)	2 (3.0)	8 (7.0)	10 (5.7)	5 (5.3)	8 (8.6)
Seizure cluster	- -	- -	1 (0.9)	1 (0.6)	- -	- -
Tonic convulsion	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Partial seizures	1 (0.8)	- -	- -	- -	1 (1.1)	- -

Source: Table RTTDB.MAA.6.12 and Study 003 CSR Table 14.3.1.5.1

Abbreviations: BID=twice daily; MedDRA=Medical Dictionary for Regulatory Activities; RTTDB=Rett syndrome in double-blind oral-treatment studies; SMQ=Standardized MedDRA Query; TEAE=treatment-emergent adverse event

Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study 003.

Subjects can be counted in multiple dose/drug columns if a different dose/drug was administered in these studies. However, these subjects are only counted once in the placebo or all trofinetide column.

Notes: The definition of TEAE can be found in each individual study's statistical analysis plan except for Study RETT-002, where adverse events with onset date/time on or after the first double-blinded study dose date/time are considered as TEAEs for the integrated analyses.

A subject may have more than one TEAE per category. A subject is counted at most once per category.

N is used as the denominator for calculating percentages for subject incidences.

[a] Subjects who received trofinetide in Study 003 are also included in the ≥200 to 500 mg/kg BID and all trofinetide columns. Subjects who received placebo in Study 003 are also included in the all placebo group.

In the RTTLT Pool, 47% had a medical history of seizures, 20% epilepsy, 2% partial seizures, 1% febrile convulsion, 1% focal dyscognitive seizures, 1% petit mal epilepsy, and 2% status epilepticus.

In total 16% (n=28) experienced a TEAE of seizure, and 24/28 had a history of seizures/epilepsy. In total 6 subjects had seizures and 1 subject had seizure cluster, considered by the Investigator to be related to study drug. Five subjects had seizures and 2 subjects had seizure cluster that led to study discontinuation. Nine subjects had serious seizure, 3 subjects had serious status epilepticus, 1 subject had serious generalised tonic-clonic seizure and 1 subject experienced sudden unexplained death in epilepsy (Table 44).

Table 44: AEs under the Convulsion SMQ by SOC and PT – RTTLT Pool

	Overall trofinetide (N=178) n (%)
Any TEAE Under the Convulsions SMQ	28 (15.7)
General disorders and administration site conditions	1 (0.6)
Sudden unexplained death in epilepsy	1 (0.6)
Nervous system disorders	28 (15.7)
Seizure	26 (14.6)
Status epilepticus	4 (2.2)
Seizure cluster	2 (1.1)
Generalised tonic-clonic seizure	1 (0.6)

Source: Table RTTLT.MAA.6.12
Abbreviations: MedDRA=Medical Dictionary for Regulatory Activities; RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; SMQ=Standardized MedDRA Query; TEAE=treatment-emergent adverse event
Notes: Adverse events are coded using MedDRA version 24.0.
A subject may have more than one TEAE for each level of summary in the given time period. A subject is counted at most once for each level of summary in the given time period.
N is the number of subjects who are eligible for AE reporting for the given dose group during the given time period, where subjects are eligible to be counted up to the minimum of (last dose date+30, death date).
N is used as the denominator for calculating percentages for subject incidences.

Adverse drug reactions

The following adverse events were proposed to be included as adverse drug reactions in SmPC section 4.8 (Table 45). These were identified by the Applicant based on safety data from Study 003.

Table 45: Adverse Reactions

MedDRA System Organ Class	Very common	Common
Nervous systems disorders		Seizure[a] Fatigue[b]
Gastrointestinal disorders	Diarrhoea Vomiting[c]	
Metabolism and nutrition disorders		Decreased appetite
Psychiatric disorders		Anxiety[d]
Investigations	Weight loss	

[a] Includes partial seizure
[b] Includes lethargy and somnolence
[c] Includes retching
[d] Includes agitation and irritability

2.6.8.3. Serious adverse events, deaths, and other significant events

Serious TEAEs were reported in 3 subjects (3%) in each treatment group in Study 003. These included bacteraemia, bronchiolitis, and urinary tract infection (all in 1 subject), COVID-19 pneumonia, and seizure (in 1 subject each) in the trofinetide group and constipation, pneumatosis intestinalis, and respiratory distress (in 1 subject each) in the placebo group (Table 46). Of these, bacteraemia, urinary tract infection, seizure, and pneumatosis intestinalis were considered related to study drug. The

outcome for all SAEs was recovered/resolved. Seizure and pneumatosis intestinalis led to discontinuation.

In the RTTDB Pool, serious TEAEs were reported in 8 subjects (5%) for the trofinetide group and 4 subjects (3%) for the placebo group (Table 46). The SOC with the highest incidence of serious TEAEs in the trofinetide group was Infections and infestations (trofinetide: 8 events in 5 subjects (3%) vs. placebo: 1 event in 1 subject (1%)); the incidence for all other SOCs was ≤2.0%. The majority of serious TEAEs were considered unrelated or unlikely related by the Investigator and reflected the comorbidities and natural history of the underlying population (RTT and largely paediatric).

Table 46: Serious AEs by PT – RTTDB Pool and Study 003

		Trofinetide			ACP-2566-003[a]	
	Placebo (N=133)	<200 mg/kg BID (N=67)	≥200 to 500 mg/kg BID (N=115)	All trofinetide (N=176)	Placebo (N=94)	Trofinetide (N=93)
Any serious TEAE	**4 (3.0)**	**4 (6.0)**	**4 (3.5)**	**8 (4.5)**	**3 (3.2)**	**3 (3.2)**
Bacteraemia	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
Bronchiolitis	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
COVID-19 pneumonia	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
Ludwig angina	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Oral candidiasis	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Pneumonia	1 (0.8)	- -	1 (0.9)	1 (0.6)	- -	- -
Streptococcal infection	- -	- -	1 (0.9)	1 (0.6)	- -	- -
Urinary tract infection	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
Altered state of consciousness	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Seizure	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
Tonic convulsion	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Pulmonary embolism	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Constipation	1 (0.8)	- -	- -	- -	1 (1.1)	- -
Pneumatosis intestinalis	1 (0.8)	- -	- -	- -	1 (1.1)	- -
Respiratory distress	1 (0.8)	- -	- -		1 (1.1)	- -

Source: Table RTTDB.MAA.6.5 and Study 003 CSR Table 14.3.1.8.1
Abbreviations: BID=twice daily; COVID-19=coronavirus disease 2019; MedDRA=Medical Dictionary for Regulatory Activities; RTTDB=Rett syndrome in double-blind oral-treatment studies; TEAE=treatment-emergent adverse event
Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study 003. Subjects can be counted in

In the RTTLT Pool a total of 39 subjects (22%) experienced at least one SAE. The percentage of subjects with SAEs was 3% at <6 weeks and 20% at ≥6 weeks.

Overall, the SOC with the highest incidence (≥2%) of serious TEAEs was Infections and infestations (13%, 23 subjects), followed by Nervous system disorders (8%, 15 subjects), Respiratory, thoracic and mediastinal disorders (4%, 7 subjects), and Metabolism and nutrition disorders (3%, 5 subjects), and Gastrointestinal disorders and General disorders and administration site conditions (each 2%, 4 subjects).

The most frequent serious TEAEs (≥1% incidence) experienced by trofinetide-treated subjects were seizure (9 subjects, 5%), pneumonia (5 subjects, 3%), urinary tract infection, dehydration, acute respiratory failure (each 4 subjects, 2%), pyrexia, rhinovirus infection, status epilepticus (each 3 subjects, 2%), vomiting, COVID-19, COVID-19 pneumonia, enterovirus infection, parainfluenza virus

infection, viral infection, aspiration (each 2 subjects, 1%). Serious TEAEs of seizure occurred in 2 (1%) subjects at <6 weeks and in 7 subjects (4%) at ≥6 weeks after the first trofinetide dose (Table 47).

The majority of serious TEAEs were considered unrelated or unlikely related by the Investigator. There were 3 subjects (2%) with serious TEAEs considered possibly related to study drug: urinary tract infection (2 subjects), bacteraemia (1 subject), dehydration (1 subject), and seizure (1 subject).

Table 47: SAEs by PT – RTTLT Pool

	Number (%) of subjects		
	<6 weeks (N=178)	≥6 weeks (N=169)	Overall (N=178)
Any Serious TEAE	**6 (3.4)**	**34 (20.1)**	**39 (21.9)**
Seizure	2 (1.1)	7 (4.1)	9 (5.1)
Pneumonia	1 (0.6)	4 (2.4)	5 (2.8)
Urinary tract infection	1 (0.6)	3 (1.8)	4 (2.2)
Dehydration	- -	4 (2.4)	4 (2.2)
Acute respiratory failure	1 (0.6)	3 (1.8)	4 (2.2)
Pyrexia	- -	3 (1.8)	3 (1.7)
Rhinovirus infection	1 (0.6)	2 (1.2)	3 (1.7)
Status epilepticus	1 (0.6)	2 (1.2)	3 (1.7)
Vomiting	- -	2 (1.2)	2 (1.1)
COVID-19	- -	2 (1.2)	2 (1.1)
COVID-19 pneumonia	- -	2 (1.2)	2 (1.1)
Enterovirus infection	1 (0.6)	1 (0.6)	2 (1.1)
Parainfluenzae virus infection	- -	2 (1.2)	2 (1.1)
Viral infection	- -	2 (1.2)	2 (1.1)
Aspiration	1 (0.6)	1 (0.6)	2 (1.1)
Anaemia	- -	1 (0.6)	1 (0.6)
Cardiac arrest	- -	1 (0.6)	1 (0.6)
Abdominal pain	- -	1 (0.6)	1 (0.6)
Gastric perforation	- -	1 (0.6)	1 (0.6)
Gastric ulcer	- -	1 (0.6)	1 (0.6)
Gastrointestinal haemorrhage	- -	1 (0.6)	1 (0.6)
Ileus	- -	1 (0.6)	1 (0.6)
Pneumoperitoneum	- -	1 (0.6)	1 (0.6)
Sudden unexplained death in epilepsy	- -	1 (0.6)	1 (0.6)
Bacteraemia	1 (0.6)	- -	1 (0.6)
Bronchiolitis	1 (0.6)	- -	1 (0.6)
Device related infection	- -	1 (0.6)	1 (0.6)
Escherichia sepsis	- -	1 (0.6)	1 (0.6)
Gastroenteritis	- -	1 (0.6)	1 (0.6)
Gastroenteritis viral	- -	1 (0.6)	1 (0.6)
Influenza	- -	1 (0.6)	1 (0.6)
Peritoneal abscess	- -	1 (0.6)	1 (0.6)
Peritonitis	- -	1 (0.6)	1 (0.6)
Pharyngitis streptococcal	- -	1 (0.6)	1 (0.6)
Pneumonia viral	- -	1 (0.6)	1 (0.6)

	Number (%) of subjects		
	<6 weeks (N=178)	≥6 weeks (N=169)	Overall (N=178)
Pyelonephritis	- -	1 (0.6)	1 (0.6)
Staphylococcal infection	- -	1 (0.6)	1 (0.6)
Streptococcal sepsis	- -	1 (0.6)	1 (0.6)
Urinary tract infection fungal	- -	1 (0.6)	1 (0.6)
Urinary tract infection pseudomonal	- -	1 (0.6)	1 (0.6)
Vaginal abscess	- -	1 (0.6)	1 (0.6)
Procedural pain	- -	1 (0.6)	1 (0.6)
Renal procedural complication	- -	1 (0.6)	1 (0.6)
Hypokalaemia	- -	1 (0.6)	1 (0.6)
Hyponatraemia	- -	1 (0.6)	1 (0.6)
Disturbance in attention	- -	1 (0.6)	1 (0.6)
Dystonia	- -	1 (0.6)	1 (0.6)
Dystonic tremor	- -	1 (0.6)	1 (0.6)
Generalised tonic-clonic seizure	- -	1 (0.6)	1 (0.6)
Pelvic fluid collection	- -	1 (0.6)	1 (0.6)
Vaginal discharge	- -	1 (0.6)	1 (0.6)
Pleural effusion	- -	1 (0.6)	1 (0.6)
Pneumonia aspiration	- -	1 (0.6)	1 (0.6)
Respiratory distress	- -	1 (0.6)	1 (0.6)

Source: Table RTTLT.MAA.6.5

Abbreviations: COVID-19=coronavirus disease 2019; MedDRA=Medical Dictionary for Regulatory Activities; RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; TEAE=treatment-emergent adverse event

Notes: Adverse events are coded using MedDRA version 24.0.

A subject may have more than one TEAE for each level of summary in the given time period. A subject is counted at most once for each level of summary in the given time period.

N is the number of subjects who are eligible for adverse event reporting for the given dose group during the given time period, where subjects are eligible to be counted up to the minimum of last dose date+30, death date.

N is used as the denominator for calculating percentages for subject incidences.

In total 3 deaths occurred while on treatment with trofinetide, during Study 005. These subjects had a fatal treatment-emergent SAE (vomiting leading to aspiration; cardiac arrest; sudden unexplained death in epilepsy), which was considered not to be related to trofinetide, by the investigator. One additional death was not treatment-emergent.

2.6.8.4. Laboratory findings

Clinical laboratory evaluation

In Study 003, shifts in chemistry parameters at Week 12 in the trofinetide group compared to the placebo group were reported for ALT (shift to high: 29% versus 6%) and AST (shift to high: 11% versus 3%). Increases in ALT and/or AST from baseline appeared relatively early in the study course, were mild, transient, and self-limited while on study drug.

There were 7 (4%) subjects in the trofinetide group and three (2%) subjects in the placebo group who had ALT ≥3×ULN. There were no instances that met Hy's Law criteria or was ≥5×ULN. There were no discontinuations due to elevated levels of ALT, AST, ALP, GGT, or bilirubin.

TEAEs of ALT increased (n=2) and hepatic enzyme increased (n=1) were reported in the trofinetide group, TEAEs did not appear to be associated with liver injury.

Similar results were observed in the RTTLT Pool for change in liver function tests from baseline and percentage of subjects with potentially clinically important results. More specifically, 7% of subjects had an ALT value ≥3×ULN and 1% had GGT value ≥3×ULN. No concomitant TEAE of jaundice or abdominal pain were reported. One subject discontinued due an AE of ALT increased, this was mild (77 U/L), resolved on follow-up and was not associated with any abnormalities in AST, GGT, ALP or bilirubin.

Vital signs

In Study 003, the mean changes at Week 12 in vital signs parameters were generally small. At any postbaseline visit, potential clinically important (PCI) decreases in systolic (≤90 mmHg and decreased ≥20 mmHg from baseline) or diastolic blood pressure (≤50 mmHg and decreased ≥15 mmHg from baseline) were reported. In total 9% versus 4% had decreased systolic BP, and 9% versus 3% had decreased diastolic BP, in the trofinetide versus placebo group. Increased pulse rate (≥120 bpm and increased ≥ 12 bpm from baseline) was reported both with trofinetide (10%) and placebo (9%).

In the RTTLT Pool 13% and 12% of subjects met the PCI criterion for decreased systolic and decreased diastolic blood pressure. In total 14% had increased pulse.

Electrocardiograms

In Study 003, the mean changes in ECG parameters were generally small and similar between the trofinetide and placebo groups except for RR interval; mean decrease was higher in the placebo group than in the trofinetide group (-14 versus -2 ms). At any postbaseline visit, QTcF >450 ms was reported in 3% of subjects in the placebo group and 1% of subjects in the trofinetide group. None of the values were >480 ms. No subjects in either treatment group had QTcF >500 ms. No subjects in the trofinetide group had QTcF change from baseline >60 ms compared with 2% of subjects in the placebo group (Table 48).

In the **RTTLT Pool**, the overall postbaseline maximum value of QTcF >450 ms was reported in 6 (3%) subjects. None of the QTcF values were >480 ms.

Table 48: Incidence of Potentially Clinically Important ECG Values – RTTDB Pool and Study 003

	Placebo n/N (%)[a]	Trofinetide			ACP-2566-003[a]	
		<200 mg/kg BID n/N (%)[a]	≥200 to 500 mg/kg BID n/N (%)[a]	All trofinetide n/N (%)[a]	Placebo n/N (%)[a]	Trofinetide n/N (%)[a]
Overall postbaseline assessment						
QTcF interval change from baseline >60 ms[b]	2/133 (1.5)	2/66 (3.0)	0/115	2/175 (1.1)	3/94 (3.2)	0/93
QTcF interval >500 ms[c]	0/133	0/66	0/115	0/175	0/94	0/93
PR interval ≥220 ms[c]	1/133 (0.8)	0/67	0/115	0/176	1/94 (1.1)	0/93
QRS interval ≥120 ms[c]	1/133 (0.8)	0/67	0/115	0/176	1/94 (1.1)	0/93

Source: Table RTTDB.MAA.11.3 and Study 003 CSR Table 14.3.4.4

Abbreviations: BID=twice daily; ECG=electrocardiogram; QTcF=corrected QT interval using Fridericia's correction method; PCI=potentially clinically important; RTTDB=Rett syndrome in double-blind oral-treatment studies

Notes: When ECGs were collected in triplicate, the average of the triplicates was considered as one assessment for the summaries.

Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study 003. Subjects can be counted in multiple dose/drug columns if a different dose/drug was administered in these studies. However, these subjects are only counted once in the placebo or all trofinetide column.

[a] Subjects who received trofinetide in Study 003 are also included in the ≥200 to 500 mg/kg BID and all trofinetide columns. Subjects who received placebo in Study 003 are also included in the all placebo group.

[b] The numerator is the number of subjects with at least one postbaseline PCI value for the given criterion and column. The denominator is the number of subjects with non-missing baseline and at least one postbaseline value for the given parameter and column.

[c] The numerator is the number of subjects with at least one postbaseline PCI value for the given criterion and column. The denominator is the number of subjects with at least one postbaseline value for the given parameter and column.

Bodyweight

The mean change in weight (kg) from baseline to Week 12 was small and similar between the trofinetide and placebo group in Study 003. At any postbaseline visit, PCI increase in weight (≥7% increase from baseline) was observed in 13% of subjects in the placebo group and 8% of subjects in the trofinetide group. At any postbaseline visit, PCI decrease in weight (≥7% decrease from baseline) was observed in 5% of subjects in the placebo group and 13% of subjects in the trofinetide group (Table 49). Of the 11 subjects in the trofinetide group with a PCI decrease in weight, 3 had a concurrent TEAE of weight decreased. None of the TEAEs of weight decreased were severe or serious.

The percentage of trofinetide-treated subjects in the RTTLT Pool with PCI changes in body weight in the long-term studies was generally higher than in Study 003. Notably, both weight decrease ≥7% from baseline (22% vs. 13% in Study 003) and weight increase ≥7% from baseline (40% vs. 8% in Study 003) occurred more frequently in the RTTLT Pool (Table 50).

Table 49: Mean Change in Bodyweight from Baseline to Week 12 - Study 003

Vital Signs Parameter Timepoint	Statistic	Placebo (N=94)	Trofinetide (N=93)
Weight (kg)			
Baseline	Mean (SE)	29.2 (1.07)	30.5 (1.31)
Change from Baseline to Week 12	n	83	73
	Mean (SE)	0.3 (0.16)	-0.1 (0.35)
PCI values n/N (%)			
Week 12: weight increase ≥7% from Baseline[a]		8/83 (9.6)	6/73 (8.2)
Overall postbaseline: weight increase ≥7% from Baseline[b]		11/86 (12.8)	7/85 (8.2)
Week 12: weight decrease ≥7% from Baseline[a]		2/83 (2.4)	6/73 (8.2)
Overall postbaseline: weight decrease ≥7% from Baseline[b]		4/86 (4.7)	11/85 (12.9)

Source: Table 14.3.3.1 and Table 14.3.3.2
Abbreviations: PCI=potentially clinically important; SE=standard error
Baseline was the latest nonmissing value prior to the first dose of study drug.
[a] The denominator was the number of subjects with nonmissing values for the given parameter, visit, and group.
[b] The denominator was the number of subjects with at least one postbaseline value for the given parameter and group.

Table 50: Mean Change in Bodyweight from baseline - RTTLT Pool

Parameter	RTTLT Pool All trofinetide (N=178)	RTTOL Pool All trofinetide (N=154)
Change from baseline, mean (SD) n		
CFB to overall postbaseline minimum[a]	-1.21 (1.911) n=174	-0.96 (1.697) n=153
CFB to overall postbaseline maximum[b]	2.04 (3.408) n=174	2.09 (3.150) n=153
Potentially clinically important values, n/N (%)[c]		
Weight decrease ≥7% from baseline	39/174 (22.4)	25/153 (16.3)
Weight increase ≥7% from baseline	68/174 (39.1)	64/153 (41.8)

Source: Table RTTLT.MAA.10.1, Table RTTLT.MAA.10.2, Table RTTOL.MAA.10.1, and Table RTTOL.MAA.10.2
Abbreviations: CFB=change from baseline; RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; RTTOL=trofinetide-treated subjects with Rett syndrome in open-label long-term extension studies; SD=standard deviation
[a] If a subject has multiple postbaseline measurements, then the minimum value is selected for the given parameter.
[b] If a subject has multiple postbaseline measurements, then the maximum value is selected for the given parameter.
[c] The numerator is the number of subjects with at least one postbaseline PCI value for the given criterion. The denominator is the number of subjects with non-missing baseline and at least one postbaseline value for the given parameter.

2.6.8.5. In vitro biomarker test for patient selection for safety

2.6.8.6. Safety in special populations

Children 2-5 years of age (Study 009)

In total 14/15 subjects experienced at least one TEAE (142 events). Overall, most common TEAEs included diarrhoea (n=12) and vomiting (n=8), COVID19 (n=7), gastroenteritis, pyrexia and seizure (n=5 each). Diarrhoea and vomiting were considered to be related by the investigator in 9 and 4 subjects, respectively. Diarrhoea was mild for 7 subjects and moderate for 5 subjects; vomiting was mild for 6 subjects and moderate for 2 subjects. Two subjects reported a severe TEAE of gastroenteritis sapovirus and seizure (1 subject each). Serious TEAEs (5 events in 4 subjects) included

dysphagia (mild), gastroenteritis sapovirus (severe), seizures (2 events, 1 moderate and 1 severe), and altered state of consciousness (mild). None of these were considered related to study drug. Two subjects discontinued due to TEAEs (one due to diarrhoea and feeding disorder, one due to vomiting).

Overall, laboratory results were generally within normal range. Shifts in ALT were reported at Week 12 (normal to high: 26.7%) and at Week 78 (normal to high: 20%). There were no shifts to high in AST. Increases in ALT and/or AST from Baseline were mild, appeared to be transient, and were self-limited.

Changes in vital signs and ECG parameters were not clinically significant. Postbaseline PCI vital signs were observed in 4 subjects for diastolic blood pressure (≤50 mmHg and decreased ≥15 mmHg from baseline) and in 2 subjects for pulse (≥120 bpm and increased ≥15 bpm from baseline). There were no potentially clinically important changes in QTcF values noted.

There was a mean (SE) increase in weight from Baseline to Week 12 of 0.2 (0.2) kg. Overall postbaseline, 13 subjects (87%) had a weight increase of ≥7% from Baseline, and 2 subjects (13%) had a weight decrease of ≥7% from Baseline. Two subjects reported TEAEs of weight decreased during the study, both during the first 12 weeks of treatment.

Age (≥5 years)

In the RTTLT Pool, the percentage of trofinetide-treated subjects with TEAEs was higher in the 5 to <12 and ≥17 years age groups than the 12 to <17 years age group, and the percentage of subjects with serious TEAEs and discontinuation TEAEs was higher in the 5 to <12 years age group than the 12 to <17 and ≥17 years age groups.

The overall most common (≥10% incidence) TEAEs in the all trofinetide group were diarrhoea, vomiting, COVID-19, pyrexia, seizure, upper respiratory tract infection, urinary tract infection and decreased appetite. TEAEs of diarrhoea had higher incidence in the 5 to <12 (89%) and ≥17 years (90%) age groups than the 12 to <17 age group (74%). Incidence appeared to decrease with older age for the TEAEs of vomiting (42%, 35%, and 23% for 5 to <12, 12 to <17, and ≥17 years, respectively), upper respiratory tract infection (13%, 12%, and 10% for 5 to <12, 12 to <17, and ≥17 years, respectively) and urinary tract infection (14%, 9%, and 3% for 5 to <12, 12 to <17, and ≥17 years, respectively). TEAEs had higher incidence in the 12 to <17 than ≥17 years age groups and the 5 to <12 years age group for pyrexia (28% vs. 13% and 12%, respectively) and decreased appetite (14% vs. 7% and 10%, respectively). TEAEs of seizure had higher incidence in the 5 to <12 years age group than in the 12 to <17 and ≥17 years age groups (20%, 7% and 7%, respectively). There were no differences in the incidence of the TEAE of COVID-19 among age groups (20%, 21% and 19% for 5 to <12, 12 to <17, and ≥17 years, respectively)

At the PT level, there were no notable (≥5%) differences in the incidence of serious TEAEs among age subgroups (5 to <12, 12 to <17, and ≥17 years) for trofinetide-treated subjects except for seizure, which occurred in 8 (8%) subjects in the 5 to <12 years age group and 1 (2%) in the 12 to <17 age group and none of the subjects in the ≥17 years age group.

Renal impairment

Overall, a single oral dose of 6 g or 8 g trofinetide in subjects with moderate renal impairment and of 12 g trofinetide in matched healthy controls was well tolerated. TEAEs were reported in 2 subjects with moderate renal impairment (ear infection, blood creatine phosphokinase increased, headache) and 4 healthy subjects (thrombocytopenia, diarrhoea, chromaturia, haematuria). There were no deaths and no serious TEAEs. There were no clinically meaningful changes in individual laboratory, vital signs, physical findings, or ECG results.

2.6.8.7. Immunological events

2.6.8.8. Safety related to drug-drug interactions and other interactions

A drug-drug interaction clinical study (ACP-2566-012) was conducted to assess the impact of trofinetide on the PK of loperamide in healthy volunteers when loperamide is administered alone (cycle 1), concomitantly with trofinetide (cycle 2), or administered 2 hours after trofinetide (cycle 3).

Overall, 10 subjects (67%) reported at least one TEAE in the study. The most common TEAEs (reported in ≥2 subjects in any PT) were dermatitis contact reported by 6 subjects (40%), constipation and diarrhoea reported by 2 subjects (13%); all other TEAEs were reported by 1 subject each (7%). All TEAEs were mild in severity and resolved during the study. Two TEAEs (constipation) were considered related to loperamide, two TEAEs (diarrhoea) were considered related to trofinetide, and one TEAE (headache) was considered related to loperamide and trofinetide. No clinically important shifts in ECG parameters were reported.

2.6.8.9. Discontinuation due to adverse events

In Study 003, overall 17% in the trofinetide group experienced TEAEs leading to discontinuation. Most common TEAEs leading to discontinuation were diarrhoea (13%), decreased appetite (3%), and lethargy and seizure (2% each) (Table 51). All of the TEAEs leading to discontinuation were considered related to study drug, except for one case of arthralgia in the placebo group.

Discontinuations in the RRTDB Pool were similar to those in Study 003 (Table 51).

Table 51: TEAEs leading to discontinuation, by PT - RTTD Pool and Study 003

		Trofinetide			ACP-2566-003[a]	
	Placebo (N=133)	<200 mg/kg BID (N=67)	≥200 to 500 mg/kg BID (N=115)	All trofinetide (N=176)	Placebo (N=94)	Trofinetide (N=93)
Any TEAE leading to study drug discontinuation or study termination	**2 (1.5)**	**2 (3.0)**	**16 (13.9)**	**18 (10.2)**	**2 (2.1)**	**16 (17.2)**
Diarrhoea	- -	- -	13 (11.3)	13 (7.4)	- -	12 (12.9)
Decreased appetite	- -	- -	3 (2.6)	3 (1.7)	- -	3 (3.2)
Vomiting	- -	- -	2 (1.7)	2 (1.1)	- -	1 (1.1)
Lethargy	- -	- -	2 (1.7)	2 (1.1)	- -	2 (2.2)
Seizure	- -	- -	2 (1.7)	2 (1.1)	- -	2 (2.2)
Frequent bowel movements	- -	1 (1.5)	- -	1 (0.6)	- -	1 (1.1)
Gastrooesophageal reflux disease	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
Weight decreased	- -	- -	1 (0.9)	1 (0.6)	- -	1 (1.1)
Altered state of consciousness	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Pulmonary embolism	- -	1 (1.5)	- -	1 (0.6)	- -	- -
Pneumatosis intestinalis	1 (0.8)	- -	- -	- -	1 (1.1)	- -
Arthralgia	1 (0.8)	- -	- -	- -	1 (1.1)	- -

Source: Table RTTDB.MAA.6.6 and Study 003 CSR Table 14.3.1.9.1

Abbreviations: BID=twice daily; MedDRA=Medical Dictionary for Regulatory Activities; RTTDB=Rett syndrome in double-blind oral-treatment studies; TEAE=treatment-emergent adverse event

Notes: The RTTDB Pool includes Studies ACP-2566-003, Neu-2566-RETT-002, and Neu-2566-RETT-001. A total of 39 subjects were enrolled in both Study RETT-002 and the pivotal Study 003. Subjects can be counted in multiple dose/drug columns if a different dose/drug was administered in these studies. However, these subjects are only counted once in the placebo or all trofinetide column.

Adverse events are coded using MedDRA version 24.0.

The definition of TEAE can be found in each individual study's statistical analysis plan except for Study Neu-2566-RETT-002, where adverse events with onset date/time on or after the first double-blinded study dose date/time are considered as TEAEs for the integrated analyses.

Overall, 43% in the RTTLT Pool experienced TEAEs leading to discontinuation. The incidence of TEAEs leading to discontinuation was higher at <6 weeks from the first dose of trofinetide compared to ≥6 weeks (26% vs. 18%).

Overall, the SOC with the highest incidence (≥2%) of discontinuation TEAEs was Gastrointestinal disorders (32%), followed by Nervous system disorders (6%), Investigations and Metabolism and nutrition disorders (each 3%) and Respiratory, thoracic and mediastinal disorders (2%). By PT, the highest incidence of discontinuation TEAEs were diarrhoea (26%), vomiting (7%), seizure (3%), weight decreased and decreased appetite (each 2%) (Table 52).

Table 52: TEAEs Leading to Discontinuation, by PT – RTTLT Pool

	Number (%) of subjects		
	<6 weeks (N=178)	≥6 weeks (N=169)	Overall trofinetide (N=178)
Any TEAE Leading to Discontinuation of Study Drug or Study Termination	**46 (25.8)**	**31 (18.3)**	**77 (43.3)**
Diarrhoea	35 (19.7)	11 (6.5)	46 (25.8)
Vomiting	5 (2.8)	7 (4.1)	12 (6.7)
Seizure	2 (1.1)	3 (1.8)	5 (2.8)
Weight decreased	3 (1.7)	1 (0.6)	4 (2.2)
Decreased appetite	4 (2.2)	- -	4 (2.2)
Lethargy	2 (1.1)	- -	2 (1.1)
Seizure cluster	1 (0.6)	1 (0.6)	2 (1.1)
Aspiration	1 (0.6)	1 (0.6)	2 (1.1)
Cardiac arrest	- -	1 (0.6)	1 (0.6)
Frequent bowel movements	1 (0.6)	- -	1 (0.6)
Gastrooesophageal reflux disease	1 (0.6)	- -	1 (0.6)
Screaming	- -	1 (0.6)	1 (0.6)
Sudden unexplained death in epilepsy	- -	1 (0.6)	1 (0.6)
COVID-19	- -	1 (0.6)	1 (0.6)
Enterovirus infection	1 (0.6)	- -	1 (0.6)
Gastroenteritis	- -	1 (0.6)	1 (0.6)
Alanine aminotransferase increased	- -	1 (0.6)	1 (0.6)
Feeding disorder	1 (0.6)	- -	1 (0.6)
Gross motor delay	- -	1 (0.6)	1 (0.6)
Agitation	- -	1 (0.6)	1 (0.6)
Breath holding	- -	1 (0.6)	1 (0.6)
Acute respiratory failure	1 (0.6)	- -	1 (0.6)
Cough	- -	1 (0.6)	1 (0.6)
Oropharyngeal pain	- -	1 (0.6)	1 (0.6)
Pneumonia aspiration	- -	1 (0.6)	1 (0.6)

Source: Table RTTLT.MAA.6.6

Abbreviations: COVID-19=coronavirus disease 2019; MedDRA=Medical Dictionary for Regulatory Activities; RTTLT=trofinetide-treated subjects with Rett syndrome in Phase 3 double-blind and open-label long-term extension studies; TEAE=treatment-emergent adverse event

Notes: Adverse events are coded using MedDRA version 24.0.

A subject may have more than one TEAE for each level of summary in the given time period. A subject is counted at most once for each level of summary in the given time period.

N is the number of subjects who are eligible for adverse event reporting for the given dose group during the given time period, where subjects are eligible to be counted up to the minimum of last dose date+30, death date.

N is used as the denominator for calculating percentages for subject incidences.

2.6.8.10. Post marketing experience

Daybue (trofinetide) was approved in the US on 10 March 2023 and made commercially available in April 2024. An estimated 1,628 patients with RTT received trofinetide commercially in the US (November 2024).

Serious cases of aspiration (n=10) and aspiration pneumonia (n=20) were spontaneously reported (10 March 2023 to 09 September 2024). Of these, 3 cases of aspiration and 10 cases of aspiration pneumonia were reported in association with vomiting. Trofinetide administration was interrupted in 4 cases due to vomiting, and the drug was withdrawn in 5 cases after aspiration/aspiration pneumonia.

Study 014 is an ongoing, phase 4, observational, real-world, prospective study based on caregiver input, in patients prescribed trofinetide in the US. No clinical sites or HCPs were involved in data collection, and AEs were not actively solicited.

At data cutoff (26 June 2024), a total of 218 patients were enrolled, and 192 had evidence of receiving at least one dose of trofinetide. Trofinetide was administered BID in most patients (60%-93% over time); approximately 2%-7% of patients received trofinetide three times per day. Other dosing regimen (once a day or four times per day) were uncommon. The percentage of subjects who achieved their target daily dose increased from Week 1 (50%; n assessed = 58) to Week 8 (77%; n assessed = 86) and then remained stable up to Month 9.

In total 60 AEs were reported in 12% of patients, 10 SAEs were reported in 3% of patients, and at least one AE leading to discontinuation was reported in 7% of patients. No fatal AEs have been reported. The most common AEs by SOC were GI disorders (8%), followed by nervous system disorders (2%), and psychiatric disorders (2%). Among gastrointestinal disorders, diarrhoea (5%) and vomiting (4%) were reported. SAEs included constipation (2%), diarrhoea, vomiting, gastroenteritis viral, pneumonia, pneumonia aspiration, dehydration (each 1%).

2.6.9. Discussion on clinical safety

For the evaluation of safety, the Applicant has used all data from the trofinetide clinical development program, with the main focus on the placebo-controlled studies (003, Rett-002, Rett-001) and open-label extension studies (004, 005) in patients with RTT.

The conducted Study Rett-002 was a dose–finding study. TEAEs were clearly higher in the 200 mg/kg BID group, with diarrhoea as the most commonly observed TEAE. No severe or serious events of diarrhoea were reported. Therefore, and based on the totality of the efficacy and safety data, the 200 mg/kg BID dose was selected for further evaluation.

Safety data were pooled across four safety pools for evaluation of different safety aspects. The RTTDB Pool and RTTLT Pool are considered as most relevant among these pools, as these include the population of primary interest, in a placebo-controlled setting (RTTDB) and with a longer follow-up duration in a largely uncontrolled setting (RTTLT). Yet, for assessment of safety vs placebo, Study 003 remains the primary study of interest, because the recommended dose of trofinetide (200-500 mg/kg) was evaluated (during a treatment period of 12 weeks). Doses of trofinetide were lower in Studies Rett-002 and Rett-001 and study duration was shorter (up to a maximum of 6 weeks). Pooling of studies Rett-002 and Rett-001 with study 003 (RTTDB Pool) may therefore result in a diluted safety sample.

Subjects who participated in study Rett-002 were also allowed to enrol in study 003, in total 39 subjects participated in both studies. There was a considerable gap between both studies (at least 34 months). From a safety perspective there are no issues concerning the participation in both studies, the strategy on handling of these data is accepted.

No AEs of special interest were defined prior to study enrolment. Based on the experience from the phase 1 and 2 studies, it is considered that for the phase 3 studies, AEs of special interest could have been defined, especially for gastrointestinal AEs. That way, AEs could have been better characterized (e.g., diarrhoea symptom subtypes incl. improvement) and data on persistence, recurrence, impact of discontinuations, dose modifications, secondary AEs etc. could have been collected more carefully.

Patient exposure

The total size of the safety database is limited, the number of patients with RTT (ages 2-45 years), exposed to the recommended dose, is lower than the general standard for clinical safety evaluation (ICH E1). In total 260 patients with RTT were exposed to at least a single dose, and 112 and 92 patients were exposed for ≥6 months and ≥12 months, respectively. Trofinetide is intended for long-term treatment, and usually long-term treatments require at least 100 patients exposed to the recommended dose for ≥12 months. In the context of a rare disease, the intended treatment population is small, and a smaller number of patients exposed in clinical trials may be expected. Nevertheless, non-clinical data provides only limited support, since exposures in animals are overall low and safety margins may not be sufficient. The adequacy of the safety database therefore also depends on the emerging safety concerns. Based on the totality of data it is considered that the common safety issues with trofinetide are well captured, although the underlying mechanism of action is still not understood. Potential off-target effects were initially not adequately evaluated, therefore a new broad *in vitro* secondary pharmacology screen was conducted upon request (refer to non-clinical assessment). Further, despite non-clinical uncertainties and suboptimal QT Study design, the risk of serious consequences due to cardiac repolarization is considered minimal (refer to PD assessment).

The striking number of dropouts (mainly due to gastrointestinal AEs) raised questions concerning the representativeness of the completer population for the randomised population, and the impact on the interpretation of the efficacy results. In addition, it remains unclear what the impact of dose modifications are on long-term efficacy (see below).

In the pivotal study weight-based banded dosing was used, which resulted in administration of doses between 200 mg/kg to 500 mg/kg. The incidence of TEAEs was similar in subjects across dose groups (96-100%), except for a lower proportion of TEAEs (81%) in the lowest dose group (150<250 mg/kg BID).

Another limitation of the safety database is the absence of any safety data in male patients with RTT or patients above the age of 20 years, while the proposed target population includes all patients with RTT (male and female of all ages). The clinical phenotype of RTT in males is distinct from the phenotype in female patients. Generally, the disease is more severe and associated with early death. Safety of trofinetide (<200 mg/kg BID) was evaluated in 60 males (patients with FXS or TBI). Based on these data the similarity in safety profile of trofinetide, for males versus females cannot be adequately interpreted. A higher percentage of females (with RTT) had TEAEs, including gastrointestinal AEs (diarrhoea and vomiting), compared to males (with FXS or TBI). Yet, it remains unclear whether observed differences in TEAEs are attributable to gender, underlying disease characteristics, age, or other confounding factors.

The majority of patients included in the studies were paediatric, and the age range in Study 003 and the RTTLT Pool was 5-20 years and 5-21 years, respectively. Study 009 included 15 patients 2-5 years of age. Data above the age of 21 years is scarce, and data above the age of 40 years is lacking. Based on the available PK/PD data, there is currently no evidence that safety would be different in adults compared to adolescents/children.

Adverse Drug Reactions

The safety profile of trofinetide is not without concern, and tolerability of trofinetide is considered poor due to the overall high number of discontinuations (17% in Study 003 and 43% in the RTTLT Pool), mainly due to gastrointestinal AEs (driven by diarrhoea and vomiting). Other AEs leading to discontinuation in Study 003 were seizure (3%), weight decreased (2%), and decreased appetite (2%). The proportions of patients and TEAEs leading to discontinuation in the RTTLT Pool are now

included in the proposed SmPC (section 4.8, summary of the safety profile). Included information provides adequate understanding of the impact of tolerability issues, with long-term treatment.

Further, trofinetide dose was modified for 35.5% of patients, and 10% reverted to their original dose following dose reduction. Dose modifications were applied in 7/16 subjects who discontinued Study003 and 10/16 had concomitant medication prior to discontinuation. Doses were typically reduced as part of the management of diarrhoea. Detailed reasons for dose reduction are not available, since investigators were not required to mention which AE led to dose reduction. The impact of dose reductions on efficacy outcomes remains uncertain. A modest change from baseline in RSBQ and CGI-I is observed in patients treated with trofinetide, irrespective of dose modifications. Patients on trofinetide requiring a dose reduction, however, seem to have a smaller change from baseline in RSBQ vs placebo (-3.3±1.8 vs 0.4±4.3), compared to those who received their planned dose (-6.0±1.2 vs -1.8±1.0). Dose reductions occurred almost exclusively in the trofinetide group (n=33), limiting the direct comparison with placebo (n=5). It cannot be excluded that efficacy is affected by dose reductions, especially if long-term dose reductions are required. Also, with longer treatment duration, the need for dose reductions increased (61% in the RTTLT Pool). The proposed SmPC section 4.2 included the recommendation to reinstate the planned dose as clinically appropriate. Considering the conclusion that efficacy has not been demonstrated this issue was not further pursued.

Based on the available data, the impact of the diarrhoea management plan seems to be insufficient, since the overall number of discontinuations was high, and diarrhoea seemed to persist. In the RTTLT Pool diarrhoea was persistent (13%) or recurred (40%) for the majority. Another 19% discontinued treatment due to diarrhoea. For 18% diarrhoea completely resolved while on trofinetide and for 9% it resolved after the last dose. Vomiting resolved while on treatment for the majority of patients (48%). In total vomiting recurred in 36% of patients, and 6% discontinued treatment due to vomiting. The persistence/recurrence of diarrhoea and vomiting are reflected in the proposed SmPC section 4.8 and this is accepted. The proportions of patients with persistent/recurrent diarrhoea/vomiting are now indicated based on the population that actually experienced respective AE (53% for diarrhoea and 36% for vomiting).

The impact of common gastrointestinal AEs (diarrhoea and vomiting) on the overall health and quality of life of patients with RTT remains uncertain. During the early engagement the patient organization expressed their concerns with regards to adverse reactions (e.g., diarrhoea) that should not compromise essential daily functioning like attending school or other activities. They considered side effects acceptable if they are manageable. Whether or not diarrhoea and vomiting are manageable/acceptable differs per individual patient/carer. Sufficient information is provided in SmPC/PIL for patients/carers to make informed decisions together with their prescribers. Diarrhoea and vomiting are included in the proposed SmPC as ADRs and details are provided with regards to the incidence, severity, persistence, etc. in clinical studies (section 4.8). Further, warnings and precautions are included, including recommendations for dose reduction or discontinuation if treatment is not tolerated (sections 4.2 and 4.4).

Bodyweight loss may have a temporal relationship with common gastrointestinal AEs, and an E-R relationship was shown. Since weight loss occurs frequently in patients with RTT, it is challenging to determine a temporal relationship with GI AEs following trofinetide treatment. No difference was found in the proportion of patients with weight loss ≥7% or AE weight decreased, among those who experienced diarrhoea or vomiting and those who did not experience these TEAEs in the RTTLT Pool (23% vs 22%). This comparison is affected by an imbalance in group sizes (n=160 with vs n=18 without diarrhoea/vomiting), which may limit the validity of conclusions. The provided data give an idea of whether diarrhoea/vomiting and weight loss occurred within the same patient, but do not enable to determine causality, sequence of events, and relatedness in time. The Applicant provided a time-to-event analysis for ⩾7% weight loss with time-updated diarrhoea and vomiting in the RTTLT

pool, upon request. Diarrhoea event status was used as a time-dependent covariate, adjusting for baseline weight z-score, age, feeding difficulties, total trofinetide exposure, and dose reduction status in Cox proportional hazard model. Overall, the analysis suggests that diarrhoea and vomiting do not increase the risk of ⩾7% weight loss.

The Applicant proposed 6 Adverse Drug Reactions to be included in section 4.8 of the SmPC: Diarrhoea, vomiting, seizure, decreased appetite, irritability and weight loss. The rationale to include these ADRs in the SmPC (section 4.8), and the strategy for selection of ADRs in general, is provided. Identified ADRs are based on a causality assessment of the total safety database in RTT patients. This is generally accepted, although some uncertainties remain (see below).

Diarrhoea, vomiting and decreased appetite are considered as ADRs. There was a significant imbalance in frequency of occurrence for diarrhoea (81% vs 19%) and vomiting (27% vs 10%) with trofinetide vs placebo. Diarrhoea was the most common AE leading to discontinuation (13% trofinetide vs 0% placebo). Decreased appetite may be associated with the occurrence of diarrhoea and vomiting. More patients with trofinetide than placebo (5 vs 2) reported decreased appetite, incidence increased with longer treatment duration (10% RTTLT Pool), and it led to discontinuation in 3% of patients (vs 0% placebo).

The potential impact and consequences of gastrointestinal ADRs are described in the proposed SmPC (section 4.8 description of adverse reactions). The onset, duration, persistence/recurrence and the overall number of patients discontinuing treatment are included.

Antidiarrhoeal treatment was used by 81% of those with diarrhoea in the RTTLT Pool, at different dosing regimens (as needed or continuous). Among those who discontinued due to diarrhoea, 85% received antidiarrhoeal medication.

Based on the totality of data on bodyweight, bodyweight loss was included as ADR upon request. The number of patients with an AE weight decreased (n=3) was considered to be underreported, considering the number of patients with ≥7% bodyweight loss (11 with trofinetide vs 4 with placebo). Per the proposed SmPC (sections 4.2 and 4.4) it is recommended to monitor the patients' weight, and modify the dose if needed. Other AEs secondary to diarrhoea or vomiting (incl. dehydration, UTI, dermatitis diaper, and aspiration) were concisely discussed. The Applicant took a conservative approach, all AEs occurring at any time after onset of diarrhoea/vomiting were considered secondary, even if not temporally associated. Whether these AEs occurred as a consequence of GI AEs therefore remains uncertain. Since the occurrence was relatively limited, these were not included as ADR. For dehydration and aspiration warnings are included in the proposed SmPC (section 4.4), this is considered sufficient.

The inclusion of seizures as ADR is agreed. Although seizures can be considered as manifestation of RTT itself, and most AEs of seizure or increase in seizures were reported as not related, an imbalance in frequency of occurrence (8 trofinetide vs 5 placebo) may imply a reasonable possibility for causality. The mechanism by which trofinetide may potentially aggravate seizures remains unknown. Seizure occurred in patients with and without history of seizures, no differences were observed in proportions of increased seizures, among those with TEAE of seizure, between trofinetide and placebo.

Previously proposed grouped terms fatigue (includes PTs lethargy, listlessness, somnolence) and anxiety (includes PTs agitation and irritability) were deleted from the list of ADRs upon request. Both were not reported as single PTs in Study 003 or the RTTDB Pool, and in the long-term safety pool (RTTLT) the incidence was low (2% for both). In general, the terms that were grouped together are not considered to have strictly the same definitions. Inclusion of single PTs as ADRs is also not considered justified, except for irritability, for which a clear imbalance was seen between trofinetide and placebo (6 vs 0). Agitation was only reported once, and no clear imbalance was seen for lethargy,

listlessness, and somnolence. Therefore, upon request, PT irritability was added to the list of ADRs. None of the subjects who experienced irritability or other psychiatric disorders (bruxism, agitation), were given concomitant medications for those indications.

The Applicant is of the opinion that there is no causality for Pyrexia and Nasopharyngitis with trofinetide, however a clear justification is lacking. An imbalance in occurrence of pyrexia was seen with trofinetide vs placebo (8 vs 4), this was less clear for (single PT) Nasopharyngitis (2 vs 1). Based on the data from the RTTDB Pool there is no dose-response effect for pyrexia (Table 43). Therefore, it is agreed that pyrexia and nasopharyngitis are not included as ADRs of trofinetide.

Serious Adverse Events, Deaths, and other significant events

Overall, the number of subjects with SAEs was low in Study 003 (trofinetide: 5 events in 3 subjects and placebo: 3 events in 3 subjects) and the RTTDB Pool (trofinetide: 12 events in 8 subjects and placebo: 4 events in 4 subjects). The overall incidence of SAEs with trofinetide was increasing with longer treatment duration in the RTTLT Pool (88 events in 39 subjects). Also without a control group for reference in this pool, it can be considered that the majority of SAEs may be attributable to RTT disease manifestations (e.g. acute respiratory failure), comorbidities (pneumonia, respiratory infections), or manifestations occurring in a merely paediatric population (viral infections).

There were 3 deaths while patients were exposed to study drug (an additional fatal case was not considered treatment-emergent). Although causality cannot be readily excluded, the narratives do not clearly point towards a causal relationship with trofinetide. The cause of death for treatment emergent cases were explained by vomiting leading to aspiration, sudden unexplained death in epilepsy, and cardiac arrest. Considering that seizures are listed as ADR, it is especially difficult to exclude causality for the fatal case associated with increase in seizures. Yet, no additional measures which can prevent or reduce the risk have been identified; the addition of a warning in section 4.4 is therefore not deemed necessary. Although it is reasonable to consider that the fatal case of aspiration was not related to trofinetide, due to the common occurrence of aspiration in RTT, treatment with trofinetide may increase the risk of aspiration due to vomiting. A warning that aspiration and aspiration pneumonia have been reported following vomiting in patients treated with trofinetide is therefore included in the proposed SmPC (Section 4.4).

In all combined studies, accidental overdose has been reported for 6 subjects per the safety database. The overdose in these cases was at most double the planned dose, was unintentional and a result of an additional trofinetide dose given due to caregivers' error (3 subjects in Study 003, 2 in Study 004, and 1 in Study 009). One subject in Study 003 reported an AE of vomiting associated with the overdose, this information is now included in the proposed SmPC (section 4.9).

Laboratory and other findings

There were overall no safety signals concerning haematology, chemistry and vital signs, except for incidences of increased ALT (7 trofinetide vs 3 placebo), decreased blood pressure (8 trofinetide vs 4 placebo), and decreased bodyweight (11 trofinetide vs 4 placebo):

The increase in ALT indicates some degree of liver cell stress, and even if trofinetide is primarily renally excreted, it may still be metabolized in the liver or interact with hepatic pathways. Yet, evidence for a causal relationship with trofinetide is insufficient at this time. Further, ALT increases were self-limited, other liver parameters were mostly normal, and there were no clinical consequences. Therefore, ALT increased is not considered an ADR of trofinetide.

There is currently no evidence for a link between decreased blood pressure and hypovolemia due to diarrhoea. Among those treated with trofinetide with decreased blood pressure (n=13), there were no AEs of dehydration, and not all had diarrhoea (n=6). Further, considering that decreased blood

pressure was not persistent, had no clinical consequences, and could also be caused by other factors (including manifestations of RTT: autonomic nervous system dysfunction, cardiac problems), there is no need to consider decreased blood pressure as an ADR of trofinetide at this time.

The overall growth patterns between the trofinetide and placebo groups seem similar, indicating that any effect of trofinetide on growth may be subtle. For weight, the majority of patients in both groups fall within the 25th to <75th percentile range across age categories on the Rett Growth Charts. Yet, these data may be impacted by the relatively high discontinuation rate and the variable (and unreported) treatment duration per age group.

The Applicant did not discuss the relevance of a percentile cut-off as requested. Since no major trends for impaired growth with trofinetide vs placebo were found based on the overlay plot, this issue is not further pursued. The impact of trofinetide treatment on failure to thrive, and pubertal development remains undiscussed, despite multiple requests. Yet, in the absence of a clear effect on growth, there is no reason to expect an impact of trofinetide on thriving or pubertal development, this issue is not further pursued.

ECG results in Study 003 did not point towards evidence of prolonged QT with trofinetide. the incidence of QTcF >450ms was limited, and higher in the placebo group vs trofinetide (3 vs 1). In the RTTLT Pool the incidence of QTcF >450ms did not increase with a longer treatment duration, and was similar to the incidence in placebo in Study 003 (3%). Cardiac abnormalities are a clinical feature of RTT, and the incidence of prolonged QT interval is increased in patients compared to age-matched healthy subjects. In Study 003 patients with clinical significant cardiovascular disease, a history of or risk factors for torsades de pointes, were excluded. Post-marketing the frequency of AEs associated with ECG WT prolongation was low (0.8%; 14 events of which 5 with medical history of prolonged QT) and there were no fatal events. Based on the available data, no risk of QT prolongation was identified. Further, RTT patients are routinely monitored by ECG, due to the risk of QT prolongation. Therefore, it is considered that, despite non-clinical uncertainties and suboptimal QT Study design due to low safety margins (see PD section), the risk of serious consequences due to cardiac repolarization is low and does not warrant further studies at present time.

Safety in special populations

Common AEs reported in patients 2-5 years of age were generally comparable to the safety profile in the population above 5 years of age. Diarrhoea and vomiting were the most common AEs reported (in 80% and 53%, respectively) and led to discontinuation in 2 patients (1 each). Vomiting occurred more frequently in patients 2-5 years of age compared to patients 5-20 years of age (in Study 003). The exposure of trofinetide is highest in the youngest patient group (refer to PK section), based on E-R relationship, the incidence of diarrhoea and vomiting increased with increasing exposure. However, the number of patients evaluated in this subset is limited.

Among age groups above 5 years, the incidence of diarrhoea seemed to be higher in the youngest (5-≤12 years) and the oldest patients (≥17-21 years), the incidence of vomiting decreased with increasing age. There seems to be no clear pattern in incidence of diarrhoea among age groups. For vomiting the imbalance in occurrence is likely due to younger children being more prone to vomiting then adolescents/adults. Diarrhoea and vomiting are both already included as ADR in the proposed SmPC (section 4.8) with frequency very common. Seizures occurred more frequently in the youngest age group (20% in 5 to ≤12 years) compared to the older age groups (7% in both ≥12-16 years and ≥17 years). Generally, the prevalence of seizures in patients with RTT is increasing with age. The incidence in the older age groups is lower compared to the natural history cohort, whereas the incidence in the younger age group is in line with this cohort. In addition, the frequency of the ADR seizure is "common" which accurately reflects the incidence seen in the youngest patients included. No additional information in the SmPC is warranted.

Safety data in patients with renal impairment was limited (n=10), and did not point towards a relevant difference. Dose adjustments are recommended for patients with moderate renal impairment and trofinetide is not recommended for patients with severe renal impairment.

Generally, administration via G-tube did not lead to differences in the safety of trofinetide compared to oral administration. Except that a higher proportion of patients with G-tube administration had diarrhoea. Frequency of vomiting was not different. Diarrhoea is already included as ADR in the proposed SmPC, further recommendations are not warranted.

Safety related to Drug-Drug interactions and other interactions

A Drug-Drug interaction study was conducted to assess the interactions between loperamide and trofinetide. In general, there were no new safety findings in patients treated with loperamide + trofinetide, except for the occurrence of contact dermatitis (reported by 6 subjects). This finding does not raise concerns, contact dermatitis was not reported during the clinical studies of trofinetide in RTT, while the use of antipropulsives, including loperamide, was common.

Post-marketing experience

Serious cases of aspiration and aspiration pneumonia (n=64) were spontaneously reported in the US between 10 March 2023 and 30 June 2025. Evaluation of causality by case was provided upon request, majority of cases was assessed as not related to trofinetide, and this seems reasonable. Since vomiting may increase the risk of aspiration a warning is now included, and this is considered appropriate. Although aspiration is common in patients with RTT, treatment with trofinetide may increase the risk of aspiration due to vomiting.

In RWE study 014 the safety profile of trofinetide is overall comparable to what has been reported in Studies 003, 004 and 005. To help manage concerns with tolerability of trofinetide, the average starting dose was commonly reported as being approximately half of the target dose and was up titrated to the recommended dose based on clinical judgement. The current SmPC does not include recommendations for a lower starting dose, since no data are currently available to evaluate the effect on tolerability and efficacy, this is accepted.

2.6.10. Conclusions on the clinical safety

The safety profile of trofinetide is not without concern; tolerability is considered poor due to the overall high number of discontinuations.

The most relevant ADRs to consider in the Benefit/Risk assessment are considered to be gastrointestinal disorders: diarrhoea and vomiting, and AEs that may be secondary to gastrointestinal disorders: including bodyweight loss, impaired growth, dehydration, and the risk of aspiration. Although gastrointestinal AEs were generally not severe or serious, their very common frequency makes them relevant for the B/R. Diarrhoea frequently led to permanent discontinuation, and probably also dose reductions, which questions whether patients received the optimal dose. In addition, diarrhoea was persistent and recurrent, which has consequences for the everyday functioning and the quality of life of patients with RTT. Especially with long-term treatment, diarrhoea may have consequences for the overall health of patients (incl. bodyweight loss, impaired growth, dehydration). For vomiting, the risk of aspiration is of special concern, which is already common in patients with RTT.

Another relevant ADR to consider in the B/R assessment is seizures, despite the natural occurrence of seizures in the RTT population. Seizures occurred more frequently with trofinetide compared to placebo, led to discontinuations, and were severe in some cases (incl. 1 fatal case). The safety profile

of trofinetide could be acceptable (with mitigation measures), if the benefit is adequately demonstrated, which is not the case.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table 53: Table SVIII.1: Summary of safety concerns

Summary of Safety Concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

2.7.2. Pharmacovigilance plan

No routine pharmacovigilance activities beyond adverse reaction reporting and signal detection have been proposed.

2.7.3. Risk minimisation measures

2.7.3.1. Routine Risk Minimisation Measures

Table 54: Description of Routine Risk Minimisation Measures by Safety Concern

Safety Concern	Routine Risk Minimisation Activities
None	Not applicable

2.7.3.2. Summary of additional risk minimisation measures

Table 55: Summary Table of Pharmacovigilance Activities and Risk Minimisation Activities by Safety Concern

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
None	Not applicable	Not applicable

2.7.4. Conclusion on the RMP

The CHMP, having considered the data submitted in the application was of the opinion that due to the concerns identified with this application, the risk management plan cannot be agreed at this stage.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

N/A

2.9. Non-Conformity of paediatric studies

N/A

2.10. Product information

In light of the negative recommendation, a satisfactory summary of product characteristics, labelling and package leaflet cannot be agreed at this stage.

2.10.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the Applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Rett syndrome (RTT) is a debilitating neurodevelopmental disorder. While the great majority of patients with RTT are females, males who meet the criteria for RTT have also been identified (Neul et al., 2019).

The incidence of RTT is reported as approximately 1 in 10,000 female live births worldwide (Petriti 2023).

In approximately 96% to 98% of patients diagnosed with classic RTT, the disease is caused by mutations in the X-linked methyl-CpG-binding protein 2 gene (MECP2) (Percy et al., 2018). MECP2 encodes human methyl-CpG-binding protein 2 (MeCP2), which modulates gene expression by binding to methylated CpG dinucleotides, primarily by activating but also by repressing transcription (Hite et al., 2009; Ip et al., 2018; Kriaucionis et al., 2003; Neul et al., 2008). The activity of the MeCP2 protein is diminished in RTT in both neurons and astrocytes (Yasui et al., 2013).

In patients with typical RTT, there is seemingly normal psychomotor development for the first 6 months of life but thereafter, the failure to reach normal developmental milestones can be observed, followed by a period of developmental regression in which there is a loss of normal use of the hands and of spoken language (Neul et al., 2014; Samaco et al., 2011).

Rett syndrome is defined by characteristic clinical features, which typically include loss of verbal and nonverbal communication and voluntary motor function, characteristic repetitive hand stereotypies, and gait problems. Loss of purposeful hand use is a defining aspect of RTT during the onset of regression, and at least 30% of individuals remain without any type of purposeful hand use (Downs et al., 2010). Other commonly observed symptoms include seizures, awake breathing disruptions, scoliosis, and intense eye communication (Neul et al., 2010). Seizures have a lifetime prevalence in RTT of around 90%. The course of seizure occurrence and remission is highly variable, with the most common pattern being waxing and waning over the lifespan. Gastrointestinal dysfunction, including constipation, gastroesophageal reflux disease, and chewing and swallowing difficulties, are observed in most patients with RTT (Baikie et al., 2014; Motil et al., 2012). Aspiration, aspiration pneumonia and co-occurring morbidities are not uncommon as are other infectious complications that can arise from a relatively immobile dependent state (Ramirez et al., 2020). Autonomic manifestations, which include abnormalities in cardiac and respiratory function, as well as in peripheral circulation, are considered to be predominantly of central nervous system (CNS) origin. Rett syndrome is also characterized by impaired growth affecting the brain and other organ systems.

3.1.2. Available therapies and unmet medical need

There are no therapies in the European Union approved specifically for the treatment of RTT. Treatment focuses on the management of each patient's symptoms and comorbidities.

The incidence of RTT is reported as approximately 1 in 10,000 female live births worldwide (Petriti 2023). There is an unmet medical need for treatments that target multiple aspects of the syndrome and improve overall systemic function.

The claimed indication is "*DAYBU is indicated for the treatment of Rett syndrome in adults and paediatric patients 2 years of age and older*."

3.1.3. Main clinical studies

The main efficacy and safety data are from pivotal **study 003**, a randomised, double-blind, placebo controlled study in girls and women with RTT aged 5 to 20 years post regression with a confirmed MECP2 mutation. Post regression was defined as having no loss or degradation within 6 months of Screening of ambulation, hand function, speech, or nonverbal communicative or social skills.

The double-blind treatment period was 12 weeks and subjects were randomized 1:1 to trofinetide (n = 93) and placebo (n = 94). Treatments were administered BID based on weight-banded dosing (6g [12 – 20 kg], 8g [>20 – 35 kg], 10g [>35 – 50 kg] and 12g [>50 kg]).

Two uncontrolled open-label extension studies provided longer follow-up data: Study 004 (n=154) up to 40 weeks, and Study 005 (n=77) up to an additional 32 months. Additional supportive studies were RETT-001, RETT-002 and study 009.

Safety data from studies 003, 004, 005 were pooled in the RTTLT Pool (n=178), for evaluation of long-term safety.

3.2. Favourable effects

After 12 weeks of trofinetide treatment a statistically significant difference was observed on the co-primary endpoints RSBQ total score and CGI-I. The least square mean (LSM) treatment difference was -3.1 for the RSBQ (95% CI [-5.7, -0.6], p= 0.0175) and -0.3 (95% CI [-0.5, -0.1], p= 0.0030) for the CGI-I.

On the key secondary endpoint CSBS-DP-IT Social Composite Score a statistically significant difference was observed in favour of trofinetide. The LSM difference was 1.0 (95% C [0.3, 1.7], p=0.0064).

The percentage CGI-I responders in a post hoc analysis, where a response is defined by having any improvement on the CGI-I (i.e., score 1, 2, or 3 at week 12), was 14% (13/93) compared to 32% (29/91) in the trofinetide group.

3.3. Uncertainties and limitations about favourable effects

Dose reductions of up to 50% of the target dose were permitted up until week 6 of the study in the event of tolerance issues (e.g., gastrointestinal issues). Therefore, functional unblinding due to diarrhoea could have impacted the results.

For all outcome measures, clinical relevance has been determined using cohen's d or a distribution based minimal clinical important difference (MCID), whereas it should have been most meaningfully determined based on clinical rather than statistical considerations and/or supportive responder rates and secondary endpoints reflecting the relevance of the effect. There is no MCID established in this condition for the co-primary endpoints. It is unclear how the numerical results on the co-primary endpoints would translate into a benefit for daily functioning of the patient.

Type I error correction ended at the key secondary endpoint, none of the remaining endpoints have been formally tested. The effect of trofinetide is not consistent across the secondary endpoints that contextualise the effects of the co-primary endpoints. Small numerical differences were observed (without nominal statistical significance).

The double-blind phase of 12 weeks is too short to draw a conclusion on a treatment effect in this long-lasting condition. In addition, maintenance of effect of trofinetide has not been established in controlled studies of adequate duration.

The study population of study 003 (female RTT patients aged 5 – 20 years of age) does not reflect the target indication (patients with RTT aged 2 years and older).

3.4. Unfavourable effects

Study 003: In total 93% of patients with trofinetide versus 54% with placebo experienced any TEAE; 3% in both groups experienced any serious TEAE; AEs were a reason for discontinuation in 17% with trofinetide and 2% with placebo.

RTTLT: In total 97% of patients experienced any TEAE; and 22% experienced any serious TEAE; AEs were a reason for discontinuation in 43% of patients.

The most common TEAEs (trofinetide vs placebo) in Study 003 included: diarrhoea (81% vs 19%), vomiting (27% vs 10%), seizures (9% vs 5%), pyrexia (9% vs 4%), decreased appetite (5% vs 2%), and irritability (7% vs 0%).

Diarrhoea and vomiting

In total 41% of patients with trofinetide had an TEAE of mild diarrhoea, 45% moderate diarrhoea, and 2% severe diarrhoea. In 13% of patients with trofinetide, diarrhoea led to treatment discontinuation.

In total 19% of patients with trofinetide had an TEAE of mild vomiting, 7% moderate vomiting, and 1% severe vomiting. In 1% of patients with trofinetide, vomiting led to treatment discontinuation.

Diarrhoea and vomiting were also the most common TEAEs in the RTTLT Pool (occurring in 85% and 37% of patients), and led to discontinuation in 26% and 7%, respectively. Diarrhoea was persistent/recurrent in 53% of patients.

Weight loss

A decrease in bodyweight (≥7% from baseline) was observed in 13% of patients in the trofinetide group vs 5% in placebo; 3% of patients in the trofinetide group had a concurrent AE of weight loss vs none of the patients in the placebo group. In the RTTLT Pool 22% of patients had a decrease in bodyweight; 8% had an AE of weight decreased.

AEs secondary to gastrointestinal AEs

Other AEs that may have occurred as a consequence of diarrhoea and/or vomiting were in Study 003 (incidence in trofinetide vs placebo): dermatitis diaper (4% vs 1%), urinary tract infection (3% vs 3%), aspiration pneumonia (2% vs 0%) and dehydration (1% vs 1%).

In the RTTLT Pool (incidence): urinary tract infection (11%), dehydration and dermatitis diaper (5% each), aspiration pneumonia (3%), aspiration (1%). Also serious cases of UTI (2%), dehydration (2%), aspiration and aspiration pneumonia (1% each) were reported, including 1 fatal case of vomiting leading to aspiration (considered not related by the investigator).

Seizures

In total, 8 (9%) patients with trofinetide reported 11 TEAEs of seizure, and 6 patients (6%) with placebo reported 6 TEAEs of seizure or partial seizures. The majority of these patients had a history of seizures/epilepsy (7 in the trofinetide group vs 5 in the placebo group). Mild seizures occurred in 3 patients in each group, and moderate seizures occurred in 5 patients with trofinetide and 2 patients

with placebo. No severe seizures were reported. One patient with trofinetide reported an SAE of seizure. Two patients in the trofinetide group discontinued due to seizures (vs none with placebo).

In the RTTLT Pool 15% of patients had an AE of seizure (24/28 had a history of seizures/epilepsy); 4% of patients discontinued due to seizure or seizure cluster. There was 1 fatal case of sudden unexplained death in epilepsy.

3.5. Uncertainties and limitations about unfavourable effects

The main uncertainty is the tolerability of trofinetide and the impact of common gastrointestinal AEs (diarrhoea and vomiting) on the overall health and quality of life of patients with RTT. Diarrhoea and vomiting were generally not severe or serious, and vomiting led to discontinuation in a limited number of patients. Yet, there are potential (serious) consequences for the overall health of patients, including weight loss, and other AEs that may develop secondary to diarrhoea or vomiting (e.g., aspiration, dehydration, UTI, dermatitis diaper). A recommendation to interrupt, modify, or discontinue treatment in case of tolerability issues was included in the proposed SmPC (sections 4.2 and 4.4). Further, a warning for the risk of aspiration following vomiting, and a recommendation to monitor patients' weight were included (proposed SmPC section 4.4).

Discontinuations due to AEs were high as well as the number of patients needing dose modifications. The impact of dose modifications on the efficacy of trofinetide remains uncertain, and the Applicant addressed this in the proposed SmPC section 4.2, together with the recommendation to consider reinstatement of the recommended dose based on clinical judgement. In addition seizures were reported as an AE, however considering that seizures are a feature of the disease, there is uncertainty about the potential effect of trofinetide on aggravating seizures in some patients with RTT.

3.6. Effects Table

Table 56: Effects Table for Daybu for the treatment of Rett Syndrome

Effect	Short Description	Unit	Trofinetide 200-500 mg/kg	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
RSBQ (co-primary)	Change from baseline in Rett Syndrome Behavior Questionnaire at week 12	LSM (SE)	-4.9 (0.94)	-1.7 (0.90)	**SoE**: LSM difference -3.1 (p= 0.0175) **Unc**: - - Clinical relevance of effect not substantiated - No support secondary endpoints that contextualise RSBQ elements/RTT core features - Impact of functional unblinding unclear - Study duration too short to establish efficacy / Maintenance of effect not substantiated	ACP-2566-003
CGI-I (co-primary)	Clinical Global Impression of Improvement score at week 12	LSM (SE)	3.5 (0.07)	3.8 (0.07)	**SoE**: LSM difference -0.3 (p= 0.0030) Post hoc responder analysis (any improvement): 32% (29/91) in trofinetide and 14% (13/93) in placebo **Unc**: - Impact of functional unblinding unclear - No support secondary endpoints that contextualise RTT core features - Study duration too short to establish efficacy / Maintenance of effect not substantiated	ACP-2566-003
CSBS-DP-IT Social Composite score (key secondary)	Change from baseline in Social Composite Score at week 12	LSM (SE)	-0.1 (0.26)	-1.1 (0.25)	**SoE**: LSM difference 1.0 (p= 0.0064) **Unc**: - Impact of functional unblinding unclear - Clinical relevance of effect not substantiated - No support secondary endpoints that contextualise RTT core features - Study duration too short to establish efficacy / Maintenance of effect not substantiated	ACP-2566-003
Unfavourable Effects						

Effect	Short Description	Unit	Trofinetide 200-500 mg/kg	Placebo	Uncertainties/ Strength of evidence	References
Diarrhoea	Incidence	%	81	19	**SoE**: high frequency; most common reason for discontinuation (13%); reason for dose modifications (exact % not reported); Consistent effect in RTTLT Pool; persistent/recurrent (53% in RTTLT Pool)	ACP-2566-003
Vomiting	Incidence	%	27	10	**SoE:** high frequency; consistent effect in RTTLT with second most common reason for discontinuation (7%). **Unc:** low discontinuation rate (1%) in Study 003.	ACP-2566-003
Weight loss	Overall postbaseline decrease in weight ⩾7%	%	13	5	**SoE:** Consistent effect in RTTLT Pool (22%); potential temporal relationship with diarrhoea **Unc:** low number of TEAEs weight loss (n=3 considered underreported); potential for simultaneous increase in growth/weight related to MoA and/or impact on growth factors	ACP-2566-003
Seizures	Incidence	%	9	5	**SoC:** occurred in patients with and without history of seizures. **Unc:** manifestation of RTT	ACP-2566-003

Abbreviations: CGI-I: Clinical Global Impression – Improvement, CSBS-DP-IT: Communication and Symbolic Behavior Scales, RSBQ: Rett Syndrome Behaviour Questionnaire, SoE: strength of evidence, Unc: uncertainty
Notes:

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Favourable effects

There are currently no treatments approved specifically for Rett Syndrome.

Although the main study 003 met its primary objective, the findings of the study do not allow for any conclusions with respect to efficacy, clinical meaningfulness and consistency of effect of trofinetide in the treatment of Rett Syndrome, as detailed in the following paragraphs.

A difference of -3.1 points on the co-primary endpoint change in RSBQ at week 12 was observed in the pivotal study 003. The clinical relevance of the observed effect sizes has not been demonstrated. The effect of trofinetide was also not consistent across endpoints that help contextualise the findings on the co-primary endpoints. With the exception of one, none of the secondary endpoints that measure RTT features support the co-primary endpoints, including several outcomes that are partially covered by the RSBQ. Moreover, the findings on the secondary endpoints are numerically small, and the relevance of these differences have not been substantiated. The observed group mean difference on coprimary endpoint CGI-I is not supported by a predefined post-hoc responder analysis which showed no clinically relevant difference between treatment groups. An additional post hoc responder analyses on the CGI-I wherein a responder was defined as having "any improvement" did show a clinically relevant difference in favour of trofinetide. However, the agreement between a responder on the RSBQ (improvement of at least 3 points) and CGI-I (any improvement) was minimal. It was not demonstrated that the findings on the co-primary endpoints would translate into a benefit for a RTT patients daily functioning, which was also concurred by the SAG-N. The double-blind treatment phase of 12 weeks is too short to establish efficacy in this condition. Finally, functional unblinding due to the high incidence of diarrhoea may have impacted the results.

RTT is a neurodevelopmental disorder characterised by a variety of symptoms. Not all RTT core features/symptoms are either covered by the endpoints evaluated in study (e.g. seizures, respiratory function, sleep function) or failed to show a benefit of trofinetide (e.g. ambulation). In light of this, the nonspecific mechanism of action, absence of evaluation of specific core RTT features and the findings of small, inconsistent, numerical differences are observed for specific RTT symptoms, the sought indication "treatment of Rett Syndrome" is not supported.

Rett Syndrome is a life-long disease requiring chronic treatment, yet maintenance of effect of trofinetide cannot be concluded. Study 003 had a double-blind treatment period of 12 weeks which is too short to claim efficacy. The open-label extension studies 004 (40 weeks) and 005 (until FDA approval) cannot be used to substantiate trofinetide long term efficacy/maintenance of effect. Both studies lack a control arm for comparison. Subjects for whom a benefit is perceived are more likely to continue treatment, thus selection bias cannot be excluded. In study 004, almost half of the subjects prematurely discontinued due to the occurrence of adverse events, hampering interpretation of effect. Thus, in addition to the lack of convincing data on short term efficacy, the long term studies are insufficient to support a claim of maintenance of effect.

The target population in the proposed indication are RTT patients from 2 years of age and older. Study 003 evaluated trofinetide in a more restricted population (girls and females with RTT aged 5 – 20 years) and the supportive studies (study RETT-001 and 009) are insufficient to claim efficacy of trofinetide in these patient subpopulations.

Study 009 in girls between the ages of 2 and 4 (regression phase) was primarily a safety study and lacks a control arm, therefore it is not possible to evaluate efficacy of trofinetide. Efficacy of trofinetide in older children (> 5 years, i.e. plateau phase) has not been demonstrated. By default this would mean that paediatric extrapolation to the regression phase would not be feasible. However, even if efficacy in the plateau phase would have been established, extrapolation to the regression phase of the condition would not be feasible. Although the underlying MECP2 mutation remains the same and symptoms may be similar across the two phases, the frequency and severity may not. Similarity in disease stages has not been demonstrated. Moreover, it is also unknown whether a similar treatment response can be anticipated. This has also been highlighted by the SAG-N, indicating that different instruments are needed to examine efficacy in the regression phase.

Proof of concept study RETT-001 was the only study which evaluated trofinetide in older adult women with RTT (16 – 45 years old). The results do not provide support for efficacy in this age group, as the doses of trofinetide evaluated are substantially lower than the final proposed posology and the total treatment duration was 4 weeks which is too short for showing a clinically meaningful effect. Efficacy was indicative with a large p-value of 0.2, increasing the risk for chance findings. Small numerical differences were observed between the placebo and trofinetide groups over the cohorts, but it was not demonstrated that these are clinically meaningful (e.g. compared to the MCID defined by the Applicant). The totality of evidence to support treatment initiation in adult patients aged > 20 years remains inconclusive. However, there is no reason to discontinue trofinetide treatment in patients who become > 20 years if they were benefiting from treatment already. However, considering that efficacy has not been demonstrated in study 003 (for any age group), the issue of extrapolation to older age groups was not further pursued.

Finally, none of the clinical studies evaluated trofinetide in male patients with RTT. The provided argumentation that there is no reason to assume difference in efficacy between male and female patients due to PK and PD similarity is insufficient. There are very limited data of male RTT patients who initiated trofinetide treatment in the ongoing RWE Lotus study, precluding any conclusions with respect to efficacy. From a pragmatic perspective, given the rarity of RTT in males, overlap in symptomatology and application of the same diagnostic criteria, CHMP considered that restriction of the indication to exclude male patients would not have been necessary, provided efficacy had been demonstrated, which was not the case.

Unfavourable effects

The most important unfavourable effects to consider are diarrhoea and vomiting. Although these AEs were generally not severe or serious, their very high frequency makes them relevant for the B/R weighing. Diarrhoea frequently led to dose reductions and/or permanent discontinuation, which questions whether patients received the optimal dose of trofinetide, and whether the efficacy and safety results are representative for the recommended dose. Further, the inability to properly manage diarrhoea may lead to significant burden for patients. Management of diarrhoea (incl. treatment with antipropulsives, halting laxatives, dose reductions) implemented in the trials was not sufficient. Vomiting may put patients at increased risk of aspiration, and diarrhoea may increase the risk of (serious) urinary tract infections. Especially if persistent/recurrent, diarrhoea and vomiting may have a significant impact on the overall health and daily functioning of patients (and their caregivers).

An important unfavourable effect is weight loss, which may have consequences for the overall growth and development with long-term treatment, and is a concern especially in a paediatric population, already at risk of growth failure. More patients with trofinetide lost weight compared to placebo, and weight loss was a reason for discontinuation. It remains uncertain whether this is

solely due to gastrointestinal AEs. Weight loss is included as an ADR. A warning to monitor bodyweight was already included in proposed SmPC.

Another consequence of gastrointestinal AEs may include dehydration and aspiration. To minimize the risk for serious consequence (incl. potentially life-threatening), a warning for the risk of aspiration was included in the proposed SmPC.

A relevant ADR to consider in the B/R assessment are seizures. The imbalance between trofinetide and placebo may imply that treatment with trofinetide may aggravate the risk of seizures, however, the potential mechanism is not known. Seizures led to discontinuation and were severe in some cases (incl. 1 fatal case). Considering that seizures occur frequently in RTT and their occurrence can increase with age, a treatment which potentially aggravates the risk of seizures deserves further investigation. No additional measures which can prevent or reduce the risk have been identified and in light of the negative B/R balance the issue has not been further pursued.

3.7.2. Balance of benefits and risks

Support for the sought indication is primarily based on the pivotal study 003. The findings of pivotal study 003 do not allow for any conclusions with respect to efficacy of trofinetide for the treatment of RTT. While statistically significant improvements in the co-primary and key secondary endpoints were shown, the clinical meaningfulness of the observed effects and consistency across endpoints has not been demonstrated. The double-blind treatment phase of 12 weeks is too short to establish efficacy and the impact of functional unblinding due to the high incidence of diarrhoea remains uncertain. In addition, the mechanism of action of trofinetide remains unspecific, some small numerical differences have been observed for certain RTT symptoms, while other specific core RTT features have not been evaluated at all. Generally, trofinetide is not well tolerated. Most important unfavourable effects (ADRs) are diarrhoea, vomiting and weight loss. Diarrhoea and vomiting occurred in the vast majority of patients, appeared difficult to manage, and frequently led to dose modification and/or discontinuation. Persistent gastrointestinal AEs may have (serious) consequences for the overall health (incl. weight loss, aspiration), thriving and quality of life of patients and caregivers. Warnings and recommendations for dose adjustment were included in the proposed SmPC as appropriate. The safety profile of trofinetide could be acceptable, if benefit is adequately demonstrated, which is not the case.

The benefit/risk of trofinetide is **negative**.

3.7.3. Additional considerations on the benefit-risk balance

SAG-Neurology

On January 16, 2026, a SAG Neurology meeting was convened upon request of the CHMP. Next to the core SAG-N members, additional experts were present. The following issues were discussed:

1. Does the SAG consider the effect sizes observed with trofinetide in improvement in the RSBQ and CGI-I at week 12 as clinically meaningful? Please take into account the impact of potential functional unblinding due to diarrhoea on these results.
2. How does the SAG consider the overall clinical relevance of the efficacy of trofinetide taking into account the totality of the results, including secondary endpoints and duration of the double-blind treatment phase?

3. Does the SAG consider that the presentation of symptoms and their severity during the regression phase in Rett syndrome is similar to the plateau phase of the disease? Can a similar response to trofinetide treatment in both stages of the disease be anticipated?
4. Does the SAG consider available efficacy data sufficient to support the use of trofinetide (to treat symptoms of Rett syndrome) in male patients and older adult patients with Rett syndrome (>20 years of age)?

The draft answers of the SAG experts were the following:

1. **Does the SAG consider the effect sizes observed with trofinetide in improvement in the RSBQ and CGI-I at week 12 as clinically meaningful? Please take into account the impact of potential functional unblinding due to diarrhoea on these results.**

Overall, most SAG-N experts are not convinced that the current results in RSBQ and CGI-I at week 12 could be considered clinically meaningful. The SAG-N experts discussed the below aspects when reaching this conclusion:

It was commented that some key domains (e.g., gastrointestinal issues and feeding, seizures) of the condition are not included in the instruments specified as endpoints which hampers reaching a conclusion on the overall efficacy of the medicinal product on Rett Syndrome. Some SAG-N experts highlighted that the favourable results on certain subdomains (e.g. hand movement, walking, fear, anxiety) should not be neglected in the context of the known unmet medical need. Other experts noted that results in the subdomains of these endpoints should not be interpreted aside the results of the overall sum score of the scale, and no control for type 1 error was applied in these exploratory analyses. Other SAG-N experts including patient's representative were of the opinion that an improvement in communication abilities (e.g. eye gazing, attention) is relevant in daily life, but was not convincingly demonstrated by the data and no difference was seen in the domains considered most important by caregivers based on data presented.

It was noted that the minimally clinically important differences (MCIDs) for these scales have not been established in the condition, making it difficult to interpret the small numerical differences. A patient representative stressed that the meaning of the small differences on the RSBQ and CGI-I for daily functioning is unknown. Some experts noted that the LSM differences from the MMRM analysis were reported, and noted the absence of reporting of absolute differences at 12 weeks. The use of Cohen's D as measure of effect size is questioned and cannot be interpreted in the absence of an MCID based on clinical parameters. Ordinal shift analysis would have been preferred for the CGI-I, which is an ordinal scale.

All experts considered 12 weeks of study duration too short a time period to draw a conclusion on treatment effect in this long-lasting condition.

Most experts were concerned about the potential for functional unblinding due to diarrhoea in particular because the frequency was unbalanced (81% of the treated participants experienced diarrhoea compared to 21% in the control group). This is particularly problematic when using subjective endpoints as in this case, in which RSBQ is caregiver-informed and CGI-I is clinician-informed.

The experts noted the relatively large number of drop-outs in the intervention arm. Adverse events such as diarrhea or lack of perceived effect may have contributed to this. This may have biased the results towards an overestimation of the treatment effect.

Patient's representatives consider that what it is clinically meaningful may vary from patient to patient but they were also uncertain whether these effects could be considered clinically meaningful. They mentioned that gastro-intestinal and feeding problems and seizures are important symptoms, but not captured in the studies.

2. **How does the SAG consider the overall clinical relevance of the efficacy of trofinetide taking into account the totality of the results, including secondary endpoints and duration of the double-blind treatment phase?**

As per the overall clinical relevance of the efficacy of trofinetide, the experts noted that absolute difference on the key secondary endpoint was small and the clinical relevance unknown. Most domains explored with the additional secondary endpoints did not show a difference between groups, despite some of these domains being part of the RSBQ. This illustrates the difficulty of interpretation of the RSBQ results. Some experts commented that for some symptoms this is not unexpected considering the 12-week study duration for the double-blind treatment phase. Other limitations discussed above in the context of the primary endpoints were also considered applicable here. For example, it was noted that the MCID of scales used for the key secondary endpoint is unknown. The large number of drop-outs in the open label extension studies 004 and 005 prohibits the interpretation of the long-term efficacy data.

One patient's representative considers that the overall results are not convincing and noted that it is unknown whether the use of this medicinal product could help to reduce the need for or affect the use of the other (non)-medical therapies (e.g. speech, sleep or epilepsy or behavioral therapies).

3. **Does the SAG consider that the presentation of symptoms and their severity during the regression phase in Rett syndrome is similar to the plateau phase of the disease? Can a similar response to trofinetide treatment in both stages of the disease be anticipated?**

The SAG-N agreed by consensus that extrapolation of the efficacy data from the plateau phase of the disease to the regression phase is not acceptable. It is unknown whether a similar treatment response can be anticipated.

The SAG-N experts expressed that the regression phase represents a very demanding and stressful period for families which may impact the answers to the instruments used as endpoints. Additionally, the SAG-N experts noted that despite some symptoms may be similar in these two phases, the developmental phase of the brain is not and therefore, the treatment effect may be different. Further, other experts added that some symptoms are similar but frequency and severity may not, which entails a difference as well.

Patient representatives agreed that the first phase of the condition comes with a psychological storm for the families including denial phase and phase for accepting the diagnosis impacting the answers in the questionnaires. Extrapolation is not acceptable.

4. **Does the SAG consider the available efficacy data sufficient to support the use of trofinetide (to treat symptoms of Rett syndrome) in male patients and older adult patients with Rett syndrome (> 20 years of age)?**

<u>Male patients</u>

The SAG-N experts agreed by consensus that symptomatology is more severe in male patients, who more often have a severe encephalopathy with epilepsy. Provided it can be concluded that the medicinal product is efficacious (i.e. if the answer to question 1 would be yes) the majority of the

SAG-N experts support extrapolation to males if they otherwise fulfill the eligibility criteria of the pivotal study. These experts acknowledged that males experience a more severe phenotype but the symptoms are similar and hence, a similar response could be anticipated for the same level of severity. Most experts agreed that more information on the effect of the medicinal product on epilepsy is required, because seizures may worsen with treatment and epilepsy is a more common and more severe symptom in male patients. Thus, these experts expressed that caution should be exercised on patients with epilepsy and it should be checked whether there is a signal on epilepsy worsening after treatment initiation.

Few experts said that based on the clinical differences between male and female and the absence of data on male patients in the current dossier, extrapolation is not possible.

Older adult patients with Rett syndrome (> 20 years of age)

Provided it can be concluded that the medicinal product is efficacious (i.e. if the answer to question 1 would be yes), the SAG-N experts noted that there are no clear identifiable reasons for the efficacy to be different above or below 20 years. However, the SAG-N experts noted that the mechanism of action is unknown and hence, it is unclear whether the effect may be different in a very different developmental time of the condition. They also noted that there are no data on the chronic use of the medicinal product and no data to support the efficacy in this patient group.

Patient and healthcare professional engagement

Patient perspective (Rett Syndrome Europe)

Rett syndrome affects every system in the body: central nervous system (around 70% have epilepsy, all have problems with communication, the majority being non-verbal), respiratory (breathing irregularities, hyperventilation, apnoea's), mobility (wheelchair dependence, motor planning difficulties), and other systems (endocrine, gastrointestinal, musculoskeletal, and cardiac).

In Europe, there is no specific treatment for Rett syndrome. The standard of care is predominantly symptomatic, such as using medications to control epilepsy. Given the complexity and breadth of symptoms, there is a clear need for treatments that target multiple aspects of the syndrome and improve overall systemic function. There is a lack of clear metrics to evaluate the effectiveness of symptomatic treatments, which hinders the ability to determine best practices. Quality of life is highly variable and closely linked to the individual's overall health status. The emotional, financial, and social burdens on families and carers are substantial and are not always sufficiently acknowledged or addressed in treatment strategies.

Desired improvements for new medicines include:

- Overall health: Enhanced systemic functioning across affected systems.
- Respiratory and sleep: Reduction in abnormal breathing patterns and improved sleep quality.
- Neurological: Fewer seizures and reduced teeth grinding (bruxism).
- Motor and communication: Better hand use, improved mobility, increased vocalizations, and more effective communication.
- Daily functioning: Enhanced ability in eating, drinking, and independent toileting.

Side effects considerations are:

- The acceptability of side effects is judged relative to the benefits.

- Side effects are acceptable if they are manageable (through slow titration and careful monitoring) and not permanently debilitating.
- A key concern is ensuring that side effects (for example, severe diarrhoea) do not compromise essential daily functions like attending school or other activities.

HCP engagement (EPNS)

Today, no specific curative treatment is available for Rett syndrome. The standard of care is symptomatic treatment. Improvements in the language and communication abilities, stereotypies and autistic features and motor disability are considered desirable benefits of new medicines. Side effects that could be not manageable would be drowsiness, decrease of eye contact, fatigability, tremor, as well as anorexia and dysphagia.

CHMP Reflection of the patient and healthcare perspective on clinical results

The feedback from the patient organisation in particular highlights the need for treatments that target multiple aspects of the syndrome. In single pivotal study 003, which is the primary source of data for the intended indication, various secondary and exploratory endpoints are included which focus on specific aspects of RTT such as hand function, motor function and communication. Respiration and sleep are partly covered by the RSBQ co-primary endpoint, but as discussed previously not evaluated in separate endpoints such as respiratory function. In addition, effects on major symptoms such as seizures are also not evaluated. Several of the secondary/exploratory endpoints in study 003 that focus on the desired improvements highlighted by the patient organisation do not show a benefit with trofinetide treatment.

From a safety perspective the patient organisation considers side effects acceptable if they are manageable. They expressed special concerns with regards to AEs (e.g., diarrhoea) that should not compromise essential daily functioning of patients. Diarrhoea was frequently occurring and despite the diarrhoea management plan, this was the most common reason for discontinuations and dose modifications. Uncertainties remain with regard to the impact on the overall health of patients (weight loss, dehydration, other secondary AEs). The impact on daily functioning (attending school or other activities) was not evaluated.

The input from patient and healthcare professional engagement is highly appreciated. Their input was considered valuable for the evaluation of CHMP's and the final conclusion on the B/R balance.

4. Recommendations

The overall benefit /risk balance of Daybu is **negative**.

Grounds for Refusal

Based on the CHMP review of the Application for trofinetide in the treatment of Rett syndrome, the CHMP considers that the **efficacy** of the above-mentioned medicinal product is not sufficiently demonstrated, and therefore recommends the refusal of granting a marketing authorisation for the above-mentioned medicinal product.

The CHMP considers that:

- The observed differences in the co-primary efficacy endpoints, assessed after 12 weeks of treatment, were too small to be of clinical relevance. Effects on other (key) secondary endpoints were inconsistent, modest and not clinically meaningful. Moreover, several key symptoms (e.g., seizures) of the Rett syndrome are not included among the endpoints. The interpretability of long-term efficacy is limited by the short placebo-controlled period and the large number of dropouts among patients with trofinetide in the extension studies.

- The population included in the study does not reflect the full spectrum of the proposed target population and available data question extrapolation from patients in the plateau phase to those in the regression phase of the disease.

Due to the aforementioned concerns a satisfactory summary of product characteristics, labelling, package leaflet, pharmacovigilance system, risk management plan and post-authorisation measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

Furthermore, following review of the available data in the context of the Applicant's claim of new active substance status, the CHMP position at the time of this report is reflected in Appendix 8.1.

5. Re-examination of the CHMP opinion of 26 February 2026

Following the CHMP conclusion that Daybu was not approvable for the treatment of Rett syndrome in adults and paediatric patients 2 years of age and older, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

5.1. Detailed grounds for re-examination submitted by the applicant:

5.1.1. Ground #1

The observed differences in the co-primary efficacy endpoints, assessed after 12 weeks of treatment, were too small to be of clinical relevance. Effects on other (key) secondary endpoints were inconsistent, modest and not clinically meaningful. Moreover, several key symptoms (e.g., seizures) of the Rett syndrome are not included among the endpoints. The interpretability of long-term efficacy is limited by the short placebo-controlled period and the large number of dropouts among patients with trofinetide in the extension studies.

5.1.1.1. Complementarity of co-primary endpoints CGI-I and RSBQ

The primary clinical evidence supporting the treatment benefit of trofinetide is derived from Study 003, a randomized, double-blind, placebo-controlled trial conducted over 12 weeks in 187 post-regression female patients with Rett syndrome aged 5 to 20 years. Efficacy was assessed using two co-primary endpoints (CGI-I score at week 12 and change from baseline to Week 12 in RSBQ score) as well as a key secondary communication endpoint, change from baseline to Week 12 in CSBS-DP-IT, along with additional clinician-reported (ClinRO) and observer-reported (ObsRO) outcome measures.

The two co-primary endpoints are described briefly below:

Clinical Global Impression - Improvement

The CGI-I is a clinician-rated categorical assessment designed to assess change over time, with Rett specific anchors designating how to determine improvement relative to Baseline. The response options scale includes: 1- Very much improved; 2- Much improved; 3- Minimally improved; 4- No change; 5- Minimally worse; 6- Much worse; 7- Very much worse.

In this study the CGI-I is scored based on anchors specific to the core features of Rett (Neul et al. 2015) but it should be remembered that it is a **global assessment** of the overall status of the patient. It not only considers patients' neurobehavioral manifestations that are included in the RSBQ, but also those that fall outside of it (e.g., seizures, gastrointestinal issues). It is not a checklist, but is an assessment of overall change, a single clinical judgment across disease areas, with a focus on changes across the core RTT manifestations. It captures a clinician's holistic judgment of patient improvement, mirroring "real-world" clinical practice, rather than just isolated manifestation scores. It takes into account the patient's total functional, emotional, and social well-being. Anchors for the CGI-I were written with explicit reference to key clinical domains that were to be considered that are relevant in Rett syndrome. These domains are:

language/communication, ambulation, hand use, social (eye contact), autonomic abnormalities, seizures, and attentiveness (Neul et al. 2015). In applying the anchors to real-life cases, the signs and symptoms of the underlying disease should be considered **as a whole**. An individual does not necessarily need to be impaired to the same degree across all signs/symptoms (Neul et al. 2015). For the CGI-I scale, the anchors provide a framework for considering the following factors related to sign/symptom severity in order to determine the global change score: duration, onset, durability of change, and the context of the sign/symptom change across the domains. The CGI-I anchors provide examples of signs/symptoms and, for scores representing important thresholds for treatment response, information on factors to differentiate between the scores.

Each rater was extensively trained on the CGI-I RTT-specific anchors by Jeffrey Neul, MD, the lead author of the publication on the use of the CGI scale in the assessment of RTT in clinical studies (Neul et al. 2015). CGI-I raters were certified after training and assessment. All attempts were made to ensure consistency of raters throughout the study period.

Rett Syndrome Behaviour Questionnaire

The RSBQ is a 45-item caregiver-completed rating scale assessing a wide range of neurobehavioral manifestations known to be impaired in RTT (Mount et al. 2002). The scale includes 45 items, including 8 subscales, whose ratings reflect the severity and frequency of symptoms. The eight subscales include:

1. General mood
2. Breathing problems
3. Hand behaviour
4. Face movements
5. Body rocking/expressionless face
6. Night-time behaviors
7. Fear/anxiety
8. Walking/standing

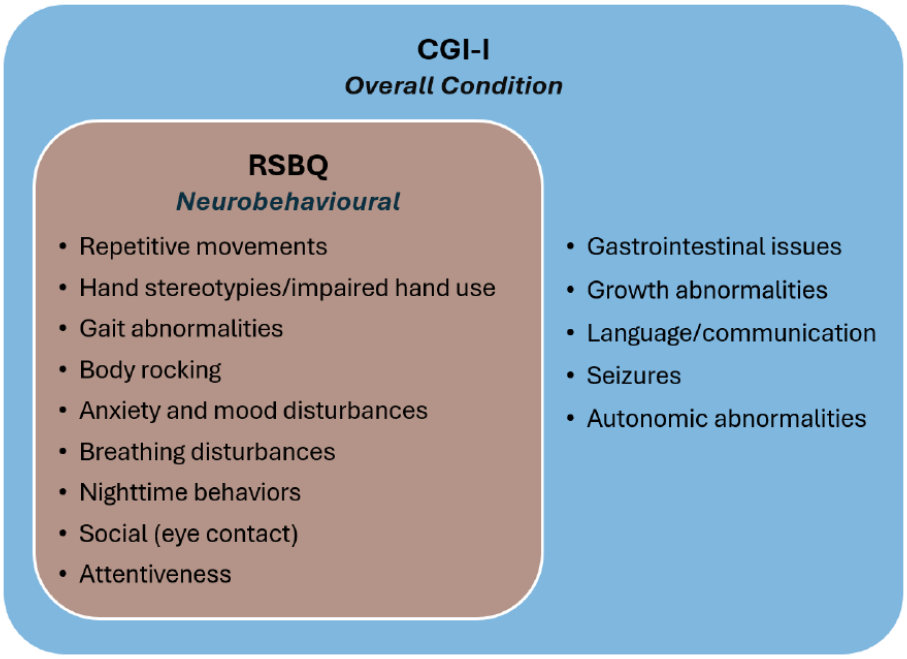
The caregiver rates items as "0" (not true as far as you know), "1" (somewhat or sometimes true) or "2" (very true or often true).

Although it was initially developed as a diagnostic tool to help differentiate females with RTT from those with other severe intellectual disabilities, the RSBQ transitioned into a clinical trial "fit-for-purpose" outcome assessment based on its reliability and validity in distinguishing between the two. Specifically, the RSBQ demonstrated high levels of internal consistency, content validity, construct validity (i.e., stable, factor structure), and discriminative validity (i.e., known groups validity) (Downs et al. 2024; Mount et al. 2002; Oberman et al. 2023). The RSBQ shows correlations with functioning, is validated across a range of ages (2 to 47 years) and is the most widely used instrument in Rett syndrome studies (Neul et al. 2023) due to its breadth as well as specificity in examining the neurobehavioural features of RTT.

Complementarity of co-primary endpoints

The CGI-I and RSBQ overlap in some, but not all, of the manifestations of Rett syndrome that they measure, and thus provide a complementary holistic assessment of the patient (Figure 25). The CGI-I provides a global disease perspective, whereas the RSBQ focuses on features/manifestations that define and distinguish Rett. Each co-primary endpoint captures distinct underlying disease constructs rather than redundantly measuring the same dimension. The results on change in RSBQ total score from Baseline confirm a treatment effect on the neurobehavioural manifestations of Rett syndrome, and the results on CGI-I confirm those treatment effects in the context of the broader patient presentation. The two co-primary endpoints are meant to be interpreted together and are both clinically relevant.

Figure 25: Diagram Illustrating the Complementarity of the Manifestations of Rett Syndrome Assessed by the RSBQ and CGI-I Scales



Sources: Mount et al. 2002 (RSBQ); Neul et al. 2015 (CGI-I)

Co-primary endpoints results – Study 003

In the pivotal study, Study 003, the mixed model for repeated measures (MMRM) was performed as the primary analysis for the co-primary and key secondary endpoints assuming missing at random. The statistically significant results on both co-primary endpoints are presented in Table 57. Various sensitivity analyses, including pattern-mixture models assuming missing not at random, were performed for each of the co-primary endpoints and confirmed the robustness of the primary analysis results and showed a consistent conclusion.

Table 57: Primary Analysis of Co-primary Endpoints CGI-I and RSBQ in Study 003 – Full Analysis Set

	CGI-I		RSBQ	
Visit	**Placebo (N=93)**	**Trofinetide (N=91)**	**Placebo (N=93)**	**Trofinetide (N=91)**
Baseline				
n	--	--	93	91
Mean (SE)	--	--	44.5 (1.26)	43.7 (1.21)

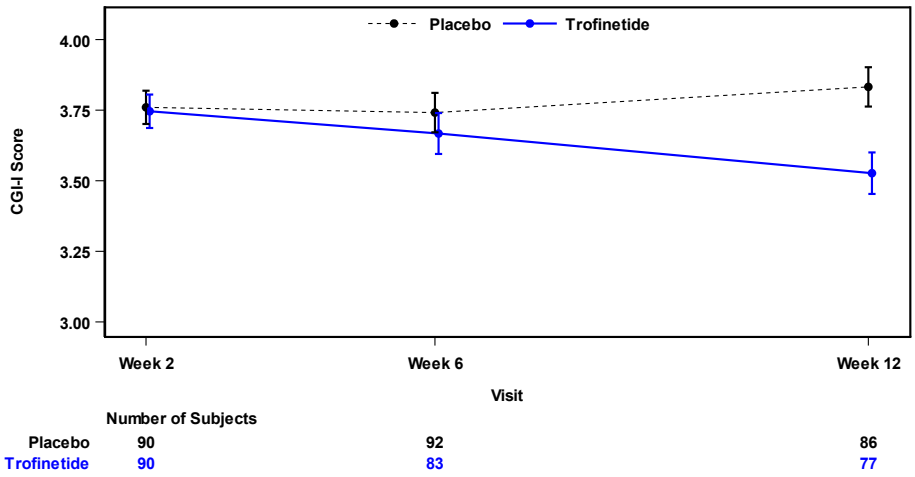
	CGI-I		RSBQ	
Visit	**Placebo (N=93)**	**Trofinetide (N=91)**	**Placebo (N=93)**	**Trofinetide (N=91)**
SD	--	--	12.2	11.52
Median	--	--	43.0	42.0
Min, max	--	--	14, 69	21, 74
Week 12				
n	86	77	85	76
Mean (SE)	3.8 (0.06)	3.5 (0.08)	42.8 (1.42)	39.9 (1.38)
SD	0.55	0.74	13.05	12.02
Median	4.0	4.0	41.0	40.5
Min, max	2, 5	2, 5	16, 69	9, 69
Change from Baseline to Week 12				
n	--	--	85	76
Mean (SE)	--	--	-1.7 (0.98)	-5.1 (0.99)
SD	--	--	9.05	8.67
Median	--	--	-2.0	-3.5
Min, max	--	--	-31, 40	-34, 10
MMRM Analysis				
LS mean (SE)	3.8 (0.07)	3.5 (0.07)	-1.7 (0.90)	-4.9 (0.94)
95% CI	(3.7, 4.0)	(3.4, 3.7)	(-3.5, 0.0)	(-6.7, -3.0)
Difference from Placebo				
LS mean difference (SE)		-0.3 (0.10)		-3.1 (1.30)
95% CI		(-0.5, -0.1)		(-5.7, -0.6)
Two-sided p-value		0.0030		0.0175
Effect size (Cohen's d)		0.47		0.37

Sources: Study 003 CSR Tables 11-6 and 11-8
Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; LS=least squares; max=maximum; min=minimum; MMRM=mixed-effects model for repeated measures; RSBQ=Rett Syndrome Behaviour Questionnaire; SD=standard deviation; SE=standard error

Co-primary endpoint results – CGI-I

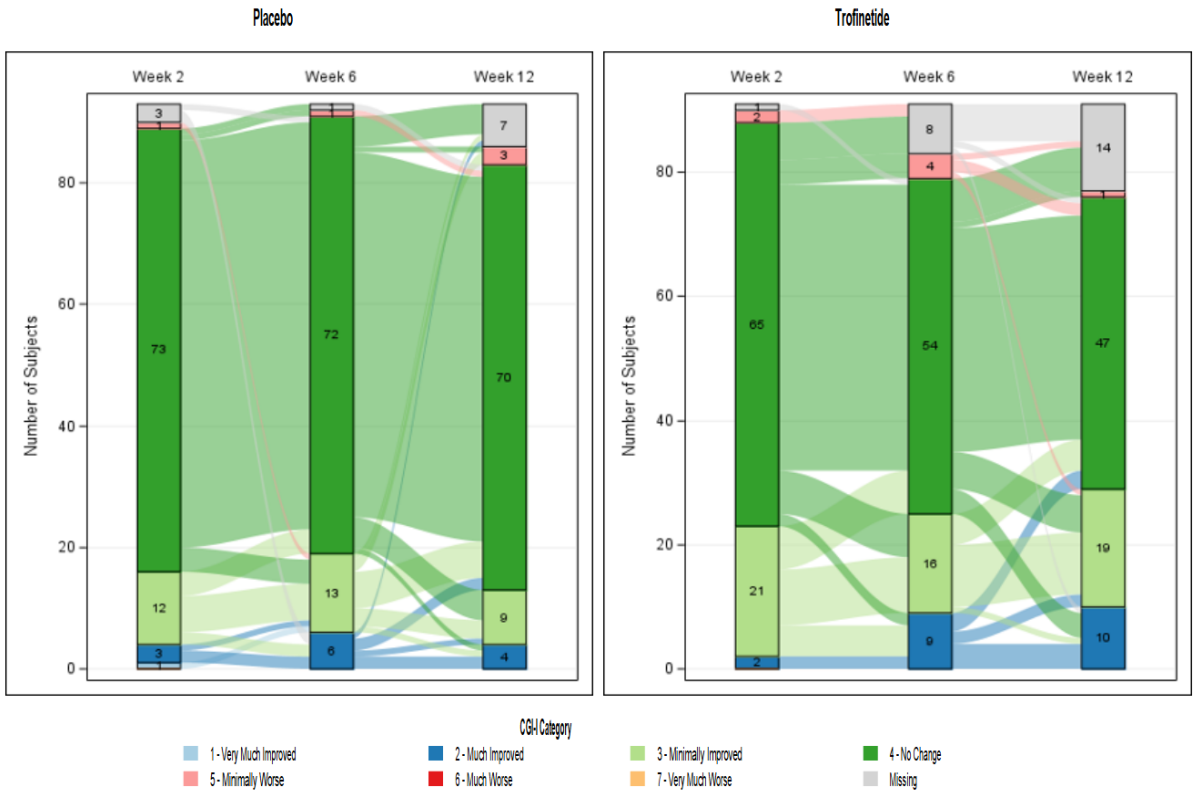
At Week 12, trofinetide-treated patients achieved a LS mean CGI-I score of 3.5, compared with 3.8 in the placebo group (MMRM LS mean difference: -0.3; p=0.0030; Cohen's d effect size: 0.47) (Table 57, Figure 26), reflecting a larger magnitude of clinician-assessed improvement observed in the trofinetide group than the placebo group after adjusting for age group, Baseline RSBQ severity category and Baseline CGI-S score. The robustness and clinical relevance of these findings were further supported by a non-parametric analysis (p=0.0035), reflecting a distributional difference between the trofinetide group and the placebo group after adjusting for age group, Baseline RSBQ severity category and Baseline CGI-S score (see Day 121 Responses Clinical MO Q130c). During the 12-week double-blind period, CGI-I scores in the trofinetide group continued to improve over time, unlike CGI-I scores for patients in the placebo group (Figure 26, Figure 27).

Figure 26: CGI-I Score by Visit (LS Mean±SE) (OC, MMRM) – Study 003 - Full Analysis Set



Source: Study 003 CSR Figure 11-3
Abbreviations: CGI-I=Clinical Global Impression-Improvement; LS=least squares; MMRM=mixed-effects model for repeated measures; OC=observed cases; RSBQ=Rett Syndrome Behaviour Questionnaire; SE=standard error

Figure 27: CGI-I Score by Visit – Study 003 – Sankey Plot



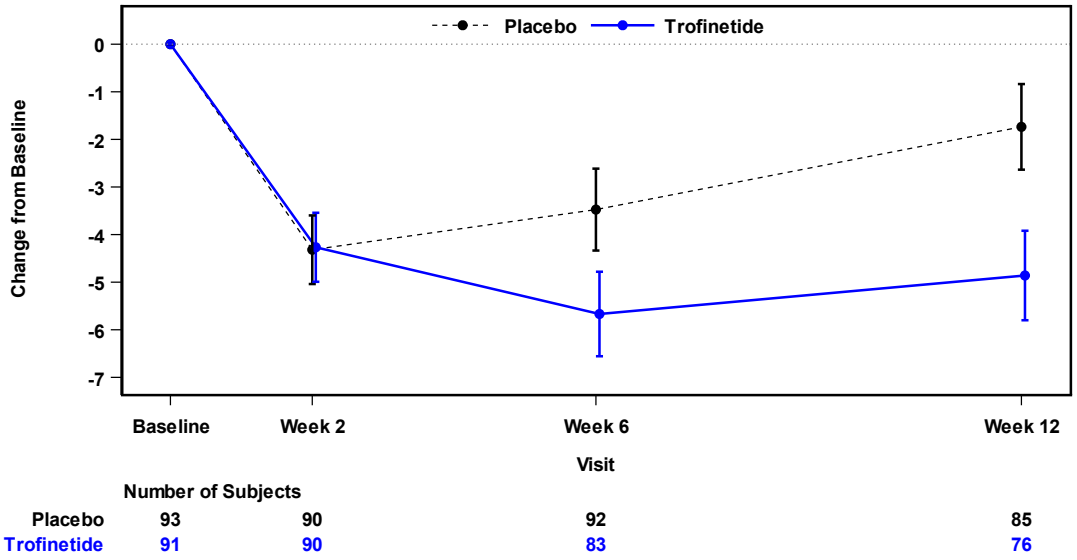
Source: Figure RX1.1
Abbreviations: CGI-I=Clinical Global Impression-Improvement

Co-primary endpoint results - RSBQ

Trofinetide treatment resulted in a least-squares (LS) mean reduction from baseline of 4.9 points in the RSBQ total score, compared with a 1.7-point reduction in the placebo group (MMRM LS mean difference: −3.1; p=0.0175; Cohen's d effect size: 0.37) (Table 57, Figure 28).

During the 12-week double-blind period, RSBQ total scores in the trofinetide group continued to improve following the initial treatment effect observed at Week 2 (Figure 28). In contrast, patients in the placebo group demonstrated an early improvement at Week 2, followed by a clear regression toward baseline. This transient placebo response at Week 2 is most plausibly attributable to expectation bias, a well-recognised phenomenon in caregiver-reported outcomes in neurodevelopmental disorders (Williams et al. 2012).

Figure 28: RSBQ Total Score Change From Baseline by Visit (LS Mean±SE) (OC, MMRM) – Study 003 - Full Analysis Set



Source: Study 003 CSR Figure 11-1
Abbreviations: LS=least squares; MMRM=mixed-effects model for repeated measures; OC=observed cases; RSBQ=Rett Syndrome Behaviour Questionnaire; SE=standard error

For completeness, and as was discussed in previous responses and presumed to be resolved, we are including in Appendix 1 a discussion of potential functional unblinding in our trofinetide studies.

5.1.1.2. MCID in Rett syndrome

During the assessment of trofinetide, the issue of establishing clinical relevance of the treatment difference in Study 003 has been raised. The Applicant has presented the discussion of clinically important differences for RSBQ for this re-examination within three perspectives:

- whether a pre-defined MCID for RSBQ was feasible prior to trofinetide Study 003
- estimates of an MCID for RSBQ with Study 003 results
- interpretation of clinical relevance beyond the MCID

Feasibility of pre-defining an MCID for RSBQ prior to Study 003

National Scientific Advice from four national competent authorities received in 2020 provided consistent perspectives that clinical relevance of results should be justified and pre-defined.

This advice was not disregarded; rather, there are inherent limitations in establishing an MCID in a rare heterogeneous neurodevelopmental disease for which no treatments have been established prior to trofinetide and for which no trials had previously been successful. Given the scarcity of treatment outcome data in RTT in general that inherently limited the ability to pre-define an MCID, which typically relies on large-scale validation across multiple trials, the Applicant believes that those difficulties should not be disregarded in the context of this severely debilitating and rare neurodevelopment disorder.

Estimates of an MCID for RSBQ with Study 003 results

Similar to national scientific advice issued prior to Study 003 results, in the pre-submission meeting with the previous Rapporteurs held on 05 November 2024 it was noted that an MCID for the RSBQ should be explained within the trofinetide MAA

The Applicant has previously provided the context of meaningful difference on the RSBQ by highlighting the value of the CGI-I, which was intentionally pre-specified as a co-primary endpoint to the RSBQ in Study 003 for the express purpose of lending interpretation of clinical meaningfulness to the RSBQ score change.

A post-hoc distribution-based method estimating a plausible MCID range of 3 to 6 points on the RSBQ has been provided (Sikirica et al. 2025) and now the Applicant has further conducted additional anchor-based analyses to estimate the MCID for RSBQ using the data from the two separate studies (Study 002 and Study 003) The CGI-I commonly serves to anchor changes in other scales in determining minimum clinically important differences (MCIDs) and can only do so because of its inherent clinical relevance. MCIDs estimated based on the two separate studies were consistent. A reduction of 3 to 7 points in the RSBQ total score has been anchored to any improvement on CGI-I (reflecting a CGI-I score ≤ 3). Furthermore, a reduction of 9 points in the RSBQ total score is the observed difference of change in RSBQ between the patients with "worsening or no change" on CGI-I (CGI-I score ≥ 4) and those with "much / very much improved" (CGI-I ≤ 2) (see results in Appendix 2).

The Applicant has used methods that are standard for the identification of MCIDs to provide a range of potential values. The Applicant's intent with these analyses is to provide evidence of within-patient changes that are of clear clinical relevance to give some context for the interpretation of threshold-based analyses. We acknowledge that as additional data become available in Rett syndrome, smaller changes may be established as being meaningful.

Interpretation of clinical relevance beyond the MCID

The fundamental concept behind MCID is to lend interpretability to a Patient-Reported Outcome/Caregiver-Reported Outcome (PRO/CaRO) score change that reflects what patients/caregivers perceive as beneficial. Therefore, if the ultimate goal is to understand what patients/caregivers perceive is clinically meaningful, and there remains a scarcity of data beyond trofinetide Study 003 to contextualize benefit via RSBQ score change in RTT, one must include other lines of evidence to interpret the relevance of trofinetide effects.

In the absence of a single pre-defined value for the MCID, responder rates should be taken into consideration to establish clinical meaningfulness, and these support the overall clinical relevance of trofinetide in RTT (see Section 5.1.1.3). For RSBQ, where no responder definition was pre-specified, we present an analysis across multiple thresholds, including thresholds of unequivocally clinically relevant changes.

5.1.1.3. Clinical meaningfulness/relevance of CGI-I results

In the pivotal clinical study, Study 003, the Applicant used co-primary endpoints of RSBQ and CGI-I that are complementary in nature. The caregiver-reported RSBQ measures specific, relevant aspects of Rett syndrome while the clinician-reported CGI-I is able to reflect whether changes in certain manifestations matter to patients in the global context of their disease.

Regarding the ground for refusal on clinical relevance, the final assessment report noted that trofinetide treated patients had a higher chance of having any improvement on the CGI-I compared to placebo, with that being considered a clinically relevant finding. Acadia concurs with the CHMP position [CHMP Assessment Report 26 February 2026 p135] that CGI-I scores of 1, 2, or 3 represent inherently clinically meaningful improvements.

Responder (CGI-I≤3) analysis showed that 31.9% (29/91) of trofinetide-treated patients improved compared with only 14.0% (13/93) in the placebo group (odds ratio [95%CI]: 2.9 [1.4, 6.1]; p=0.0042 (Table 58).

The percentage of patients judged to be "much / very much improved" on CGI-I (CGI-I ≤ 2) was numerically higher in trofinetide group (11.0%) than in placebo (4.3%) (p=0.0955) with the odds of achieving this in the trofinetide group being ~3 times the odds in the placebo group.

Table 58: CGI-I Score at Week 12 - LS Mean (SE) and Responder Analyses - Study 003 - Full Analysis Set

CGI-I Analyses at Week 12	Trofinetide (N=91)	Placebo (N=93)	Treatment Group Comparison	p-value	Cohen's d
MMRM					
LS Mean (SE)	3.5 (0.07)	3.8 (0.07)	LSM Difference (95% CI) -0.3 (-0.5, -0.1)	0.0030	0.47
Responder Analysis n (%)					
Responder: CGI-I ≤3 (MN)	29 (31.9)	13 (14.0)	Odds Ratio (95% CI) 2.9 (1.4, 6.1)	0.0042	N/A
Responder: CGI-I ≤2 (MN)	10 (11.0)	4 (4.3)	Odds Ratio (95% CI) 2.8 (0.8, 9.3)	0.0955	N/A

Source: Study 003 CSR Table 11-8 and Trofinetide MAA Summary of Clinical Efficacy Tables 2.7.3-21 and 2.7.3-22

Abbreviations: CGI-I=Clinical Global Impression–Improvement; CI-confidence interval; LS=least squares; MN=missing imputed as nonresponders; N/A=not applicable; SE=standard error

5.1.1.4. Clinical meaningfulness/relevance of RSBQ results

MCID

The final assessment report noted on page 175 that "There is no MCID established in this condition for the co-primary endpoints. It is unclear how the numerical results on the co-primary endpoints would translate into a benefit for daily functioning of the patient". The final assessment report also commented that "clinical relevance is usually determined based on a predefined MCID (based on clinical rather than statistical considerations) and/or supportive responder rates and secondary endpoints reflecting the relevance of the effect".

Agreement on a single value as the minimal clinically important difference on the RSBQ is not necessary in order to establish clinical relevance as responder analyses demonstrate the clear difference between trofinetide and placebo at thresholds that are unequivocally clinically meaningful. Methods to estimate MCID can inform this process by providing estimates of the minimal clinically important difference that allow us to identify the range of values that are clearly clinically meaningful.

The Applicant has previously submitted distribution-based MCIDs for RSBQ ranging from 3 to 6 to the EMA (see final EMA assessment; and Sikirica et al., 2025). The Applicant has now conducted additional analyses to estimate MCID for RSBQ using CGI-I as the anchor. Three approaches were applied to data from Study 002 and Study 003: a between-patient score change approach, a within-patients score change approach, and the method of 95% limits of upper agreement (Franceschini et al. 2023).

Study 002 was a Phase 2, 6-week, randomized, double-blind, placebo-controlled, dose-ranging study evaluating the safety and tolerability of oral trofinetide (50, 100, or 200 mg/kg BID) in 82 female children and adolescents with RTT. Study 003 served as the pivotal trial. In Study 002, all 82 patients had both a change in RSBQ score and a CGI-I score available at Day 54. In Study 003, 161 patients had both change from baseline in RSBQ score and CGI-I score available at Week 12. MCIDs estimated based on the two separate studies were consistent. Anchored to the category of "any improvement" (CGI-I score ≤3), the between-patient approach yielded MCID estimates of 6 RSBQ points in Study 002 and 5 points in Study 003. Using the within-patient approach, the corresponding estimates were 6 points and 7 points, respectively. The 95% limits of upper agreement method produced estimates of 3 points for Study 002 and 4 points for Study 003 (see results in Appendix 2). These estimated MCIDs ranging from 3 to 7 points improvement in RSBQ, anchored to CGI-I categories of 'any improvement', are consistent with the previously submitted distribution-based estimates, which ranged from 3 to 6 points. Furthermore, an improvement of 9 points in RSBQ score is the observed difference of change in RSBQ between the patients with "worsening or no change"on CGI-I (CGI-I score ≥ 4) and those with "much / very much improved"(CGI-I ≤ 2) in both Study 002 and Study 003 providing a "high bar" for clinical meaningfulness.

Regardless, as will be demonstrated in the following section, patients treated with trofinetide had a higher likelihood of achieving improvements in RSBQ total scores compared with placebo across a broad range of score reductions, including and in excess of thresholds anchored to CGI-I categories of 'minimal improvement' and 'much/very much improved'. Further details are provided below (Figures 29 to 31, Table 64).

Responder analysis

As stated above, the Applicant previously submitted distribution-based MCIDs in the range of 3 to 6 points. The newly estimated MCIDs, anchored to CGI-I categories of "any improvement," range from 3 to 7 points. An improvement of 9 points in RSBQ score is the observed difference of change in RSBQ between the patients with "worsening or no change" on CGI-I (CGI-I score ≥4) and these with "much / very much improved"(CGI-I ≤2).

When these clinically important difference thresholds are applied in a responder analysis, the odds of achieving a 6-point and 9-point improvement in the trofinetide group (35.2% and 27.5%, respectively) were 2.4 (95% CI: 1.2 to 4.9) and 3.2 (95% CI: 1.4 to 7.2) times the odds in the placebo group (20.4% and 11.8%, respectively) (p = 0.0159 and 0.0056). When a more stringent threshold of 12 points, exceeding one standard deviation of the RSBQ total score, was applied, the odds of response in the trofinetide group (20.9%) were 2.8 (95% CI: 1.1 to 6.8) times those observed in the placebo group (9.7%) (p = 0.0261) (Table 64).

In the absence of a single pre-defined MCID value for the RSBQ, and consistent with CPMP's guideline on multiplicity (CPMP, 2002), applying multiple responder definitions (i.e., different thresholds) to the same dataset can be used to illustrate differences in the proportion of patients reaching thresholds that are clearly clinically relevant.

In Section 5 of the CPMP "Points to Consider on Multiplicity Issues in Clinical Trials", the guideline states:

If the "responder" analysis is not the primary analysis it may be used after statistical significance has been established on the mean level of the required primary variable(s), to establish the clinical relevance of the observed differences in the proportion of "responders". When used in this manner, the test of the null hypothesis of no treatment effect is better carried out on the original primary variable than on the proportion of responders.

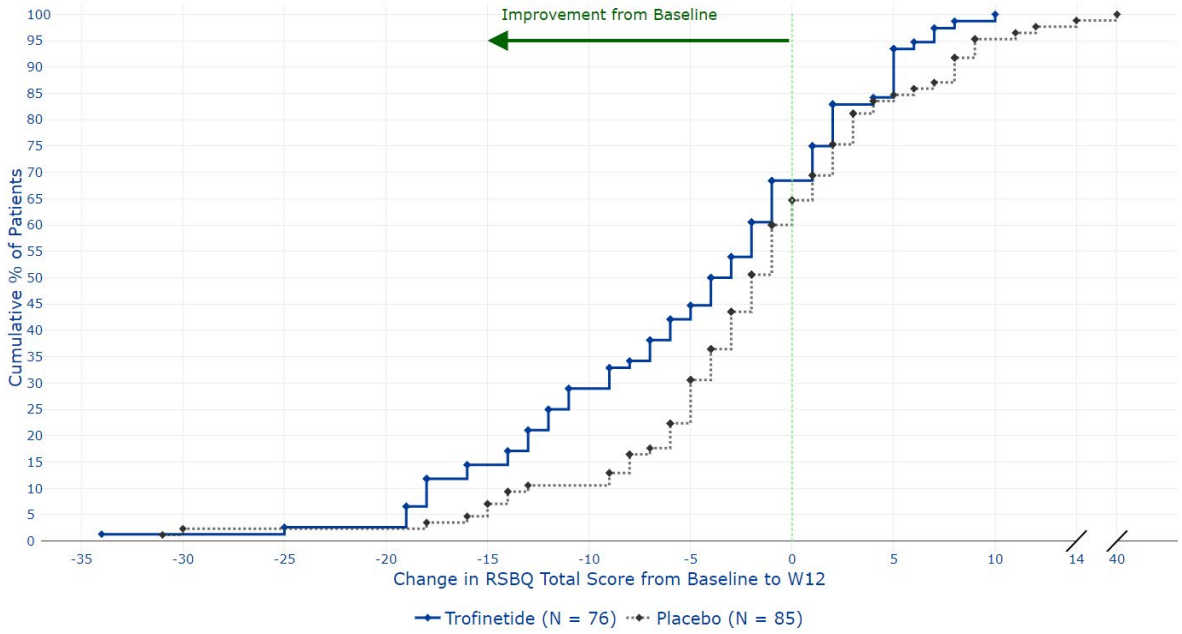
As previously stated in Section 5.1.1.1, the change from Baseline to Week 12 in the RSBQ total score was statistically significantly greater in the trofinetide group compared with the placebo group.

The Applicant now presents the percentage of patients achieving RSBQ improvement from Baseline to Week 12 across a broad range of thresholds by treatment. A broad range of thresholds is presented for two reasons. First, responder definitions were not pre-specified and we appreciate that CHMP would have concerns of data-driven selection of a threshold if only one was presented. Secondly, in the absence of an agreed MCID, the range is presented in order for CHMP to consider changes they consider to be of clinical importance for their assessment.

Across the full observed range of change in RSBQ total scores from Baseline to Week 12, the percentage of trofinetide-treated patients achieving a point-reduction threshold is consistently higher than placebo. This is illustrated in Figure 29 by the non-crossing empirical cumulative distribution function (eCDF) curves of RSBQ change, which offers a comprehensive and transparent characterization of the outcome distribution and shows that, regardless of which point-reduction is chosen as a criterion, the percentage of trofinetide-treated patients meeting this criterion is always higher than the percentage of placebo-treated patients. When considering patients with missing values at Week 12 as "non-responders" (not meeting the response threshold), the cumulative percentages of patients with RSBQ total score reduction of at least 1 to

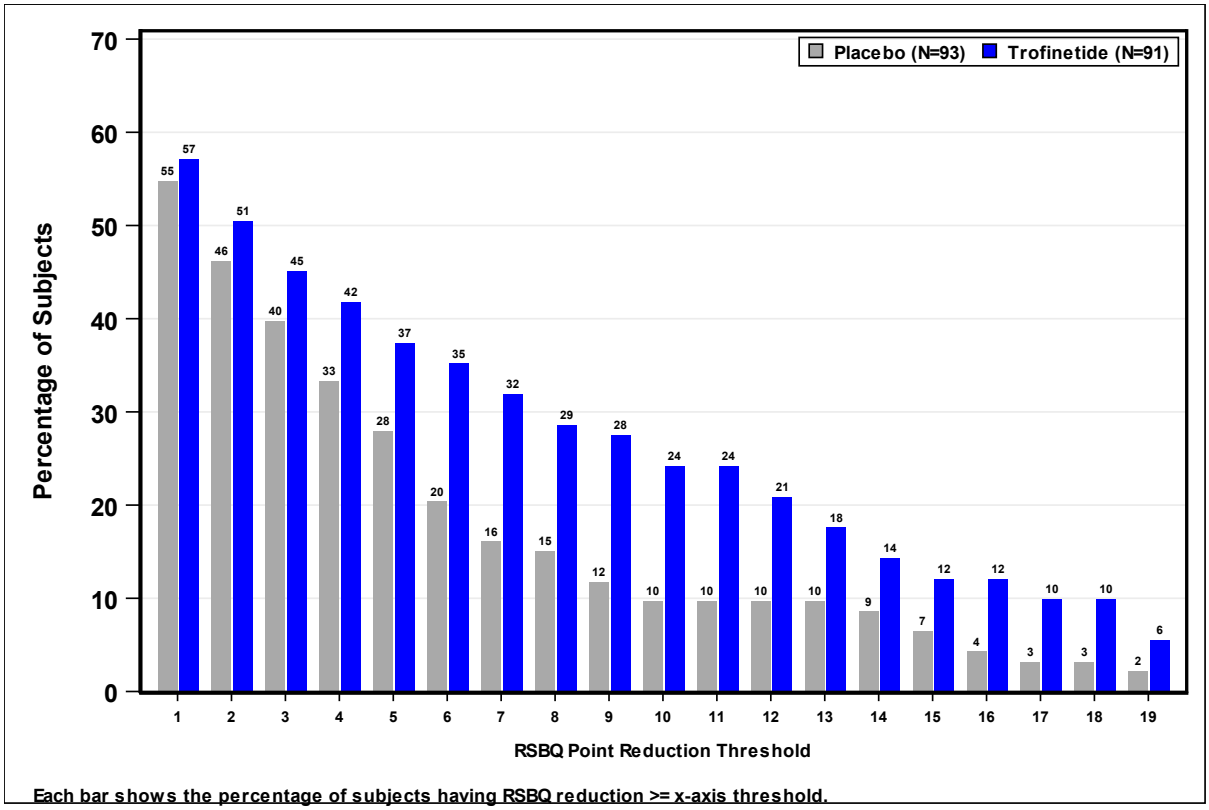
19 points by treatment group are presented in Figure 30. The results clearly show that trofinetide benefited more patients than placebo across a wide range of thresholds, well above the range of the estimated MCIDs, underscoring its clinical relevance to this disease population.

Figure 29: Study 003 Empirical Cumulative Distribution Function of Change in RSBQ Total Score from Baseline to Week 12 (OC) - Full Analysis Set



Source: CHMPah110.2
Abbreviations: OC=observed cases; RSBQ=Rett Syndrome Behaviour Questionnaire; W12=Week 12
Note: The vertical dotted line indicates no change from Baseline in RSBQ.

Figure 30: Study 003 Percentage of Subjects Achieving RSBQ Total Score Reduction of at Least 1 to 19 Points at Week 12 (MN) - Full Analysis Set

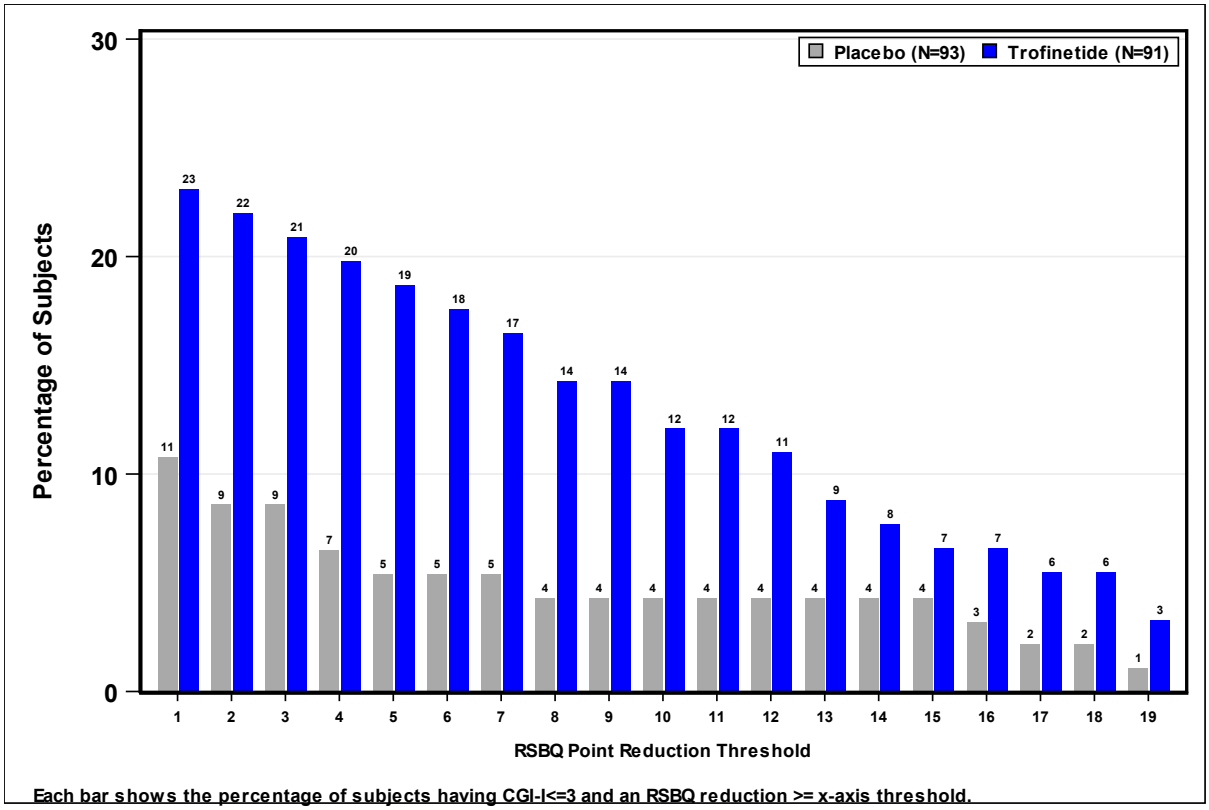


Source: CHMPah124.4
Abbreviation: MN=missing imputed as not meeting the criterion; RSBQ=Rett Syndrome Behaviour Questionnaire

Given the RSBQ and CGI-I are complementary assessments, the percentage of patients who were classified as at least 'minimally improved' on the CGI-I **and** had RSBQ reduction of at least 1 to 19 points are presented by treatment group in Figure 31. Consistent with the results shown in Figure 30, trofinetide benefited more patients when considering both RSBQ and CGI-I improvements and the differences over placebo across a wide range of RSBQ thresholds are even more prominent. Detailed comparisons between the treatment groups evaluating odds ratios and rate differences across various RSBQ-reduction thresholds are presented in Table 59. These results further demonstrate that the difference between treatment groups is highly unlikely due to chance alone and is consistent across a wide range of threshold.

Moreover, the response analysis demonstrates the clinical relevance of the RSBQ data observed in Study 003. Using thresholds that are clearly clinical meaningful, such as 9 points and above on the RSBQ, there is a clear difference between trofinetide and placebo in the percentage of subjects with clinically meaningful benefit.

Figure 31: Study 003 Percentage of Subjects Achieving CGI-I≤3 and RSBQ Total Score Reduction of at Least 1 to 19 Points at Week 12 (MN) - Full Analysis Set



Source: Figure CHMPah124.5
Abbreviations: CGI-I=Clinical Global Impression-Improvement; MN=missing imputed as not meeting the criterion; RSBQ=Rett Syndrome Behaviour Questionnaire
Note: CGI-I ≤3 includes very much improved, much improved, and minimally improved

Table 59: Percent Subjects Meeting Various Response Thresholds by Treatment Group

Responder Criteria	Placebo (N=93) n (%)	Trofinetide (N=91) n (%)	Odds Ratio & 95% CI (Trof / PBO) OR (LCL, UCL)[1]	p-val[1]	Rate Diff & 95% CI (Trof - PBO) Diff (LCL, UCL)[2]	p-val[3]
>=3-point RFB in RSBQ at W12	37 (39.8)	41 (45.1)	1.3 (0.7, 2.5)	0.3683	5.3 (-8.9, 19.2)	0.4636
>=6-point RFB in RSBQ at W12	19 (20.4)	32 (35.2)	2.4 (1.2, 4.9)	0.0159	14.6 (1.5, 27.1)	0.0221
>=9-point RFB in RSBQ at W12	11 (11.8)	25 (27.5)	3.2 (1.4, 7.2)	0.0056	15.7 (3.8, 26.9)	0.0064
>=12-point RFB in RSBQ at W12	9 (9.7)	19 (20.9)	2.8 (1.1, 6.8)	0.0261	11.2 (0.3, 21.8)	0.0329
>=3-point RFB in RSBQ and CGI-I in (1, 2, 3) at W12	8 (8.6)	19 (20.9)	3.0 (1.2, 7.3)	0.0178	12.3 (1.9, 22.8)	0.0194
>=6-point RFB in RSBQ and CGI-I in (1, 2, 3) at W12	5 (5.4)	16 (17.6)	3.9 (1.3, 11.2)	0.0123	12.1 (2.6, 21.9)	0.0102
>=9-point RFB in RSBQ and CGI-I in (1, 2, 3) at W12	4 (4.3)	13 (14.3)	3.9 (1.2, 12.6)	0.0234	10.0 (0.9, 19.2)	0.0203
>=12-point RFB in RSBQ and CGI-I in (1, 2, 3) at W12	4 (4.3)	10 (11.0)	2.9 (0.8, 9.6)	0.0908	6.6 (-1.9, 15.3)	0.0926

Source: RAM1.1 and RAM1.3
Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; LCL=lower confidence limit; PBO=placebo; RFB=reduction from Study 003 Baseline; RSBQ=Rett Syndrome Behaviour Questionnaire; Trof=trofinetide; UCL=upper confidence limit; W12=Week 12
Note: Subjects with missing values are considered as non-responders..
1 Based on logistic regression response adjusted for age group, baseline RSBQ severity, and planned treatment as factors. For RSBQ-only responders, the model also include Baseline RSBQ score as a covariate
2 Adjusted difference using the weighting scheme of Cochran-Mantel-Haenszel (CMH) and stratified Newcombe 95% confidence interval.
3 Based on CMH test for treatment-group comparison adjusted by age group and baseline RSBQ severity categories.

The Applicant thereby shows that patients treated with trofinetide had a higher likelihood of achieving improvement in RSBQ total scores compared with placebo across multiple thresholds, including thresholds anchored to CGI-I categories of 'minimal improvement' and 'much/very much improved'.

5.1.1.5. Patient vignettes and clinicians' notes

Patient vignettes

The Applicant provides two patient vignettes to contextualize clinically relevant RSBQ improvements for the re-examination rapporteurs. These are strictly illustrative and are not intended to substantiate efficacy, whether through qualitative descriptions or quantitative data.

Patient Vignette #1: This patient enrolled in Study 003 with Baseline clinical assessment of limitations in verbal communication, unsteady gait, and stereotypic hand movements. Reported change from from Baseline to Week 12 on the RSBQ was -6 and the CGI-I score at Week 12 was 3 (Minimally improved). Clinician notes (redacted) on the CGI-I score describe observed changes and functional impact in the core domains of Purposeful Hand Use, Spoken Language, Gait, and Stereotypies.

Patient Vignette #2: This patient enrolled in Study 003 with Baseline clinical assessment of no meaningful speech, impaired walking with frequent stops, no purposeful hand use, minimal eye contact, frequent hand to mouth movements, and irregular breathing patterns. Reported change from Baseline to Week 12 on the RSBQ was -11 and the CGI-I score at Week 12 was 2 (Much improved). Clinician notes (redacted) on the CGI-I score describe observed changes and function impact in the core domains of Purposeful hand use, Alternes/Attention, Gait, and Eye gaze.

Clinicians' notes on CGI-I ratings in Study 003

While a written rationale for each CGI-I rating was not required, investigators sometimes included notes to explain their ratings, especially when there was a clinical change. All clinicians' notes from Study 003 are provided in an appendix and these notes illustrate the kind of change that clinicians found noteworthy in their patients.

5.1.1.6. Clinical relevance to patients, caregivers, and clinicians based on external sources and literature

Introduction

The Applicant has evaluated the available data sources (qualitative and quantitative) that can inform the question of meaningful change with the RSBQ. To this end, we classify the sources below as "external" studies that are not related to the trofinetide development program.

The Applicant provides these sources because they have measured or considered longitudinal change on the RSBQ. These are summarized in the table below (Table 60) describing their contribution to this topic, and an overall summary is provided in several bullets below.

Table 60: External Sources Informing the Question of Meaningful Change in RSBQ

External Source	Short description	Contribution to Clinical Relevance

Natural History Data	20 years of Rett and RSBQ Natural History data (Australia)	Provides baseline context that the RSBQ score does not have meaningful changes with best supportive care in the Rett population over time.
CHOP COE manuscript	Retrospective study of real-world trofinetide use (US)	6 months of RW trofinetide use measured RSBQ, CGI-S, CGI-I and Caregiver perspectives on meaningful improvement.
EU Delphi Consensus	Delphi panel with 48 EU Rett specialists	Strong consensus was achieved on all 6 statements defining meaningful response to treatment, including RSBQ
McGraw et al	Caregiver perceptions on meaningful RSBQ item improvements (US)	Describes how caregivers view improvements on RSBQ items and impact on daily activity and/or benefit for the caregivers
PFDD meeting	Caregiver feedback on most troublesome concerns	Provides context for the meaningfulness of RSBQ items

Abbreviations: CGI-I=Clinical Global Impression-Improvement; CGI-S=Clinical Global Impression-Severity; CHOP=Children's Hospital of Philadelphia; COE=Center of Excellence; EU=European Union; PFDD=Patient-Focused Drug Development; RSBQ=Rett Syndrome Behaviour Questionnaire; RW=real world; US=United States

Applicant summary explaining how external sources support interpretation of treatment effects on the Rett Syndrome Behaviour Questionnaire (RSBQ).

- The available external qualitative and quantitative evidence characterizes longitudinal change on the RSBQ and provides Patient, Caregiver and Clinician perspectives for whether and how much change is expected over time.
- Natural history data establish the expected trajectory of RSBQ scores under best supportive care, providing a benchmark against which treatment-associated change can be judged.
- Real-world clinical experience links observed RSBQ reductions and clinician measures (e.g., CGI-I/CGI-S) with caregiver-reported improvement, supporting that observed score reductions reflect clinically observable improvement.
- Clinician Delphi consensus includes a large sample of EU Rett Specialists and establishes what constitutes a "meaningful response to treatment," thereby reducing uncertainty about how to interpret score changes.
- Caregiver qualitative research clarifies why changes in specific RSBQ items matter in daily life (function, participation, caregiver burden), strengthening content validity and the patient-/caregiver-relevance of measured change.

- Caregiver feedback identifies the most troublesome concerns of RTT caregivers. These map to 31 items of the 45-item RSBQ, confirming that the RSBQ is a relevant instrument to assess their concerns.

Together, these external sources triangulate evidence (natural history, clinician judgment, and caregiver experience) to justify that change on the RSBQ can be interpreted as clinically meaningful rather than measurement variability.

Natural History brief context

The Australian Rett Syndrome Database (AussieRett) was established in 1993 and is population-based and longitudinal with multiple waves of data collection. It is one of the largest longitudinal studies collecting RSBQ scores. In an analysis done by Downs et al. (2026), the trajectory of the RSBQ total score was investigated using data from a full sample of 298 female participants whose median age at the first questionnaire was 8.7 years. In the overall population (n=298), there was a slight decline in mean RSBQ score with increasing age, mainly in adulthood. However, it should be noted that in the 5 to 20 years subgroup (n=214), there was very little change (-0.47) in mean RSBQ for up to 4 years after the first observation. Also, the baseline RSBQ score for the 5 – 20 years old cohort was similar to baseline RSBQ score in the trofinetide development program. These results from the analysis of AussieRett data show that patients don't improve nor show reductions in RSBQ scores over a long period of time without treatment.

Children's Hospital of Philadelphia Center of Excellence – Real-World Clinical Experience

This study (not sponsored by Acadia) reports a chart review of cohort of 55 patients who received trofinetide treatment and were followed over a six-month period (Prange et al. 2026). While the data are in patients treated with trofinetide, these data are presented to support the meaningfulness of change over time on the RSBQ, not to quantify improvement on treatment with trofinetide. Patients ranged in age from 14 months to 38 years, include 50 females and 5 males, and included 50 subjects with RTT and 5 subjects with another MECP2-related neurodevelopmental disorder. The main outcomes assessed included the RSBQ, CGI-I and CGI-S after 3 months and 6 months of treatment. In addition, complementary qualitative open-ended caregiver reports were systematically collected to provide caregiver insights into treatment effects. These broad open-ended questions asked caregivers if they 'thought trofinetide was improving any aspect of their child's life and if so, to describe those improvements'. Clinicians then reviewed caregiver comments and categorized them into appropriate domains. The authors note that they used the RSBQ, CGIs as 'relatively objective anchors' to which they could compare the 'more subjective caregiver reports of improvement'. Of those who completed (62% completed) RSBQ at 3 months, 60.9% had a reduced score on the RSBQ, 17.4% had no change, and 21.7% had an increased score. At 6 months, 73.9% had a reduced RSBQ total score and the remainder (26.1%) had a worsening score. The mean RSBQ change from baseline was 2.9 points at 3 months and 4.4 points at 6 months. On the qualitative side, 75.9% of patients were reported by caregivers to experience at least one improvement, with an average of 2.8 reported improvements per patient who reported benefits. The most frequently reported domains of improvement included engagement and eye contact, gait, hand function, communication, mood, reduced stereotypies, improved use of Tobii eye-gaze communication devices, and decreased breathing dysregulation. The study demonstrates the majority of caregivers were saying that trofinetide was improving at least one meaningful aspect of their child's life.

This pending publication is the most current real-world evidence for the meaningfulness of reductions in RSBQ score in clinical practice. As with any RWE collected from routine clinical

practice, there are limitations and biases. Real-world evidence (RWE) from routine clinical practice provides high generalizability but suffers from lower internal validity compared to randomized controlled trials (RCTs). Key limitations include significant selection bias, missing assessments, and the inability to control for confounding factors.

EU Delphi Consensus on Rett syndrome in Europe

The EU Delphi Panel Consensus Report was previously submitted to the EMA as part of the Day 181 responses. The Agency noted that Domain C - meaningful response to treatment – is key to the discussion regarding the uncertain clinical relevance of the observed effects in study 003. The CHMP questioned the relevance of these results, specifically noting that the majority of responders were general practitioners (GPs) whose experience in treating and assessing patients with RTT was deemed unclear. Therefore, the Applicant is resubmitting and clarifying a couple of aspects of this report as it is a key external source reducing the uncertainty of the clinical relevance question. The Applicant understands the specialist-only perspective provides more credibility. Therefore, those results are presented below in Table 61. There were 48 Rett specialists from 8 different EU countries that met the criteria to participate, and there were consistent and substantially high (>75% threshold) levels of agreement on the statements related to meaningful response to treatment. The Applicant is also clarifying the original intent of the broader clinician group. The clinicians included in the overall survey were confirmed based on their involvement in the clinical management of Rett, not based on their specialty. This makes sense, mainly in smaller European countries, that lack broad existence of or access to Rett specialists and where general practitioners are directly involved in the care of Rett patients (Cherchi et al. 2023). All physician groups, including pediatric neurologists, achieved the pre-defined consensus levels (at least 75%) on the 6 statements considering 'Defining meaningful response to treatment'.

Table 61: EU Clinician Consensus With Statements Defining a Meaningful Response to Treatment - Delphi Consensus Report - Percent of Clinician Specialists* With Agreement on Specific Statements

No.	Statement	Agreement among specialists
36	A meaningful treatment response in RTT includes improvements in any core symptom	94%
37	Even small or incremental improvements in RTT symptoms can significantly enhance quality of life for both patients and caregivers	94%
38	Even small or incremental improvements (e.g., 1-3 points on the RSBQ) may be clinically meaningful	92%
39	Even small or incremental improvements (e.g., 1-3 points on the RSBQ) may indicate better long-term quality of life	92%
40	Even small or incremental improvements (e.g., 1-3 points on the RSBQ) may indicate better long-term survival	79%
41	Meaningful benefits should be defined by caregivers of RTT patients, and patients who are able to self-advocate	88%

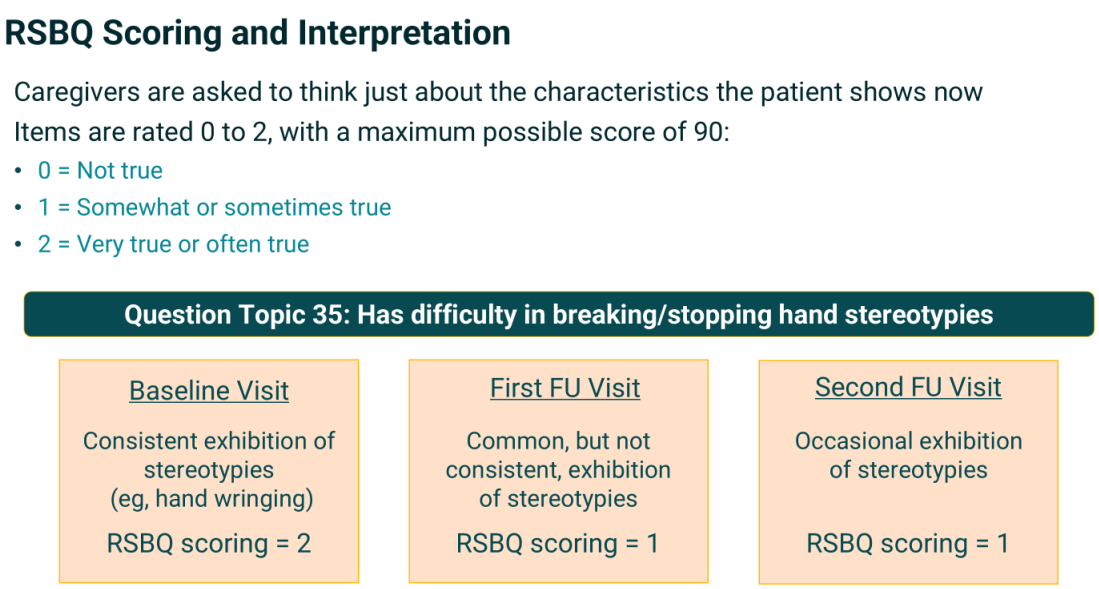
Source: EU Delphi Consensus_Specialists_Rett syndrome_Updated Specialist_results

* Note: Paediatric neurologists (N=20), Paediatric developmental or neurodevelopmental specialists (N=18), Clinical geneticists (N=4), Paediatric epileptologists (N=6)

In the context of even small improvements in the RSBQ being clinically meaningful, it should be kept in mind that the 3-point scale used to assess each item in the RSBQ may mask a substantial

clinical improvement. For example, if a child "consistently" (i.e., meaningfully more than twice a week) wrang their hands at baseline (so, scored a 2); then at first follow-up visit was down to only twice a week (scored a 1); then at second follow-up visit was down to twice a month the rating would still stay at '1' (Figure 32). Hence, a single point improvement may very likely denote a meaningful clinical improvement.

Figure 32: RSBQ Scoring and Interpretation



Abbreviations: FU=follow-up; RSBQ=Rett Syndrome Behaviour Questionnaire

McGraw et al. – Caregiver Study on meaningful RSBQ improvements

McGraw and colleagues provide direct insights about the patient experience and clinical relevance of the RSBQ from Rett caregivers. By conducting interviews (semi-structured qualitative study) with Rett caregivers, they assessed what caregivers consider "meaningful change" in the symptoms covered by the 45 items on the RSBQ. Caregivers reviewed the RSBQ and defined meaningful change as an improvement in symptoms that are bothersome or troublesome, limit daily life or create health risks. Further, they also describe an interconnectedness between items on the RSBQ where improvements are meaningful when they unlock new abilities (e.g., hand stereotypies jeopardize ability to focus and school performance or play with a toy) or reduce barriers to participation and communication. In other words, caregivers emphasize that meaningful change reflects improvements in several key areas:

- **Physical health** (less pain, fewer injuries, improved sleep)
- **Psychological and Social Consequences** (less anxiety, frustration, and isolation; ability to engage with people and in activities)
- **Fine and Gross Motor Skills** (enables play, self-feeding, stability and independence)
- **Communication** (Improvements like vocalizing needs, faster use of eye-gaze devices, and focus are contingent on better motor function, head control, or eye gaze)

Even minor improvement in non-communication symptoms (e.g., less body rocking) can lead to important improvement in communication (e.g., ability to focus on a person or eye-gaze device) The Applicant provides a table in Appendix 4 organized and adapted based on the text from McGraw and the supplement. The table shows each RSBQ item, its associated theme, and caregivers description of why the change on the item is meaningful to the patient. Combined with the finding from McGraw that each domain can have real-functional consequences in daily life, even a 1-point reduction of frequency can point to meaningful decrease of the daily activity impact, or just the worry about the consequences.

Patient-focused drug development meeting

The IRSF and the Rett Syndrome Research Trust co-hosted a Rett Syndrome Externally Led Patient-Focused Drug Development (PFDD) meeting on 11 March 2022. The goal of the meeting was to systematically gather perspectives from caregivers and families with the hope that the 'report will be used to guide therapeutic development and inform [regulatory] benefit-risk evaluations when assessing therapies to address Rett syndrome.' The proceedings from the meeting, called the Voice of the Patient Report, summarized the results of live caregiver surveys conducted during the meeting. The report, the meeting transcript, a document containing the comments submitted through an online portal, and a recording of the meeting can be found at: https://rettpfdd.org.The report provides context for understanding the clinical meaningfulness of the benefit of treatment for RTT. The surveys assessed the most troublesome current or past Rett-related health concerns of caregivers and the most important aspects that new therapies should address. The aspects of RTT that caregiver respondents ranked as the three most important for a new therapeutic to improve upon were communication/speech impairment, impaired hand use or repetitive hand movements, and mobility or balance difficulties (IRSF, 2022). Clinicians involved in research in intellectual/developmental disabilities mapped the items of the RSBQ to these top caregiver concerns, noting that 31 of the 45 items of the RSBQ mapped to the top 5 observable behavioral and emotional domains identified by the caregivers as areas in need of treatments (Table 62). The RSBQ has only one item related to communication. For this reason, the CSBS-DP-IT was added as the key secondary endpoint and the RTT-COMC was added as another secondary endpoint. Both scales showed improvement with trofinetide compared to placebo (see Section 5.1.1.7).

Table 62: Top 5 Observable Behavioural and Emotional Manifestations Identified by Caregivers of Individuals With RTT Mapped to Items of the RSBQ

RSBQ Number	RSBQ Item Description
Caregiver Concern: Communication/speech impairment	
31	Uses eye gaze to convey feelings, needs, and wishes
Caregiver Concern: Impaired hand use/repetitive hand movements	
18	Does not use hands for purposeful grasping
20	Hand movements are uniform and monotonous
24	Restricted repertoire of hand movements
35	Has difficulty in breaking/stopping hand stereotypies
4	Makes repetitive movements involving fingers around the tongue
40	Tendency to bring hands together in front of the chin or chest
27	Has wounds on hands as a result of repetitive hand movements
3	Makes repetitive hand movements with hands apart
43	Amount of time looking at objects is longer than the time spent holding or manipulating them
Caregiver Concern: Mobility/balance	
23	Although can stand independently, tends to lean on objects or people
39	Walks with stiff legs
Caregiver Concern: Breathing irregularities	
5	There are times when the breath is held
1	There are times when breathing is deep and fast
6	Air or saliva is expelled from the mouth with force
19	Swallows air
25	Abdomen fills with air and sometimes feels hard
Caregiver Concern: Emotional/behavioral problems	
2	Spells of screaming for no apparent reason during the day
14	Abrupt changes in mood
16	Times when appears miserable for no apparent reason
22	Screams hysterically for long periods of time and cannot be consoled
29	There are times when irritable for no apparent reason
30	Spells of inconsolable crying for no apparent reason during the day
7	Spells of apparent anxiety/fear in unfamiliar situations
9	Seems frightened when there are sudden changes in own body position
38	Spells of apparent panic
13	Spells of screaming for no apparent reason during the night
37	Spells of laughter for no apparent reason during the night
42	Spells of inconsolable crying for no apparent reason during the night
26	Spells of laughter for no apparent reason during the day
33	Rocks self when hands are prevented from moving

Source: Doshi et al. 2022
Abbreviations: RSBQ=Rett Syndrome Behaviour Questionnaire; RTT=Rett syndrome

5.1.1.7. Secondary endpoints

Communication

Impairments in communication and social interaction represent core and highly impactful features of Rett syndrome, as mentioned by caregivers (see Section 5.1.1.6).

There was no consensus measure of communication in RTT. The CSBS-DP-IT Social Composite Score was chosen as the prespecified key secondary endpoint because its items are appropriate for the abilities of patients with RTT, because it had been used previously in RTT studies, and because it had shown evidence of sensitivity to change in RTT (Neul et al. 2022). The items rated (for example, 'Does your child let you know that he/she needs help or wants an object out of reach?'

and 'Does your child nod his/her head to indicate yes?') are clinically meaningful at face value and based on data collected from caregivers on what is meaningful to them.

There was a statistically significant improvement observed on the CSBS-DP-IT Social Composite Score. Whereas placebo-treated patients demonstrated worsening from baseline (-1.1 points), trofinetide-treated patients showed near-stabilisation (-0.1 points), resulting in a statistically significant between-group difference (LS mean difference: 1.0; 95% CI: 0.3, 1.7; p=0.0064; Cohen's d effect size: 0.43).

The findings on another secondary endpoint, the RTT-COMC, support the findings on the key secondary communication endpoint. The RTT-COMC is a functional assessment of ability to communicate and is inherently clinically relevant. Subjects are presented with a standard set of objects, pictures and drawings or symbols and are required to communicate choices. Better ratings are given for more developmentally advanced abilities. The anchors that were developed were based on existing, validated measures of early language development and social communication skills (e.g., Vineland Adaptive Behavior Scales, Mullen Scales of Early Learning, Bayley Scales of Infant and Toddler Development – 4th Edition) with specific and applicable adaptations for RTT (e.g., use of real objects, photographs of real objects, and picture symbols to make choices). The RTT-COMC demonstrated a nominally significant treatment effect in favour of trofinetide (LS mean difference placebo–trofinetide: -0.3; p=0.0257).

Given the impact that impaired communication has on caregivers, these significant findings in the CSBS-DP-IT and RTT-COMC support the clinical relevance of trofinetide treatment to patients and caregivers.

Other secondary endpoints

In addition to the prespecified co-primary and key secondary endpoint, eight other secondary clinician-reported (ClinRO) and observer-reported (ObsRO) outcome measures were evaluated. Of the other secondary endpoints, the RTT-COMC achieved statistical significance (see above) and six others showed LS mean differences favouring trofinetide, although these did not reach nominal statistical significance (Table 63).

In support of the narrowed indication, it is noteworthy that the endpoints favouring trofinetide included neurobehavioral manifestations of Rett such as hand function (RTT-HF) and ambulation (RTT-AMB). Only two measures, the Rett Syndrome Clinician Rating of Verbal Communication (RTT-VCOM) and the CGI-S, did not favour trofinetide and indeed these measures showed essentially no change from baseline for either treatment group.

Table 63: Analysis of Secondary Endpoints in Study 003 - Full Analysis Set

	Trofinetide group comparison (PBO-TROF)		
	LS mean difference (SE)	**2-sided p-value**	**Effect size (Cohen's *d*)**
Key secondary endpoint			
CSBS-DP-IT SCS	1.0 (0.37)	0.0064	0.43
Other secondary endpoints			
QoL rating of ICND	0.1 (0.13)	0.2507	0.19
RTT-HF	-0.1 (0.11)	0.3649	0.14
RTT-AMB	-0.1 (0.10)	0.2114	0.19
RTT-COMC	-0.3 (0.15)	0.0257	0.36
RTT-VCOM	0.0 (0.08)	0.9799	0.00
CGI-S	0.0 (0.05)	0.5304	0.10
RTT-CBI	-0.8 (1.40)	0.5855	0.09
ICND	-4.5 (4.67)	0.3376	0.22

Sources: Study 003 CSR Tables 11-10 and 11-12
Abbreviations: CGI-S=Clinical Global Impression-Severity; CSBS-DP-IT=Communication and Symbolic Behavior Scales Developmental Profile(TM) Infant-Toddler Social Composite Score; ICND=Impact of Childhood Neurologic Disability Score; LS=least squares; PBO=placebo; QoL=quality of life; RTT-AMB=Rett Syndrome Clinician Rating of Ambulation and Gross Motor Skills; RTT-CBI=Rett Syndrome Caregiver Burden Inventory; RTT-COMC=Rett Syndrome Clinician Rating of Ability to Communicate Choices; RTT-HF=Rett Syndrome Clinician Rating of Hand Function; RTT-VCOM=Rett Syndrome Clinician Rating of Verbal Communication; SE=standard error; TROF=trofinetide

5.1.1.8. Interpretability of long-term efficacy

Regarding the limited interpretability of long-term efficacy, the final assessment report noted that the short placebo-controlled period and the large number of dropouts among patients with trofinetide in the extension studies limits the interpretability of long-term efficacy. The Applicant acknowledges the preference for a longer placebo-controlled duration. However, a 12-week placebo-controlled period was deliberately selected to take into account the burden placed on patients and caregivers who often live far from trial sites and the challenges of bringing these fragile patients with multiple co-morbidities to the sites, as well as the consideration of prolonged placebo exposure in patients with Rett syndrome. The Applicant maintains that the duration was adequate to detect and substantiate treatment effects across both co-primary and key secondary endpoints, which was sustained in the open-label extension studies, as further explained below.

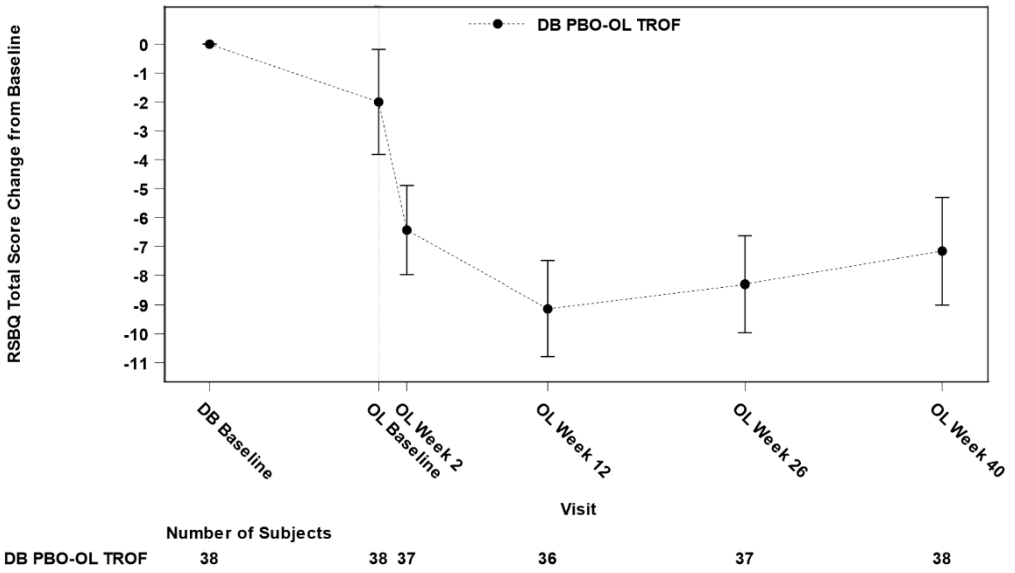
During the 12-week double-blind period, RSBQ total scores in the trofinetide group improved through Week 12 compared to Baseline following the initial treatment effect observed at Week 2. In contrast, patients in the placebo group exhibited an early improvement at Week 2, followed by a clear regression toward baseline. This transient placebo response is most plausibly attributable to expectation bias, a well-recognized phenomenon (Williams et al. 2012) in caregiver-reported outcomes in neurodevelopmental disorders that will also affect the treatment arm, at least initially. Consistent with these findings, CGI-I scores in the trofinetide group demonstrated continued improvement over time, whereas patients in the placebo group did not improve. This study design is further supported by natural history evidence indicating minimal spontaneous improvement in RSBQ scores and skills in untreated individuals over both short and long time periods (see below and Neul et al. 2026), reinforcing the appropriateness of a shorter placebo-controlled period.

Subjects who completed Study 004

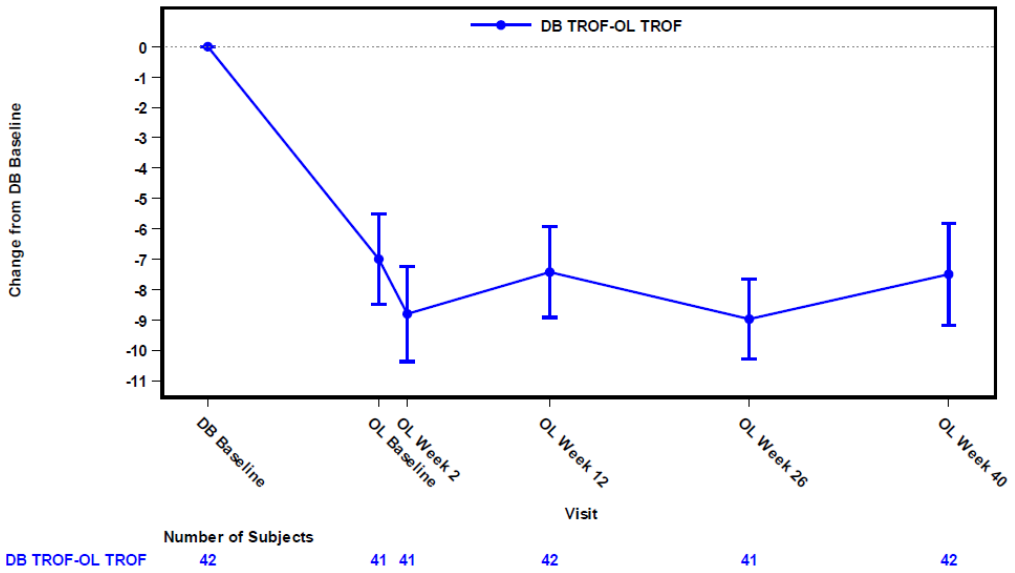
An analysis looking at the subpopulation who continued through to the end of the study permits an evaluation of that group's maintenance of effect over time. Dropouts did occur in Study 004 for a variety of reasons, most commonly adverse events. As the Applicant has reported, patients who did complete the open-label extension showed sustained improvement on the RSBQ (Figure 33). This group constitutes 45% of the enrolled Study 003 population and achieved substantially improved (rollovers from placebo group) and sustained (rollovers from trofinetide group) benefit as assessed by the RSBQ compared to the mean efficacy results reported at Week 12.

Figure 33: RSBQ Total Score Change From Double-Blind Baseline by Visit (Mean+/-SE) and Treatment Assignment in Study 003 - OL Study 004 Completers with Week 40 RSBQ Scores

A Study 004 Completers - Placebo in Study 003



B Study 004 Completers - Trofinetide in Study 003

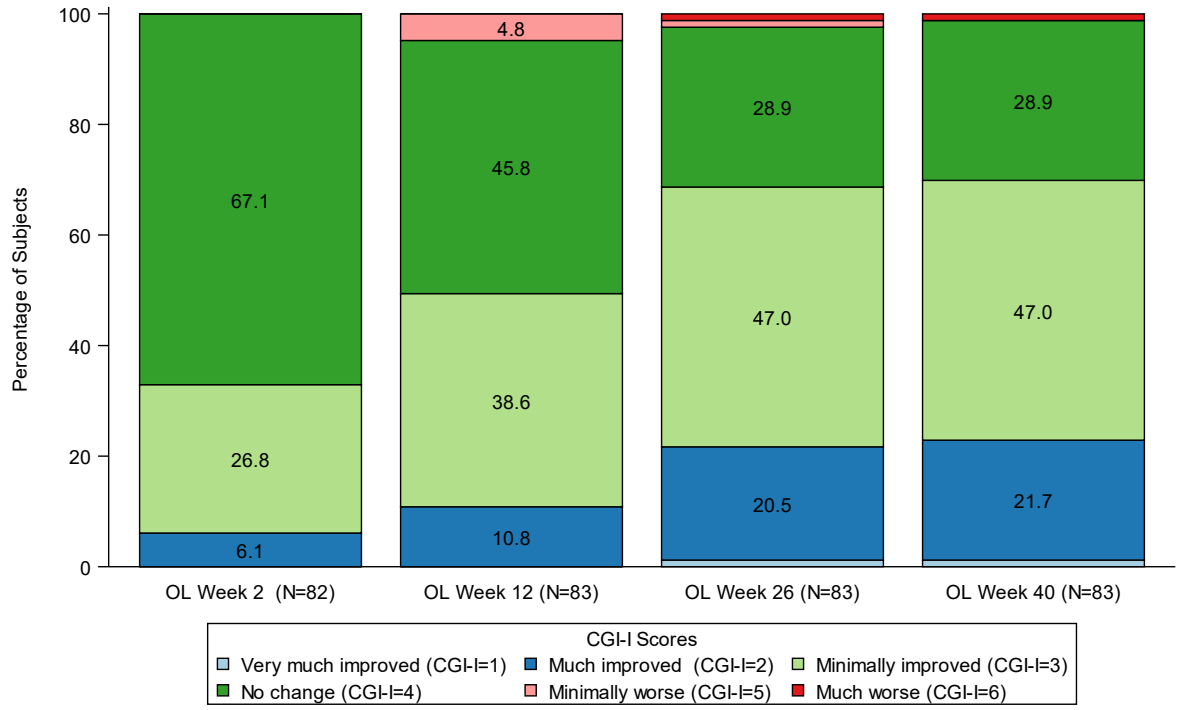


Sources: Study 004 CSR Figures AH1.1.2.3 and AH1.1.2.4
Abbreviations: DB=double-blind; OL=open-label; PBO=placebo; RSBQ=Rett Syndrome Behaviour Questionnaire; SE=standard error; TROF=trofinetide
Note: In Figure A, the reference line indicates the start of trofinetide treatment

The patients who completed Study 004 do not differ appreciably from the Study 003 population in their demographic or baseline characteristics (mean age, age at Rett Syndrome diagnosis, RSBQ or CGI-S scores at Baseline) (Data in Appendix 5, Table A-5.1) and frequency of diarrhea and vomiting (Appendix 5, Table A-5.2).

Among the Study 004 completers, the number of patients showing improvement relative to the open-label baseline (CGI-I score of 1, 2, or 3) increased across visits, with 27 (32.9%) patients at Week 2, 41 (49.4%) at Week 12, 57 (68.7%) at Week 26 and 58 (69.9%) at Week 40 (Figure 34). Among all patients who improved at any time in Study 004, more than 64% maintained improvement at the subsequent visit. At the end of Study 003 12-week placebo-controlled period, 29 trofinetide patients had improvement on CGI-I score. Among them, 17 (58.6%) continued on 40-week treatment during the open-label extension with CGI-I improvement (compared to the double-blind baseline) sustained.

Figure 34: CGI-I Responders by Visit - Study 004 Completers - Safety Analysis Set



Source: CHMPah131.4
Abbreviation: CGI-I-Clinical Global Impression-Improvement

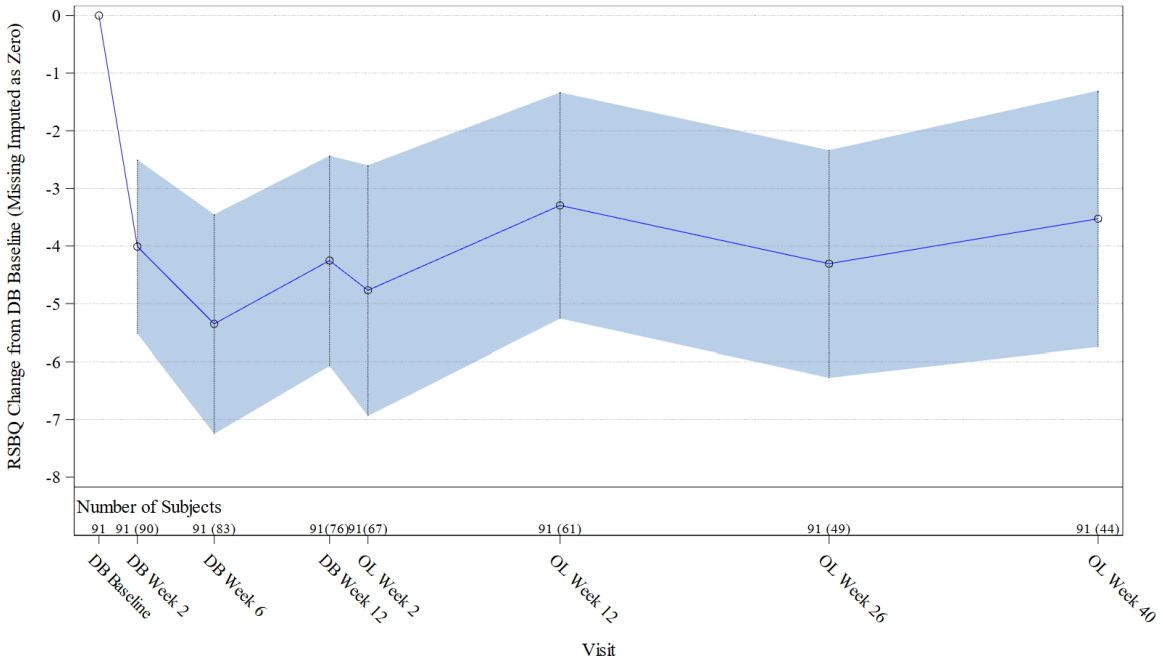
Thus, the data show that subjects who do continue trofinetide treatment (responders) continue to show clinically meaningful benefit. These results are notably distinct from the minimal improvement in RSBQ scores seen over a comparable period in untreated patients (see below, Downs et al. 2026).

RSBQ scores with missing data imputed as zero

As an additional approach to present long-term efficacy, change from double-blind baseline in RSBQ was plotted across all Study 003 and Study 004 visits for subjects who were in the trofinetide group in Study 003. For subjects with missing data, it was assumed that no change

from baseline had occurred. Accordingly, missing change from baseline values were imputed by random sampling from a normal distribution with mean zero and standard deviation of 9.5. In the 40-week open-label Study 004, the mean change from baseline in RSBQ remained below -3 with the upper bound of the 95% confidence interval below -1 at all visits (Figure 35). Together with Figure 33, this shows that, not only in subjects who completed Study 004, but also in the more conservatively-selected population for which all missing data are imputed as zero, sustained efficacy is detectable at Week 40.

Figure 35: RSBQ Total Score Change From Double-Blind Baseline by Visit in Patients Treated With Trofinetide in Study 003 - Studies 003 and 004 (Missing Imputed as Zero)



Numbers inside parentheses represent the numbers of subjects with non-missing data.

Source: Figure CHMPah 134
Abbreviations: DB=double blind; OL=open label; RSBQ=Rett Syndrome Behaviour Questionnaire
Notes:
Missing Studies 003 and 004 data were imputed as no-Change from Double-blind Baseline
The blue line in the figure represents the mean with shaded band indicating the 95% confidence interval.

Modeling of 24-week duration

The Applicant acknowledges that the design of the Study 003 does not permit a direct evaluation of 24-week placebo-controlled data, and therefore primary understanding of longer-term efficacy is gleaned through the existing open-label data in Study 004. As most discontinuations in Study 003 and Study 004 were due to adverse events rather than lack of efficacy, it is possible as a supportive assessment to model the treatment difference between trofinetide and placebo at Week 24 using a range of assumptions applied to existing double-blind and open-label data from Study 003 and Study 004.

Methodology is described in greater detail in Appendix 6, but briefly: all available RSBQ data for trofinetide were used (including the 12-week double-blind data from Study 003 and data from the beginning portion of open-label Study 004) with missing data imputed; after Study 003, modeled data are used for patients in the placebo arm. For the placebo group, the Applicant applied three analytical approaches:

- Method 1 (missing data imputation): Week 14 and Week 24 RSBQ change from baseline values for placebo subjects were extrapolated based on each subject's observed linear trajectory over 12 weeks in Study 003.
- Method 2 (more conservative missing data imputation): Week 14 and Week 24 RSBQ change from baseline values for placebo subjects were imputed by random sampling from a normal distribution with mean zero and standard deviation of 9.5.
- Method 3 (most conservative: missing data imputation and change adjustment): Missing data for placebo were imputed per method 1 to inform SE and trofinetide missing data imputation, after which the average placebo change from baseline was reduced to 0.

After imputing the missing data for placebo subjects, it was assumed that trofinetide subjects with missing RSBQ change from baseline values (due to early discontinuation from Study 003 or Study 004 or non-participation in Study 004) followed a similar trajectory to placebo subjects (see Appendix 6 for details). This assumption is considered conservative, as very few trofinetide patients discontinued in either Study 003 or the open-label extension trial Study 004 for lack of efficacy.

Utilizing these approaches, the estimated treatment differences at Week 24 are -3.91 for Method #1, -3.10 for Method #2, and -2.70 respectively (Table 64).

While these modeling approaches can only be supportive and do not substitute for prospectively generated randomized, double-blind, placebo-controlled data, they indicate a persistent treatment difference beyond week 12 between trofinetide and placebo under a range of assumptions for placebo arm trajectory.

Table 64: Estimated Change from Baseline in RSBQ at Week 24 - Studies 003 and 004 - Full Analysis Set

	LSM (SE) Change from Baseline at Week 24		Treatment Group Comparison (TROF - PBO)		
	PBO (N=93)	TROF (N=91)	LS Mean (SE)	95% CI	p-value
Method #1	1.21 (0.787)	-2.70 (0.811)	-3.91 (1.13)	(-6.13, -1.70)	0.0005
Method #2	0.03 (1.018)	-3.07 (1.168)	-3.10 (1.55)	(-6.14, -0.07)	0.0452
Method #3	0 (0.787)	-2.70 (0.811)	-2.70 (1.13)	(-4.92, -0.49)	0.0169

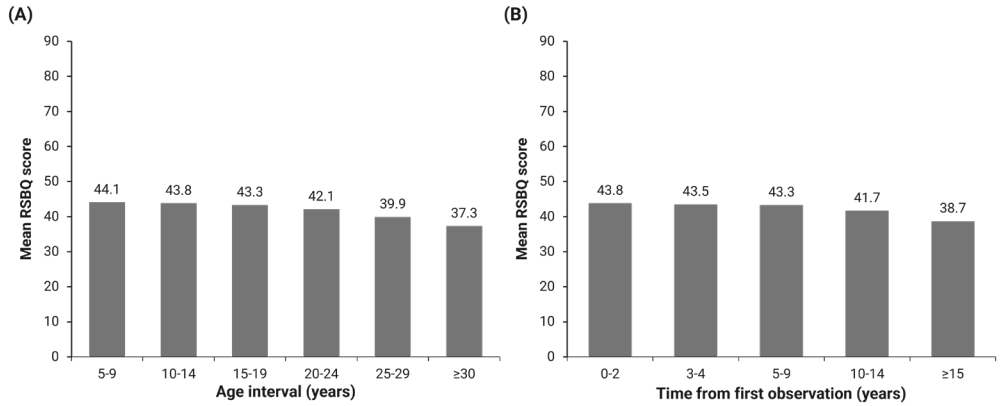
Source: CHMPah136.1 and CHMPah136.2
Abbreviations: LSM=least squares mean; PBO=placebo; RSBQ=Rett Syndrome Behaviour Questionnaire; SE=standard error; TROF=trofinetide

Natural history of Rett syndrome

To address concerns over the clinical meaningfulness of the long-term efficacy of the responses observed with trofinetide, the Applicant has conducted a broad literature analysis of the natural history of patients with Rett syndrome which includes the RSBQ which confirms and reinforces the notion that very little improvement in manifestations is observed over time in the natural history of Rett, which contrasts with the magnitude and durability of improvements observed in the double-blind and open-label extension studies. A summary is presented below.

In the Australian cohort (N=298 overall) 214 patients with RTT aged 5 to 20 years, there was very little change in mean RSBQ for up to 9 years after the first observation (Downs et al. 2026). As well, in the full sample of 298 female participants with genetically confirmed RTT, there was very little change in mean RSBQ for up to 9 years after the first observation, with a 0.47-point cumulative change in the 4 years since first observation (Figure 36) (Downs et al. 2026).

Figure 36: Mean RSBQ Total Score at the Beginning of the Age Interval in 214 Children and Adults With RTT who Were 5 to 20 Years Old at the Time of the First Questionnaire, by (A) Age Interval, and by (B) Time Interval From First Observation



Source: Downs et al. 2026
Abbreviations: RSBQ=Rett Syndrome Behaviour Questionnaire; RTT=Rett syndrome

These natural history findings provide an important benchmark for interpreting both short-term placebo-controlled effects and longer-term open-label observations in Rett syndrome. Accordingly, improvements and sustained benefits observed over a 12-week placebo-controlled period and further open-label treatment in a population predominantly older than 5 years are interpretable as clinically meaningful.

Comprehensive data package

The Applicant acknowledges that securing any form of regulatory approval hinges on a favorable benefit-risk (B/R) assessment. Moreover, the Applicant maintains that the arguments presented in this submission are intended to thoroughly address the grounds for refusal under the current regulatory framework. At the same time, the Applicant recognizes that the responsibility ultimately lies with the CHMP to determine, once a positive B/R assessment is achieved, whether the application package is sufficiently "comprehensive." This topic was also discussed during the consultation with the re-examination Rapporteurs and the EMA on April 13th.

Recognizing the limited opportunities for communication throughout the re-examination process, the Applicant proactively included justifications for satisfying the criteria for Conditional Marketing Authorisation (CMA) from the outset. This approach ensures that CHMP can evaluate these justifications should they conclude that the MAA package does not fully meet comprehensiveness requirements.

A draft protocol synopsis for the proposed study "Long-term, 24-Month, Prospective, Non-interventional Phase 4 Study to Evaluate the Effectiveness and Tolerability of Trofinetide (Daybu) in Patients With Rett Syndrome in Europe" is provided.

5.1.2. Benefit/Risk conclusion by the Applicant

The Applicant remains steadfast in its belief that the benefit risk balance of trofinetide is overwhelmingly positive in RTT, a severely debilitating and rare neurodevelopment disorder currently without an approved therapy in Europe. Our data demonstrate clinically meaningful improvements exceeding any reasonable threshold of clinical relevance, alongside a manageable safety profile devoid of serious risks.

Appendices

Appendix 1 Functional unblinding

Diarrhoea was the most common adverse event in the trofinetide treatment groups in Study 003 (80.6%) but was also common in the placebo treatment group (19.1%). Because of the frequency of the adverse event of diarrhoea in Study 003, the question of the potential of functional unblinding and its possible effect on the efficacy data has been raised. These concerns have focused more on the RSBQ than on the CGI-I.

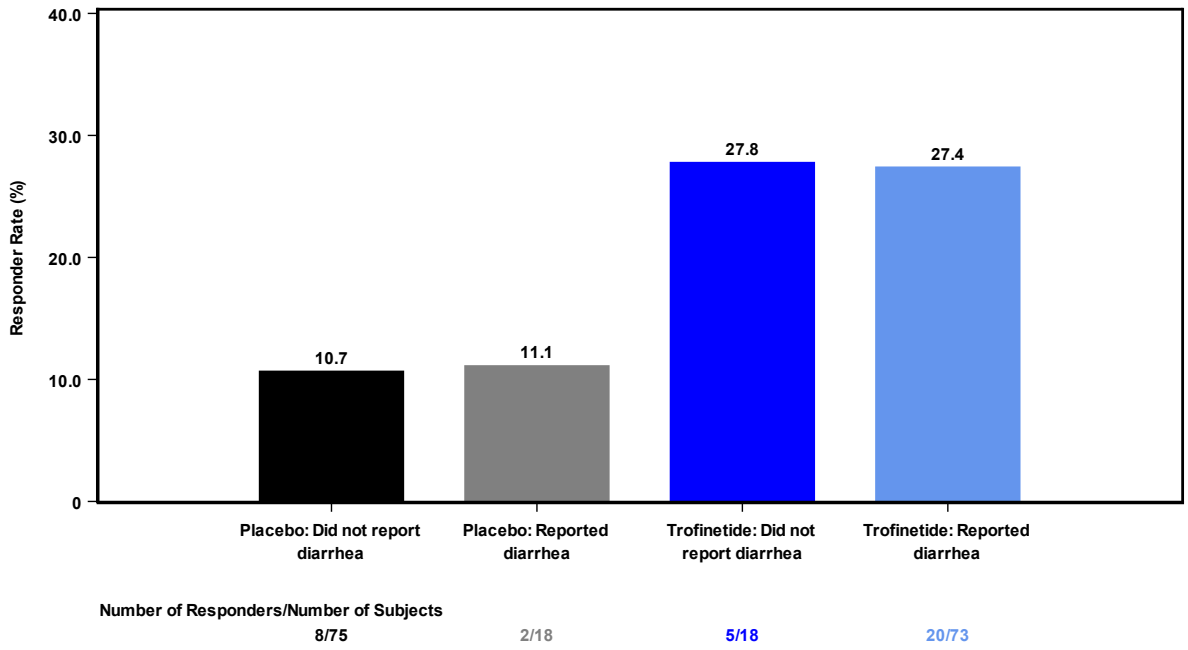
Multiple lines of evidence indicate that if functional blinding did occur, it was not of a magnitude that decisively influenced the results.

The Applicant has conducted and submitted several post-hoc analyses to evaluate the potential impact of functional unblinding on treatment effect in an effort to investigate the question with different approaches. These different approaches (summarized below) consistently demonstrate that the observed improvements in Study 003 co-primary outcome are attributable to treatment assignment (trofinetide vs placebo) rather than the presence or absence of diarrhoea, indicating that the results of Study 003 were not driven by functional unblinding due to the presence of diarrhoea.

Summary of Analyses to Evaluate the Potential Impact of Functional Unblinding

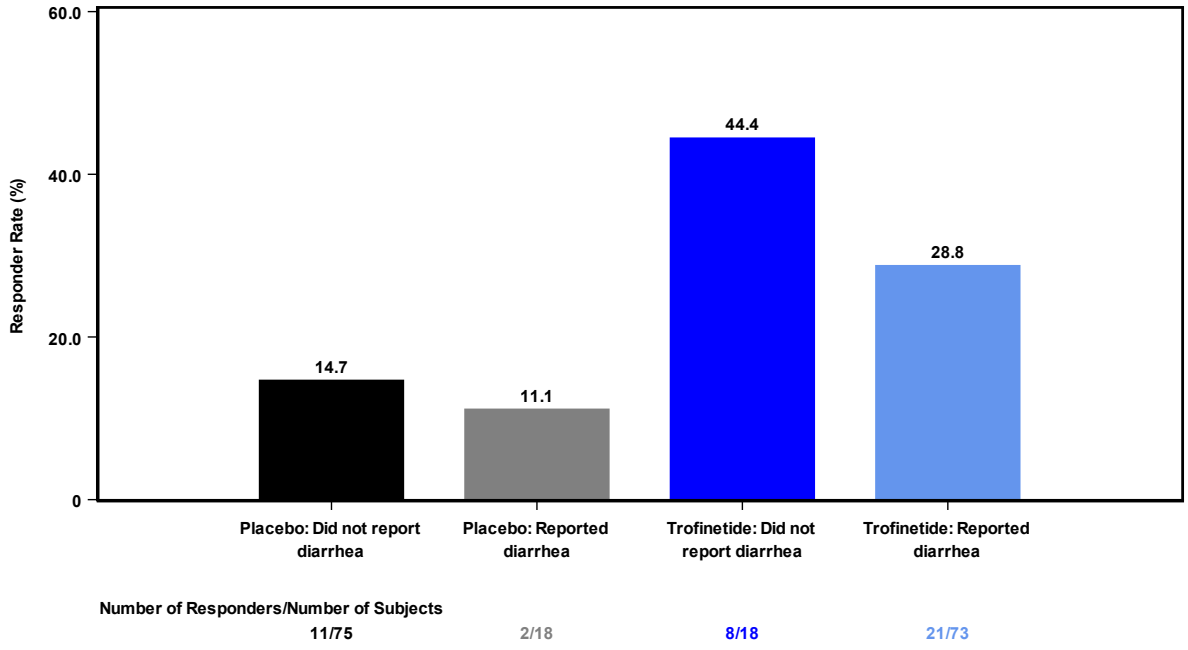
- LS mean (SE) changes of RSBQ total score change from Baseline to Week 12 were similar for subjects with and without diarrhoea in the placebo group (-2.3 [2.01] and -1.7 [1.04]) and for subjects with and without diarrhoea in the trofinetide group (-5.1 [1.01] and -4.0 [2.17])
- LS mean (SE) changes of the CGI-I score at Week 12 indicates that improvement in subjects with diarrhoea was slightly less than in subjects without diarrhoea for the trofinetide group (3.6 [0.09] vs 3.4 [0.14], respectively) and similar in the placebo group (3.7 [0.17] vs 3.8 [0.07]), respectively
- Proportion of responders (≥20% improvement in RSBQ total score change from Baseline to Week 12) was the same for subjects in the trofinetide group regardless of diarrhoea status (27.8% in group with no diarrhoea vs 27.4% in group with diarrhoea (Figure A-1.1;)
- Proportion of CGI-I responders (CGI-I score of 1, 2, or 3) was lower in subjects with diarrhoea compared to subjects without diarrhoea in the trofinetide group (28.8% vs 44.4%) (Figure A-1.2;)
- Responder analyses by diarrhoea status using composite responder definitions (RSBQ ≥20% Change from Baseline to Week 12 *and* a CGI-I score ≤3 at Week 12) (). Improvement was better in the trofinetide group compared to the placebo group, regardless of whether the subject had diarrhoea or not.
- Responder analysis by diarrhoea status using composite responder definitions (RSBQ ≥30% Change from Baseline to Week 12 *and* a CGI-I Score ≤2 at Week 12) (). Improvement was better in the trofinetide group compared to the placebo group, regardless of whether the subject had diarrhoea or not.

Figure A-1.1 Proportion of Subjects with 20% or More Reduction (Improvement) in RSBQ Total Score Change from Baseline to Week 12 by Diarrhoea Adverse Event Status (MN) - Study 003 - Full Analysis Set



MN=missing imputed as non-responders
Source: Study 003 CSR Figure 11-10

Figure A-1.2 Proportion of Subjects with CGI-I=1, 2, or 3 at Week 12 by Diarrhoea Adverse Event Status (MN) - Study 003 - Full Analysis Set



MN=missing imputed as non-responders
Source: Study 003 CSR Figure 11-9

- Sensitivity analyses were conducted by adding a diarrhoea-by-visit interaction term to the primary MMRM models for RSBQ change-from-Baseline total score and CGI-I). Diarrhoea

was included as a time-varying indicator that took a value of 0 for assessments occurring before a subject's first diarrhoea event and 1 for assessment occurring after the first diarrhoea event. Including this interaction term allows the potential impact of diarrhoea on outcomes to vary by visit. Results from these models are summarized in Table 65 alongside the corresponding primary model results for comparison.

Table 65: Sensitivity Analysis Evaluating Potential Impact on Coprimary Endpoints Caused by Events of Diarrhoea - Study 003 - Full Analysis Set)

Analysis	Dependent Variable	MMRM Effect Variables	Difference (Trofinetide - Placebo) at Week 12			
			LS Mean	95% CI	p-value	Effect Size
Primary	RSBQ TS Change from Baseline	Treatment, Visit, Treatment-by-Visit Interaction, Age Group, Baseline RSBQ Severity Category, Baseline RSBQ TS, Baseline RSBQ TS-by-Visit Interaction	-3.1	(-5.7, -0.6)	0.0175	0.37
Sensitivity	RSBQ TS Change from Baseline	Primary MMRM model **+ Diarrhoea Indicator-by-Visit Interaction term**	-3.4	(-6.5, -0.2)	0.0365	0.40
Primary	CGI-I	Treatment, Visit, Treatment-by-Visit Interaction, Age Group, Baseline RSBQ Severity Category, Baseline CGI-S, Baseline CGIS-by-Visit Interaction	-0.3	(-0.5, -0.1)	0.0030	0.47
Sensitivity	CGI-I	Primary MMRM model **+ Diarrhoea Indicator-by-Visit Interaction term**	-0.3	(-0.6, -0.1)	0.0073	0.53

CI=confidence interval; LS=least-squares; TS=total score.
Source: Study 003 Tables 14.2.1.1, 14.2.2.1, CHMPxQ28c.2-rsbq-mmrm-dia-vis-003, CHMPxQ28c.3-cgii-mmrm-dia-vis-003

Note that in both sensitivity analyses, the diarrhoea-by-visit interaction term was not statistically significant (p-value=0.5162 and 0.4275 for RSBQ and CGI-I models respectively), indicating no evidence that diarrhoea systematically shifted RSBQ or CGI-I results at any visit. Importantly, the Week 12 treatment effect remained statistically significant for both coprimary endpoints (RSBQ total score and CGI-I) after adjustment for this additional term, with similar effect sizes.

- A mediation analysis was conducted with diarrhoea as a mediator in the indirect pathway with the same covariates used in the Study 003 RSBQ primary analysis. For the RSBQ, this mediation analysis yielded a total effect estimate of -3.28 points, which is decomposed into natural direct effect (-3.44 points) and natural indirect effect (0.15 points) (Table 66). The 0.15-point natural indirect effect through diarrhoea is small, non-significant (p-value=0.8771) and pointed to the opposite direction of treatment effect. This indicates that any potential mediation through diarrhoea is negligible and there is no statistical evidence that diarrhoea status caused an inflation of the treatment effect. The p-value 0.0359 of natural direct effect of -3.44 points indicates that the RSBQ treatment effect at Week 12 is still statistically significant without mediation effect from diarrhoea.

Table 66: Mediation Analysis on Change in RSBQ Total Score from Baseline at Week 12: Natural Direct Effect and Natural Indirect Effect Estimate – Study 003

Treatment Effect (Trofinetide – Placebo)	Estimate	Standard Error	Wald 95% Confidence Limits	P-value
Total Effect	-3.28	1.308	-5.85, -0.72	0.0121

Natural Direct Effect (NDE)	-3.44	1.637	-6.65, -0.23	0.0359
Natural Indirect Effect (NIE)	0.15	0.994	-1.79, 2.10	0.8771

Source: Table SR_causalmed_RSBQ_OC

- For CGI-I, mediation analysis results also show that there is no statistical evidence that diarrhoea status inflated treatment effect of trofinetide on Week 12 CGI-I scores (Table 67).

Table 67: Mediation Analysis on CGI-I Score at Week 12: Natural Direct Effect and Natural Indirect Effect Estimate – Study 003

Treatment Effect (Trofinetide – Placebo)	Estimate	Standard Error	Wald 95% Confidence Limits	P-value
Total Effect	-0.33	0.101	-0.53, -0.13	0.0010
Natural Direct Effect (NDE)	-0.37	0.125	-0.62, -0.13	0.0030
Natural Indirect Effect (NIE)	0.04	0.075	-0.11, 0.19	0.5952

Source: Table SR_causalmed_CGII_OC

Consistent with other post-hoc analyses conducted and presented by the Applicant, these sensitivity and mediation analysis results provide further support that diarrhoea status did not cause functional unblinding that drives the Study 003 outcomes.

Consistency with Study Neu-2566-RETT-002 (Study 002)

The results on the CGI-I and RSBQ observed in the Phase 2 Study 002 at the 200 mg/kg dose were consistent with the results of Study 003. Study 001, the only study of trofinetide in RTT conducted before Study 002, investigated lower doses of trofinetide (35 mg/kg BID and 70 mg/kg BID) compared to placebo. In Study 001 diarrhoea frequency was comparable in placebo (15%) and trofinetide (21%) groups, meaning that while Study 002 was being conducted there was no expectation that the presence of diarrhoea signified treatment with trofinetide (i.e., functional unblinding in Study 002 based on TEAEs of diarrhoea would not be expected).

Clinical considerations against the influence of functional unblinding

In a severe debilitating disorder with many manifestations, trofinetide demonstrated statistically significant improvement on two different assessments (RSBQ and CGI-I), taken from two different vantage points (caregiver and clinician).

Functional unblinding is a possibility in many if not most clinical trials. It is natural in all clinical trials for participants, caregivers, and investigators to have an opinion on which treatment a subject is receiving. However, both caregiver raters and clinician raters were informed that they were blinded to treatment assignment.

CGI-I Raters and the Question of Functional Unblinding

The CGI-I rating differs from the RSBQ rating in two major ways: 1) the rating is by the clinician rather than by the caregiver and 2) the score is a single global rating rather than a rating of many separate items, thus evaluating domains that are not evaluated by the RSBQ as described previously.

In scoring the CGI-I, clinicians are instructed to render a single, global assessment regarding whether the signs and symptoms of RTT have improved, remained unchanged, or worsened. Since it is a global measure, it is also more likely than on the RSBQ, that the presence of diarrhoea would influence the clinician rater in the opposite direction, causing a bias against trofinetide treatment.

Appendix 2 Anchor-based Minimal Clinically Important Difference Analyses

In response to the comment regarding the submitted MCID for RSBQ not based on clinical rather than statistical considerations, the Applicant has now conducted additional analyses to estimate the MCID for RSBQ using CGI-I as the anchor. Three approaches were applied to data from Study 002 and Study 003: a between-patient score change approach, a within-patients score change approach, and the method of 95% limits of upper agreement (Franceschini et al. 2023). The between-patients score change MCID is the mean RSBQ total score change of patients who improved on the CGI-I minus the mean RSBQ total score change of patients with no change or worsening. The within-patients score change MCID is the mean RSBQ total score change of patients who improved. The method of 95% limits of upper agreement MCID is the mean RSBQ total score change – 1.96 standard error of the score change of stable patients (defined as patients with CGI-I ≥ 4; no patients in Study RETT-002 had CGI-I score of 5 or more [Figure A-2.1] and only 4 patients in Study 003 had CGI-I score of 5 [Figure A-2.3]). Please note six additional anchor-based methods were also described in Franceschini et al. (2023), three of which are ROC-based. These ROC-based methods were not implemented due to the low correlation between RSBQ and CGI-I. The social comparison approach requires patients rating themselves "a little better", "about the same", or "a little worse", which is not applicable to Study 002 and Study 003. The Applicant also did not implement the responsiveness statistic approach due to the large standard deviation relative to the mean change in RSBQ score, or the method of 95% limits of lower agreement approach because the reduction in RSBQ score is considered as improvement, to Study 002 and Study 003.

MCIDs estimated based on the two separate studies were consistent. Anchored to the category of "any improvement" (CGI-I score ≤3), the between-patient approach yielded MCID estimates of 6 RSBQ points in Study 002 and 5 points in Study 003. Using the within-patient approach, the corresponding estimates were 6 points and 7 points, respectively. The 95% limits of upper agreement method produced estimates of 3 points for Study 002 and 4 points for Study 003 (Table 68; see details below). These estimated MCIDs, anchored to CGI-I categories of 'any improvement', are consistent with the previously submitted distribution-based estimates, which ranged from 3 to 6 points. Furthermore, an improvement of 9 points in RSBQ is the observed difference of change in RSBQ between the patients with "worsening or no change"on CGI-I (CGI-I score ≥ 4) and these with "much / very much improved" (CGI-I ≤ 2) in both Study 002 and Study 003 (Table 68) providing a "high bar" for clinical meaningfulness.

Table 68: Anchor-based Estimation of MCID For RSBQ (Trofinetide and Placebo Combined, Observed Case)

	CGI-I Score[1]		
	No Change or Worsening (≥ 4)	Any Improvement (≤ 3)	Much or Very Much Improvement (≤ 2)
Study 002: Change from Day 14 in RSBQ Total Score at Day 54			
N	33	49	11
Mean (SD)	-0.4 (7.17)	-6.0 (8.53)	-8.6 (8.44)
Difference vs "No Change or Worsening" (95% CI)		-5.65 (-9.24, -2.06)	-8.24 (-13.51, -2.98)
p-value		0.0024	0.0029
Study 003: Change from Baseline in RSBQ Total Score at Week 12			
N	119	42	14
Mean (SD)	-2.0 (8.7)	-6.8 (9.1)	-10.1 (10.7)
Difference vs "No Change or Worsening" (95% CI)		-4.81 (-7.92, -1.69)	-8.05 (-13.02, -3.07)
p-value		0.0027	0.0017
[1]CGI-I scores at Day 54 for Study 002 and at Week 12 for Study 003 were used for the analyses. Anchor-based analysis using the within and between-patients score change approach (Franceschini, 2023; Klukowska AM 2024)			

Sources: CHMPah127.1 and CHMPah127.2

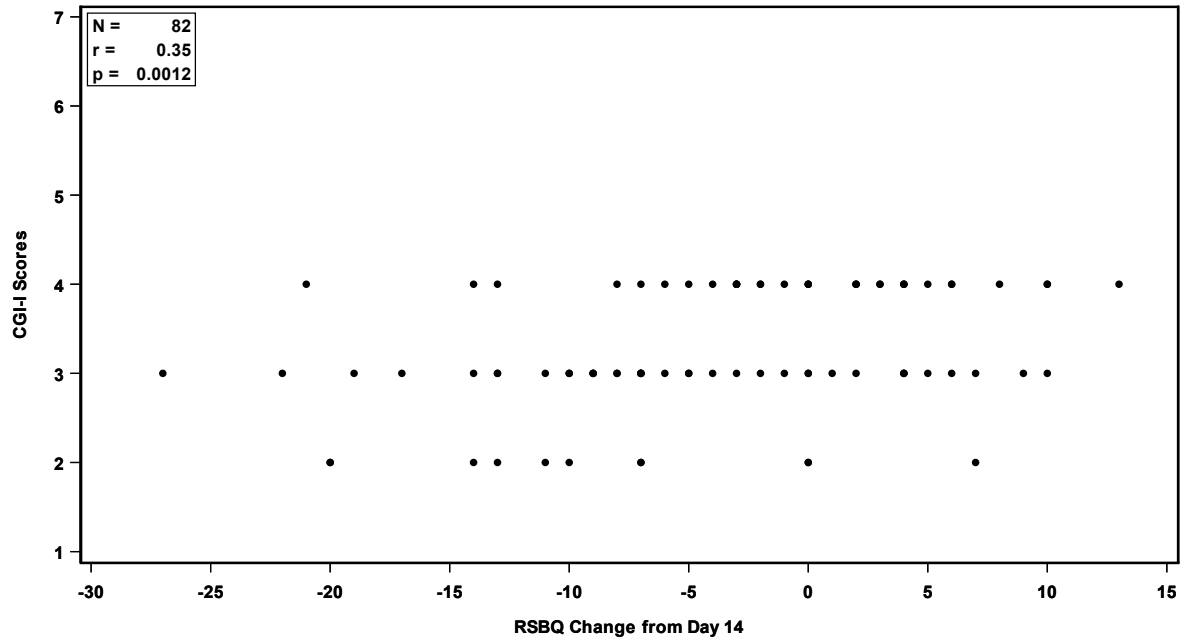
Abbreviations: CGI-I=Clinical Global Impression-Improvement; CI=confidence interval; RSBQ=Rett Syndrome Behaviour Questionnaire; Study 002=Study Neu-2566-RETT-002; Study 003=Study ACP-2566-003; SD=standard deviation

MCIDs estimated from data in Study 002

Study 002 was a Phase 2, 6-week, randomized, DB, placebo-controlled, dose-ranging study of the safety and tolerability of oral treatment with trofinetide (50 mg/kg BID, 100 mg/kg BID, or 200 mg/kg BID) in 82 female children and adolescents with RTT. A total of 82 patients had both change in RSBQ score and CGI-I score at Day 54 with correlation coefficient of 0.35 (p-value=0.0012) between RSBQ and CGI-I (Figure A-2.1). The mean (SD) changes in RSBQ score at Day 54 for patients with 'no change or worsening" on CGI-I is -0.4 (7.17), from which the method of 95% limits of upper agreement MCID was calculated as -0.4 – 1.96*7.17/sqrt(33) = -2.84 (rounding to -3). The mean (SD) changes in RSBQ score at Day 54 for patients with 'any improvement" on CGI-I is -6.0 (8.53), from which the within-patients score change approach MCID was derived as -6. The between-patients score change MCID is –5.65 (rounding to -6) (Table 68). A greater improvement in RSBQ score was associated with greater CGI-I improvement (Figure A-2.2). An improvement of 9 points in RSBQ is the observed difference of change in RSBQ

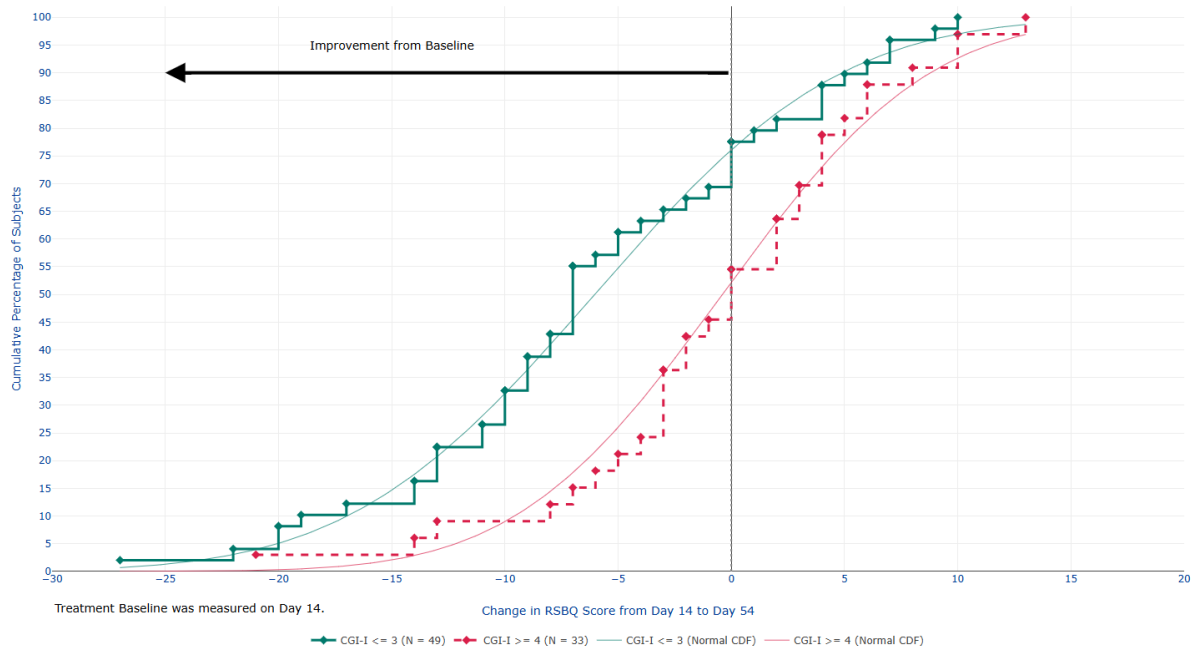
between the patients with" worsening or no change" on CGI-I (CGI-I score ≥ 4) and these with "much / very much improved"(CGI-I ≤ 2) (Table 68).

Figure A-2.1 Correlation Between CGI-I and Change in RSBQ Total Score from Day 14 at Day 54 (OC) in Study 002 - mITT Population - All Treatment Groups Combined



Source: CHMPah138.2
Abbreviations: CGI-I=Clinical Global Impression-Improvement; mITT=modified intent to treat; OC=observed cases; RSBQ=Rett Syndrome Behavioural Questionnaire

Figure A-2.2 Empirical Cumulative Distribution Function of Change in RSBQ Total Score from Day 14 at Day 54 (OC) by CGI-I Category – Study 002 - mITT Population



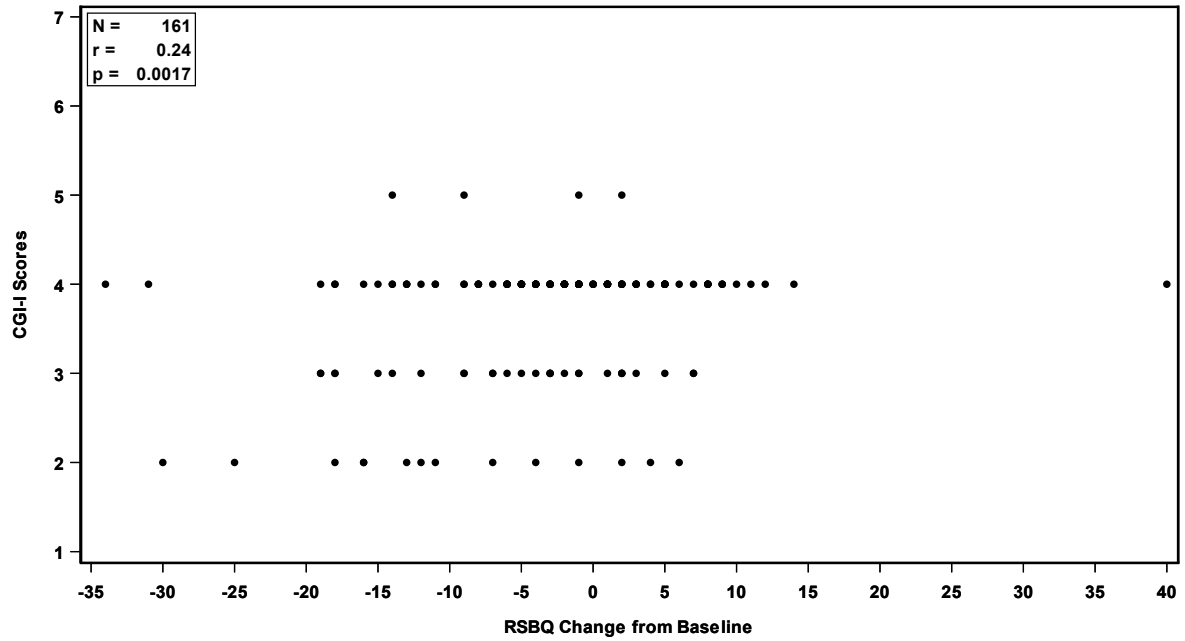
Source: CHMPah140.1
Abbreviations: CGI-I=Clinical Global Impression-Improvement; mITT=modified intent to treat; OC=observed cases; RSBQ=Rett Syndrome Behavioural Questionnaire

MCIDs estimated from data in Study 003

A total of 161 patients in Study 003 had both change from baseline in RSBQ score and CGI-I score at Week 12 with correlation coefficient of 0.24 (p-value=0.0017) between RSBQ and CGI-I (Figure A-2.3). The mean (SD) changes in RSBQ score at Week 12 for patients with 'no change or worsening" on CGI-I is -2.0 (8.7), from which the method of 95% limits of upper agreement MCID was calculated as -2.0 – 1.96*8.7/sqrt(119) = -3.56 (rounding to -4). The mean (SD) changes in RSBQ score at Week 12 for patients with 'any improvement" on CGI-I is -6.8 (9.1), from which the within-patients score change approach MCID was derived as -6.8 (rounding to -7). The between-patients score change MCID is –4.81 (rounding to -5) (Table 68). A greater percentage improvement in RSBQ score was associated with greater CGI-I improvement (Figure A-2.4). An improvement of 9 points in RSBQ is the observed difference of change in RSBQ between the

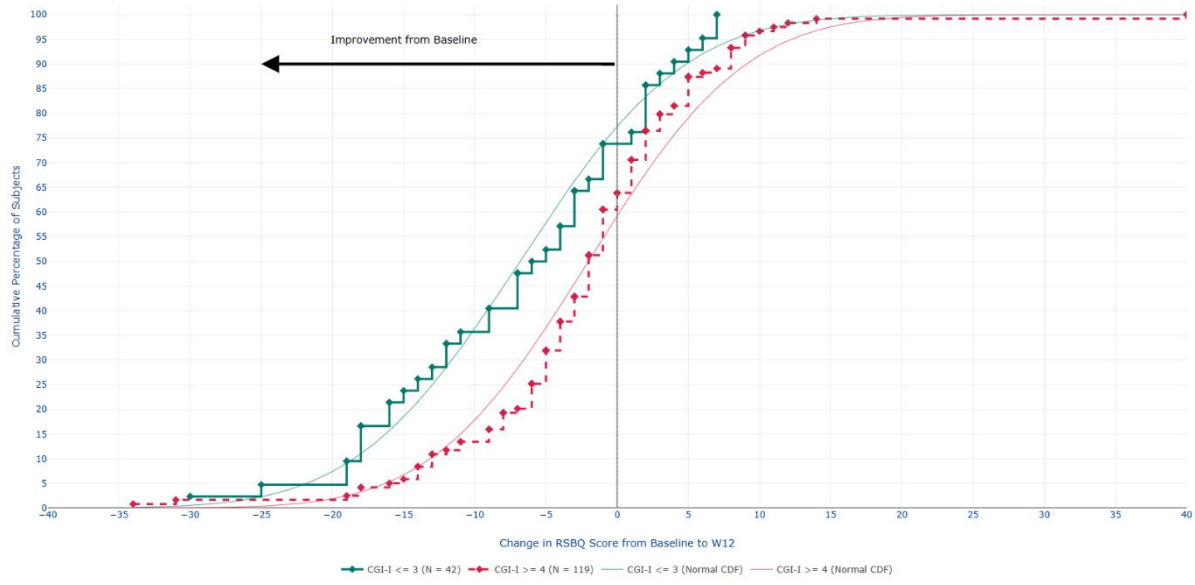
patients with "worsening or no change" on CGI-I (CGI-I score ≥ 4) and these with "much / very much improved" (CGI-I ≤ 2) (Table 68).

Figure A-2.3 Correlation Between CGI-I and Change from Baseline in RSBQ Total Score at Week 12 (OC) in Study 003 - Full Analysis Set - All Treatment Groups Combined



Source: CHMPah138.1
Abbreviations: CGI-I=Clinical Global Impression-Improvement; OC=observed cases; RSBQ=Rett Syndrome Behavioural Questionnaire

Figure A-2.4 Study 003 Empirical Cumulative Distribution Function of Change in RSBQ Total Score from Baseline to Week 12 (OC) by CGI-I Category – Full Analysis Set



Source: CHMPah141.1
Abbreviations: CGI-I=Clinical Global Impression-Improvement; OC=observed cases; RSBQ=Rett Syndrome Behavioural Questionnaire

Responder analysis of Study 003 data based on the estimated MCIDs

When these clinically important difference thresholds are applied in a responder analysis, the odds of achieving a 6-point and 9-point improvement in the trofinetide group (35.2% and 27.5%, respectively) were 2.4 (95% CI: 1.2 to 4.9) and 3.2 (95% CI: 1.4 to 7.2) times the odds in the placebo group (20.4% and 11.8%, respectively) (p=0.0159 and 0.0056). When a more stringent threshold of 12 points, exceeding one standard deviation of the RSBQ total score, was applied, the odds of response in the trofinetide group (20.9%) were 2.8 (95% CI: 1.1 to 6.8) times those observed in the placebo group (9.7%) (p=0.0261).

Appendix 3 All Investigator Comments on CGI-Ratings in Study 003

Treatment Group	Subject Number	Visit Week	CGI-I Score	CGI-I Score	Investigator Comments
Placebo		2	1	1 very much improved	Better nonverbal communication; better gait; able to stand still instead of rocking back and forth; and family very enthusiastic
Trofinetide		12/ET	2	2 much improved	Seizure decreased frequency and mood improved by report and exam
Placebo		2	2	2 much improved	Mother states is clearly much more attentive and maintains eye contact now up to 30 seconds to a minute with dad (and mom); and has had no seizures in last two weeks. Continues to make (forced) choices between items. No major changes in gross and fine motor.
Placebo		6	2	2 much improved	Continues to be improved with attention/eye contact (30sec - minute) and has not had any seizures since July; also continues to make forced choices between two items (improved from baseline; choices are more consistent and faster)
Trofinetide		12/ET	2	2 much improved	No seizures even during menses since study started
Trofinetide		2	2	2 much improved	Mother states that pt has become more attentive; now paying attention to pt'sfavorite show; "Frozen"; for 5-10 minutes instead of <5 minutes and also pays more attention on interaction with family. Furthermore; pt has started to walk around the house (on own; without support) for several minutes at a time. Also; sleeps mostly through the night as opposed to before when ptwould wake in middle of night. Seizure frequency and duration remains same; as does (largely absent) fine motor function.
Trofinetide		12/ET	2	2 much improved	More attentive with people longer duration of connection with them when they engage; Also is walking better; more straight
Trofinetide		12/ET	3	3 minimal improvement	Parents note sustained but subtle improvements in using Tobii to answer questions, play games, spell words interactively. Still having some irritability that developed since trial but better.
Trofinetide		12/ET	3	3 minimal improvement	Sustained increase in reaching with left hand. More attempts to grab cup. Sustained decrease in nighttime screaming spells.

Treatment Group	Subject Number	Visit Week	CGI-I Score	CGI-I Score	Investigator Comments
Placebo		12/ET	3	3 minimal improvement	More calm X 4 weeks - by report. Reaching more often - by report. Hearing more word approximations: "I'm done"; "om" for Mom; echolalia.
Trofinetide		2	3	3 minimal improvement	More verbal engagement: volunteers words. More social engagement. Less repetitive hand movements. More purposeful use of potty. Fewer wet pull-ups at school. More intelligible to non-family members. Still has frequent HV/BH. Fewer Rett spells
Trofinetide		6	3	3 minimal improvement	Continued stable improvements in social engagement & responsiveness. Nothing new. More easily frustrated though. Shorter fuse × 3–4 weeks. Can't wait as long for things. More screaming fits when something doesn't work.
Trofinetide		12/ET	3	3 minimal improvement	Has continued to have mild improvement in social engagement & response time to questions compared to baseline.
Placebo		12/ET	3	3 minimal improvement	More alert. Making choices more consistently.
Placebo		2	3	3 minimal improvement	No seizures since May 2nd. Seems more alert, but also taking more naps – typically 1–2 naps, but since seizure #2 had additional 1–2 quick naps (1–2 min long/day), random times of day. More likely if stimulating activities. Making eye choices more quickly & more often. More choreiform arm movement. No Δ in hand use. Possibly is babbling more in response to being spoken to. Improvements have been noticed at home & school. Uncertain whether this is just back to baseline from before seizures got under control, but mom feels: definite communication is better than the subject has ever seen.
Placebo		6	3	3 minimal improvement	More babbling; new sounds. Improved grasping
Placebo		6	3	3 minimal improvement	More aware; if name called will respond more directly. Family weighing reality currently that gains seem minimal compared to baseline; may have overestimated gains at last visit.
Trofinetide		12/ET	3	3 minimal improvement	Improvement in attention. Questionable rare attempts at words.

Treatment Group	Subject Number	Visit Week	CGI-I Score	CGI-I Score	Investigator Comments
Trofinetide		6	3	3 minimal improvement	Increased hand use; more engaged; more babbling while walking and uses one word. Based on moms report. The engagement and communication occur while walking outside. Dad was initially skeptical but agrees with minimal improvement, who is an attorney; says it is subjective
Trofinetide		12/ET	3	3 minimal improvement	More attentive; looks at elmo; notices new things in the house; fixates on household appliances; watches from behind in the car. These are new. Nothing else new.
Placebo		12/ET	3	3 minimal improvement	Mom thinks improved; but have bigger house so more to do; also in school now. Plays with peers. More interactive; more demanding; communicates what the subject likes; dislikes; more interested things. Grab and holds things more often. Calmer; hands not in mouth as often- examiner observed
Trofinetide		2	4	4 no change	Subtle changes: possibly occasional less right-hand stereotypy while eating/using left hand. Possible less waking/screaming early in night. More restful sleep.
Trofinetide		6	4	4 no change	Continued subtle changes in hand use. Might reach more often with left hand at meals. Possibly waking less and doesn't fuss as much.
Placebo		2	4	4 no change	Possibly has had better eye contact, more engaged. More consonant vowel babbling, with word approximations.
Trofinetide		2	4	4 no change	Possibly was more engaged for approximately 1 week. Possibly making choices more quickly.
Trofinetide		6	4	4 no change	Perhaps more alert. Said "stop" to babysitter while getting hair brushed. No Δ in chorea. No Δ in hand function. Mild loose stools.
Trofinetide		12/ET	4	4 no change	Possibly more alert, less need for napping, but not a significant change.
Placebo		2	4	4 no change	Parents report has been responding more quickly to requests/instructions. No other major changes.
Placebo		12/ET	4	4 no change	No change
Trofinetide		2	4	4 no change	Mother notes some improved alertness and eye contact and happier
Trofinetide		12/ET	4	4 no change	A little less interactive today; breath holding; tired. Reaching a little less; but mom has not noted any significant changes at home. Seems stable to mom. Getting up into stander again finally post hip surgery

Treatment Group	Subject Number	Visit Week	CGI-I Score	CGI-I Score	Investigator Comments
Trofinetide		2	4	4 no change	The patient appears more alert and the father reports that the subject vocalizes more.I scored 4 and not 3 because the status more likely reflects change fluctuations than a real response. On note is that the pt had AEs and restarted the full dose only 3 days ago.
Trofinetide		6	4	4 no change	Family reports more vocalization; less breath holding and more social interaction; but no other clear change
Trofinetide		2	4	4 no change	Also; parents note no change
Placebo		6	4	4 no change	Today during eval mom notes increase in the subjects' looking around more; shifting gaze more quickly to assess surroundings. Turns head trying to get moms attention. Has seemed more alert and turns to brother when the subject hears him walking down the stairs- this latter could be a random fluctuation.
Placebo		12/ET	4	4 no change	Hands to mouth now; but non purposeful; more agitated; tremors. Bowel movements have changed; titrated by mom; has had bouts of large volumes if watery stool; intermittently. Never had anything like it before. Parents state if these changes; noted in first sentence; are due to the study drug; they would not see any benefit; also do not like to see the subject agitated. They note large volume of study drug and the subject hates it.
Trofinetide		2	4	4 no change	Walking on flat feet; not toe walking but no significant change in functional ability to walk; stand; balance.
Placebo		2	4	4 no change	Unusually calm yesterday; but has these days in the past. For me; the subject is brighter and more communicative; happier; more content. Mom feels 4 is appropriate. This is the 3rd time i have seen the subject and I am questioning if the subject is a 3. Equivocal so scored as 4
Placebo		6	4	4 no change	Communicating more with 1:1 aide at school; now has school routine again; seems to benefit her skill level.; Made forced choice today first time. 2/2. We did not attempt a third time due to lack of cooperation. But inconsistent and mom feels all are normal fluctuations; the subject has gained 5.1 kg since screening 7/8. Spending more time at moms house and they feed the subject more- the subject consistently asks for food and that communication is rewarded.; Rett university coach/advocate re-wrote and got the 1:1 placed.

Treatment Group	Subject Number	Visit Week	CGI-I Score	CGI-I Score	Investigator Comments
Placebo		12/ET	4	4 no change	No change last seven days; no change since starting the study drug
Trofinetide		12/ET	5	5 minimally worse	Not eating well. Cries when offered food. No choking. Parents thinking about a GTube.
Placebo		2	5	5 minimally worse	Had breakthrough seizures from 11/7-11/9 with no obvious trigger. AED dose was increased. Prior to this; no seizure since 8/2020
Trofinetide		6	5	5 minimally worse	Parent report of worse breathing dysrhythmia / gasping x2–3 weeks. Daily episodes of agitation/screaming since -2 days before IMP. More episodes of trembling while standing up x2–3 weeks.
Placebo		12/ET	5	5 minimally worse	With new cyanosis associated with breath holding
Placebo		12/ET	5	5 minimally worse	Decreased eating; worse breath holding

Appendix 4 RSBQ Items and Link to Caregiver Reported Meaningful Improvements based on McGraw et al. 2023

RSBQ Item	Theme impacted by meaningful improvement	Caregiver description of why RSBQ item improvement is meaningful (why it's relevant and how it impacts daily activities)
1. There are times when breathing is deep and fast (hyperventilation)	PhysHealth	Less damage to lungs or brain; less disruptive eating; normalized breathing at night means more sleep and less caregiver worry that child will stop breathing
2. Spells of screaming (…) during the day	Psych-Social	Improved emotion and communication (in public settings), reduced frustration
3. Makes repetitive hand movements with hands apart	PhysHealth	Less hand wounds/injuries, less rocking if hands don't need to be prevented from moving
4. Makes repetitive movements involving fingers around tongue	F&G Motor	Less interference with fingers & tongue allows more focus on tasks or teacher and school performance
5. There are times when breath is held	PhysHealth	Less worry that child stops breathing, or passes out
6. Air/Saliva is expelled from mouth with force	No Theme	Not mentioned
7. Spells of apparent anxiety/fear in unfamiliar situations	Psych-Social	Happier child and psychological improvements, allows potential for improved social connection
8. Grinds teeth	PhysHealth	Less teeth damage, (less caregiver worry there is risk)
9. Seems frightened when there are sudden changes in own body position	Psych-Social	Reduced anxiety/fear to child
10. There are times when parts of the body are held rigid	PhysHealth	Less injury (to muscles / body) from falls
11. Shifts gaze with a slow horizontal turn of head	Comm	Faster communication; Ability to respond via eye gaze and communicate with others
12. Expressionless face	Comm	Improved ability to smile and make facial expressions enables ability for communication
13. Spells of screaming (…) during the night	Psych-Social	Improves sleep, rest, energy and physical health for child (and for the whole family)
14. Abrupt changes in mood	Psych-Social	More predictable social interactions - interact with community, restaurants, movie, grocery store
15. There are certain days/periods where she performs much worse than usual	Psych-Social	Child being happier and improved performance in school or therapy
16. There are times when she appears miserable for no apparent reason	Psych-Social	Child being happier
17. Seems to look through people into the distance	Psych-Social	More ability to focus on tasks or people
18. Does not use hands for purposeful grasping	Psych-Social, F&G Motor	May enable self-feeding; Less frustration means more psychologically stable;
19. Swallows air	PhysHealth	Less stomach pain
20. Hand movements are uniform and monotonous	PhysHealth	Engage in activities that give them joy (play more with toys) gain independence, make choices, access the environment
21. Has frequent naps during the day	No Theme	N/A - Not mentioned (perhaps related to sleep)
22. Screams hysterically for long periods of time and cannot be consoled	Psych-Social	Less screaming means more ability for child and family to leave the house
23. Although can stand independently tends to lean on objects or people	PhysHealth, F&G Motor	Means less chance for falls / injury and less physical requirement for caregivers; more independence; more ability for therapy; less medical equipment to travel
24. Restricted repertoire of hand movement	F&G Motor	Expands chances to do what she enjoys or wants (play with a toy)
25. Abdomen fills with air and sometimes feels hard	PhysHealth	Less stomach pain
26. Spells of laughter (…) during the day	No Theme	N/A - Not bothersome or troublesome occurrence, as caregivers enjoy their child's laughter
27. Has wounds on hands as a result of repetitive hand movements	PhysHealth	Less hand wounds/injuries, less rocking if hands don't need to be prevented from moving
28. Makes mouth grimaces	No Theme	Not mentioned
29. There are times when she is irritable for no apparent reason	No Theme	Not mentioned
30. Spells of inconsolable crying (…) during the day	Psych-Social	Improved emotion and psychological stability; happier child, potential for communication
31. Uses eye gaze to convey feelings, needs and wishes	Psych-Social Comm	Improvement enhances social connections with peers or other kids

RSBQ Item	Theme impacted by meaningful improvement	Caregiver description of why RSBQ item improvement is meaningful (why it's relevant and how it impacts daily activities)
32. Makes repetitive tongue movements	No Theme	Not mentioned
33. Rocks self when hands are prevented from moving	PhysHealth	Less rocking when hands are not prevented from moving
34. Makes grimacing expressions with face	No Theme	Not mentioned
35. Has difficulty in breaking/stop hand stereotypies	PhysHealth	Engage in activities, grasping, eating, improves potential for communication via reaching or pointing
36. Vocalises for no apparent reason	No Theme	N/A - Not bothersome or troublesome occurrence, as caregivers enjoy their child's voice
37. Spells of laughter (…) during the night	No Theme	N/A – While good sleep is important, this not always a negative occurrence, as caregivers can enjoy their child's laughter
38. Spells of apparent panic	Psych-Social	Greater ability for family child to leave the house, visit community
39. Walks with stiff legs	PhysHealth, F&G Motor	Walk or climb stairs more easily; More independence and more ability for therapy; less medical equipment to travel
40. Tendency to bring hands together in front of chin or chest	F&G Motor	Ability to grasp food, toy, object instead of other hand
41. Rocks body repeatedly	PhysHealth, Comm	ability to establish eye gaze; less monitoring and restraining required to prevent harm or injury (like falling off couch); less restraining means more independence
42. Spells of inconsolable crying (…) during the night	PhysHealth, Psych-Social	improves sleep, rest, energy and physical health for child (and for the whole family)
43. The amount of time spent looking at objects is longer than time spent looking/manipulating them	F&G Motor	Enables child to play more with toys or objects that give them joy
44. Appears isolated	Psych-Social	Child is having more fun, more engaged with others communicating with peers
45. Vacant 'staring' spells	Psych-Social	Helps caregivers recognize child is more engaged, by focusing on tasks or people
(…) = for no apparent reason		

Themes: PhysHealth=Preserving physical health and well-being; Psych-Social=Improve psychological and social impacts; F&G Motor=Improve Fine and Gross Motor Ability; Comm=Improve Communication; No theme= not mentioned

Appendix 5 Study 004 Completers

Table 69: Baseline Disease Characteristics in Study 003 – Safety Analysis Set and Study 004 Completers

	Study 003 safety analysis set			Study 004 completers		
	PBO (N=94)	**TROF (N=93)**	**Total (N=187)**	**DB-PBO-OL TROF (N=39)**	**DB TRO-OL TROF (N=45)**	**Total (N=84)**
Age (years)						
n	94	93	187	39	45	84
Mean (SE)	10.9 (0.47)	11.0 (0.49)	10.9 (0.34)	10.4 (0.66)	10.9 (0.66)	10.7 (0.47)
Age at Rett syndrome diagnosis (years)						
n	94	93	187	39	45	84
Mean (SE)	2.6 (0.21)	2.2 (0.11)	2.4 (0.12)	2.5 (0.24)	2.2 (0.17)	2.4 (0.15)
Derived Baseline RSBQ total score						
n	94	93	187	39	45	84
Mean (SE)	44.4 (1.25)	43.8 (1.18)	44.1 (0.86)	44.0 (2.14)	45.3 (1.76)	44.7 (1.36)
SD	12.13	11.42	11.76	13.34	11.79	12.47
Baseline CGI-S score						
n	94	93	187	39	45	84
Mean (SE)	4.9 (0.08)	4.9 (0.08)	4.9 (0.06)	4.8 (0.14)	4.9 (0.12)	4.9 (0.09)

Source: Tables CHMPah129.1, CHMPah129.2, and CHMPah129.3

Table 70: Frequency of Diarrhea and Vomiting in Study 004 Completers during the Double-blind and Open-label Study Periods Compared with All Subjects in the Safety Analysis Set

	Safety Analysis Set		Study 004 Completers Subgroup	
Preferred term	**PBO (N=94)**	**TROF (N=93)**	**PBO (N=39)**	**TROF (N=45)**
TEAEs in Study 003				
Diarrhoea	18 (19.1)	75 (80.6)	5 (12.8)	33 (73.3)
Vomiting	9 (9.6)	25 (26.9)	3 (7.7)	8 (17.8)
	PBO (N=85)	**TROF (N=69)**	**PBO (N=39)**	**TROF (N=45)**
AEs in Study 004[a]				
Diarrhoea	71 (83.5)	44 (63.8)	31 (79.5)	27 (60.0)
Vomiting	29 (34.1)	15 (21.7)	7 (17.9)	6 (13.3)

Sources: Tables CHMPah130.2A and CHMPah130.5B

Abbreviations: AE=adverse event; MedDRA=Medical Dictionary for Regulatory Activities; PBO=placebo; PT=preferred term; TEAE=treatment-emergent adverse event; TROF=trofinetide

Notes: Adverse events were coded using MedDRA version 24.0.
A TEAE is an adverse event with onset date on or after the first study dose date and no later than last study dose date + 30. A subject may have more than one TEAE per preferred term (PT) and a subject is counted at most once per PT. The number of subjects per treatment group is used as the denominator for calculating percentages for the subject counts.

[a] Adverse events included TEAEs and events that began during Study 003 and were still ongoing at the Baseline visit of Study 004.

5.1.3. CHMP position on the Ground for re-examination #1:

The existence of a treatment effect on symptoms of Rett syndrome has been already accepted in the first CHMP Opinion. Due to the high frequency of diarrhoea in Study 003 (80.6% trofinetide vs 19.1% placebo), potential functional unblinding could have challenged the credibility of the estimates of this effect. However, reassuringly, subgroup analyses in patients with/without diarrhoea and sensitivity analyses adding a diarrhoea-by-visit interaction term to the primary MMRM models indicate no anti-conservative impact of diarrhoea on the efficacy results. The effect sizes were minimally changed for the co-primary endpoints RSBQ and CGI-I compared to the primary analysis, supporting the validity of the estimates and their use for regulatory decision making.

A longer discussion is dedicated below to the clinical relevance of this effect and on long-term efficacy.

Clinical relevance of co-primary endpoints in the proposed narrowed indication

Following the grounds for refusal issued by CHMP in their initial opinion, and since some of the key symptoms of Rett syndrome (e.g. seizures) were not included in the clinical efficacy endpoints, the Applicant proposed to amend the indication to treat neurobehavioural manifestations (later in the procedure changed to "neurobehavioural symptoms") of Rett syndrome rather than the full spectrum of the disease. Thus, complementarity, but also suitability of the co-primary efficacy endpoints, RSBQ and CGI-I, to evaluate efficacy were re-assessed in the context of the newly proposed indication.

In the Applicant's view, the co-primary efficacy endpoints in the Phase 3 program (RSBQ and CGI-I) map to the complex neurobehavioral manifestations of RTT.

It is agreed that the RSBQ (Rett Syndrome Behaviour Questionnaire) is designed to evaluate behavioural symptoms associated with RTT, since it explicitly excludes other comorbid or systemic symptoms such as epileptic seizures or gastrointestinal complications. The RSBQ is caregiver-rated and assesses the Rett-specific behavioural phenotype in 8 distinct domains: general mood, breathing problems, hand behaviours, face movements, body rocking/expressionless face, night-time behaviours, fear/anxiety, walking/standing. While RSBQ is considered informative in the context of the restricted indication, the SAG-N experts confirmed that the scale is used by the clinicians in the field for routine clinical follow-up, but it has not been validated for regulatory purposes despite being used in clinical trials. This was taken into account in the assessment.

The RTT-specific CGI-I is clinician-rated and provides a holistic, overall measure of a patient's clinical improvement, focusing on 7 core clinical domains of RTT: language/communication, ambulation, hand use, social (eye contact), autonomic (breathing), attentiveness and seizures. Thus, the CGI-I also includes the evaluation of seizures but does not assess these separately. As the CGI-I provides a holistic, overall measure of a patient's clinical improvement, it is impossible to pinpoint which individual symptoms are improved, and thus the domains driving the CGI-I results could actually be outside the scope of the currently proposed label. Furthermore, individual behavioural symptoms are not assessed, making the CGI-I a potentially less suitable endpoint for the newly proposed indication.

Taken together, with regard to the suitability of the co-primary endpoints for the evaluation of efficacy, it is agreed that the RSBQ is designed to evaluate neurobehavioural symptoms associated with RTT, while the CGI-I provides a global measure of a patient's clinical improvement, which is at least partially due to symptoms that are within the scope of the currently proposed label. However, it is accepted that these co-primary endpoints offer different perspectives on behavioural aspects of Rett syndrome. It was also agreed by SAG-N experts by consensus that the co-primary endpoints sufficiently comprise neurobehavioural manifestations of the Rett syndrome in the plateau phase.

The term proposed by the Applicant (i.e. "neurobehavioural") was considered appropriate and acceptable, as it is familiar to clinicians in the field and is thought to adequately describe the symptoms

assessed by the co-primary endpoints. The wording of the indication also takes into account that, given the early endpoint assessment at 12 weeks and the mechanism of action being unclear, trofinetide is considered to provide only symptomatic treatment of current neurobehavioural symptoms (rather than improving disease manifestations caused by disrupted neuroanatomical structures).

Whether the observed changes after short-term treatment are clinically relevant in the context of a symptomatic treatment was re-assessed during this re-examination.

To support the clinical relevance of the effect size of RSBQ (-3.1), the Applicant has supplemented the distribution-based MCID estimates with MCID values derived using anchor-based methods (using the CGI-I as the anchor). MCID values derived from Study 002 and Study 003 using multiple statistical methods were reported. The estimated MCID values ranged from 3 to 9 points improvement in the RSBQ score, depending on data and methods used.

Anchor-based methods are better suited to establish a MCID, as they use information that is taken to establish the presence or absence of clinically relevant changes at an individual-patient level. However, the anchor-based analyses presented have some methodological limitations. First, the relation of the score to the anchor is not clear. The applicant describes the CGI-I score and RSBQ as complementary when discussing the scores as co-primary endpoint, however, the CGI-I needs to capture aspects of disease severity measured by RSBQ to be suitable as an anchor to derive an MCID for RSBQ. Second, the MCID should have been defined a priori.

Even if the proposed MCID of 3-7 points change in RSBQ was agreed to, the observed reduction in RSBQ score of 3.1 still indicates a benefit only at the lower end of this uncertain range of the proposed clinical relevance. Overall, the SAG-N experts found it difficult to determine the clinical meaningfulness of the observed changes. Some experts were of the opinion that any change – even if small – could positively impact the patient's daily life, while other experts indicated that observed effect sizes are at or below the MCID, acknowledging the methodological shortcomings in the approach to determine the MCID within the same study population. Statistical significance is mainly driven by 2 of 8 individual domains (body rocking/expressionless face and fear/anxiety), which both favour trofinetide in a nominally statistically significant manner. Reassuringly and in line with these findings, the remaining domains also favour trofinetide, though to an even lesser extent. Although robust support from other clinical endpoints and/or clinical efficacy studies that would facilitate the confirmation of the clinical relevance of this result is lacking, the key secondary (demonstrated to be statistically significant) and the majority of other secondary endpoints in Study 003 are directionally aligned with the co-primary endpoints.

The Applicant provided responder analyses for several responder definitions for different values of RSBQ RFB at Week 12 (3, 6, 9, 12 points) as well as a combination of RSBQ RFB of at least 3, 6, 9, 12 points and improvement of CGI-I at Week 12. The values used as threshold for the RSBQ RFB covered the range of the MCID values derived by the applicant. The responder analyses are in line with the primary analysis and there is a nominally statistically significant treatment effect that is consistent over a range of RSBQ thresholds (>=6-point, >=9-point and >=12-point reductions from baseline in the trofinetide group at Week 12; >=6-point: 35.2% (32/91) vs 20.4% (19/93), >=9-point: 27.5% (25/91) vs 11.8% (11/93), >=12-point: 20.9% (19/91) vs 9.7% (9/93)).

Additionally, the Applicant referred to several external data sources to try and define what constitutes a clinically meaningful change with the RSBQ, such as 20 years natural history data from Australia (Downs 2026), a US retrospective study of 6 months trofinetide use (Prange 2026), EU Delphi Panel Consensus Report, qualitative study exploring how caregivers viewed changes in the symptoms covered in the RSBQ (McGraw 2023). While these reports are associated with numerous uncertainties and thus of limited use for determining what the clinically meaningful effect is, they shed some light that even numerically small improvements in communication, mobility and functional hand use are meaningful and might improve quality of life, at least from the perspective of caregivers. The natural history data from Australia

suggests that on a group level, significant spontaneous improvements in the RSBQ over time are not likely.

While at the individual level any difference in CGI-I can be intrinsically seen as clinical relevant, the reported mean treatment difference of -0.3 in the co-primary endpoint CGI-I represents a rather limited effect and is also difficult to interpret, in particular considering that CGI-I does not exclusively cover the symptoms that are in the revised indication wording. Responder (any improvement, CGI-I ≤3) analyses showed that there were more responders in the trofinetide group compared to placebo [32% vs 14%], which was considered a clinically relevant finding. The provided patient vignettes and investigator comments on CGI-ratings provided by the Applicant are acknowledged, but qualitative descriptions are deemed of limited value to substantiate efficacy.

Taken together, the observed mean difference in RSBQ score of 3.1 points indicates a small effect on neurobehavioural symptoms of Rett syndrome. While the -0.3 points difference in CGI-I is with unclear relevance in the assessment of individual symptoms of Rett syndrome, taking into account the views of some SAG-N experts, it is concluded that even small improvements could impact the patient's daily life. The overall assessment of the clinical relevance of the efficacy profile of Daybu will take into account the totality of evidence, including the secondary endpoints (see below).

Support from other clinical endpoints

There was a statistically significant difference in favour of trofinetide for the key secondary endpoint CSBS-DP-IT Social composite score (LS mean difference: 1.0; 95% CI: 0.3, 1.7; p=0.0064), assessing communication and pre-linguistic skills in young children (12 to 24 months of age) and older children with developmental delay. Though the treatment effect size in this endpoint is considered minimal, a demonstrated statistically significant treatment effect also in this key secondary endpoint is in support of the conclusion of a treatment effect as seen in both of the co-primary endpoints. . SAG-N experts considered this 1-point change observed small and probably not clinically meaningful in this key secondary endpoint. The other secondary or exploratory endpoints had not been foreseen for further confirmatory testing following the key secondary endpoint. On a nominal level, one of the other secondary or exploratory endpoints was statistically significant (RTT-COMC) only. This was also discussed by the SAG-N experts, indicating that secondary outcomes addressing symptoms in the same domains as the RSBQ, did not show nominally statistically significant differences, except for one. On the other hand, those secondary endpoints were directionally consistent with the co-primary and the (key) secondary endpoints (nominally significant improvement in one secondary endpoint, non-significant improvement in five secondary endpoints and no difference up to rounding in two secondary endpoints), therefore being in directional consistency with the co-primary endpoints. Thus, when considering communication and social interaction as core behavioural feature in Rett syndrome, there were improvements in the communication scale (CSBS-DP-IT: including emotion, eye gaze, communication and gestures) and ability to communicate choices (RTT-COMC), but the extent of the effect sizes is of unclear relevance for these secondary endpoints, and there was no difference at all in verbal communication (RTT-VCOMB). In addition, other relevant functional scales (motor function: RTT-AMB; hand function: RTT-HF) did not show nominally statistically significant improvement. No change in severity has been observed on the CGI-S, nor any nominally statistically significant improvement in quality of life (QoL rating of ICND) or caregiver burden (RTT-CBI). One patient representative acknowledged that the absence of impact on the quality-of-life (one of the secondary outcomes) adds uncertainty about whether the differences on the primary outcomes could be truly considered as clinically meaningful. In summary, tested behavioural domains in Rett syndrome were statistically significantly improved in several endpoints – importantly, including all the endpoints high in the hierarchy - (RSBQ: body rocking/expressionless face and fear/anxiety; CSBS-DP-IT; and on a nominal level RTT-COMC), while the remaining behavioural domains showed trends of improvement (without statistical significance) or were unchanged.

SAG-N experts agreed by consensus that all subscores of the co-primary endpoints except the one(s) quantifying seizures (even if it was discussed that they could eventually impact neurobehavioural manifestations or symptoms) and all secondary endpoints, capture – to a certain extent – aspects in connection with neurobehavioural manifestations or symptoms.

In conclusion, while the MCID for RSBQ cannot be considered established, the totality of evidence is sufficient to conclude that an effect of clinical relevance on the neurobehavioral symptoms of Rett syndrome has been sufficiently demonstrated.

CMA consideration

It is acknowledged that the Applicant expressed their readiness to consider a conditional marketing authorisation (CMA) in case the CHMP considered the benefit-risk positive and the data not comprehensive and provided proactively justifications for satisfying the CMA criteria. However, having considered the totality of evidence, the CHMP considered that the data is comprehensive for the claimed indication of symptomatic treatment and therefore fulfilling the CMA criteria is not necessary.

CHMP conclusion

In this re-examination, the target indication was modified to *treatment of* ***neurobehavioural manifestations*** *(and later "****symptoms****") of Rett syndrome*, explicitly excluding key aspects of Rett syndrome, such as seizures. The co-primary efficacy endpoints RSBQ and CGI-I offer different perspectives on the disease. The observed effect size is numerically small, and clinical relevance of this amount of change was debated. However, the effect is considered to be established and as the calculations of MCID are also compatible with the effect being (on the lower end) of clinical meaningfulness, as the CGI-I can be seen as intrinsically meaningful, as the effect size is considered relevant by some SAG-N experts and given the directional consistency in the secondary endpoints, the observed effect size is considered acceptable in this clinical setting with no specific alternatives. The point is resolved.

5.1.4. Ground #2

The population included in the study does not reflect the full spectrum of the proposed target population and available data question extrapolation from patients in the plateau phase to those in the regression phase of the disease.

5.1.4.1. Applicant response

Concerning the sought indication 'treatment of Rett syndrome", the Applicant acknowledges that in the clinical development program of trofinetide, key clinical symptoms (including seizures) were not assessed separately from the use of the CGI-I instrument as (co-) primary outcome measure and therefore proposes to narrow the indication to 'treatment of neurobehavioral manifestations of Rett syndrome".

Previous	New
Daybu is indicated for the treatment of Rett syndrome in adults and paediatric patients 2 years of age and older.	Daybu is indicated for the treatment of neurobehavioural manifestations of Rett syndrome in adults and paediatric patients 5 years of age and older (see Section 5.1).

Neurobehavioural manifestations

In the context of the narrowed indication, "neurobehavioral manifestations of Rett syndrome" refers to the core functional, behavioral and emotional grouping of signs and symptoms that occur together and characterize RTT. Specifically, the severe impairments in communication, purposeful motor function (e.g., hand use, mobility), and behavioral regulation are the core features that characterize the 'classic' RTT. In addition, several supportive criteria including breathing disturbance when awake, abnormal muscle tone, inappropriate laughing/screaming spells, teeth grinding, and others are also extremely common (Neul et al. 2010; Percy et al. 2010). Furthermore, the evolution of the recognition of Rett neurobehavioural abnormalities has expanded to include anxiety-like symptoms, mood instability, disruptive behavior, and repetitive and perseverative behaviors (Barnes et al. 2015; Buchanan et al. 2019; Robertson et al. 2006). The complexity and diversity of these neurobehavioral manifestations of RTT's clinical presentation have been systematically characterized as the RTT behavioral phenotype (Mount et al. 2001; Mount et al. 2002, Mount et al. 2003).

Although multiple standardized but non-RTT-specific instruments (Anxiety, Depression, and Mood Scale [ADAMS], Developmental Behaviour Check-list [DBC], etc.) have been used to study RTT, they were deemed not adequate for RTT because of the severe deficits in communication and motor function (Oberman et al. 2023). The publication by Mount et al. 2002 describes the RSBQ as the first disorder-specific instrument for evaluating the behavioural phenotype associated with RTT. In order to distinguish RTT, this term excludes other comorbid or systemic symptoms such as epileptic seizures, gastrointestinal complications or other severity related manifestations that should be considered outside the scope of this behavioural phenotype (i.e., "neurobehavioral manifestations" as defined here). Those issues are considered separate medical domains (managed with established and standardized approaches).

The Applicant deliberately selected co-primary efficacy endpoints in the Phase 3 program that map to the complex neurobehavioral manifestations of RTT. The RSBQ was used as a caregiver-reported primary measure because it is a validated, fit-for-purpose instrument that assesses the specific Rett behaviors and manifestations across 8 domains: general mood, breathing problems, hand behaviours, face movements, body rocking/expressionless face, night-time behaviors, fear/anxiety, walking/standing (Mount et al. 2002). Although the RSBQ was originally developed to differentiate RTT from children with severe intellectual disabilities, it has since been extensively validated for clinical trial use – demonstrating strong internal consistency, content and construct validity, and the ability to discriminate RTT's characteristic features. It effectively quantifies the key neurobehavioral impairments that caregivers observe and have a negative impact on daily life.

We paired the RSBQ with the CGI-I scale (modified with RTT-specific anchors) as the other co-primary endpoint. The CGI-I is a clinician-rated global assessment of change that provides a holistic, overall measure of a patient's clinical improvement, ensuring we capture additional RTT specific improvements in the patient's overall condition that might not be fully reflected by a single questionnaire. Notably, the RTT-specific CGI-I used in the Phase 3 program was developed with a special attention to 7 core clinical domains of RTT, 6 of which are neurobehavioral (language/communication, ambulation, hand use, attentiveness, social (eye contact) and autonomic (breathing) (Neul et al. 2015) to keep evaluators focused on changes in the key neurodevelopmental and behavioral symptoms.

Together, these two endpoints offer complementary perspectives: the RSBQ evaluates individual domains of neurobehavioral function in detail, while the CGI-I integrates those changes into an overall impression of clinical benefit. The Applicant acknowledges that some comorbid or systemic symptoms could not be specifically evaluated in our efficacy studies for trofinetide using RSBQ and CGI as endpoints. By using the phrase "neurobehavioral manifestations," we ensure the indication precisely covers the aforementioned key CNS-driven symptoms that trofinetide was shown to improve, without implying efficacy on non-neurobehavioural problems that the study endpoints do not specifically address. Together, these two endpoints offer complementary perspectives: the RSBQ evaluates

individual domains of neurobehavioral aspects in detail, while the CGI-I integrates those changes into an overall impression of clinical benefit. This dual approach was discussed with regulators and experts during trial design, and the instruments are well-recognized in Rett syndrome research. Additionally, several publications related to the RSBQ describe the use of the questionnaire to assess neurobehavioral manifestations of RTT.

- In a publication by Percy et al. (2023), they state that "…The Rett Syndrome Behaviour Questionnaire (RSBQ) assesses the severity of neurobehavioral problems from the perspective of the caregiver and is one of the most widely used measures due to the specificity of its psychometric profile to the core features of RTT…" Neurobehavioral symptoms of RTT include breathing abnormalities, abnormal movements such as stereotypies, fine motor and gross motor abnormalities, language and communication, anxiety, depression, and sleep abnormalities.
- Sato et al. (2026): the authors refer to RSBQ as "… a 45-item caregiver-completed scale that assesses a wide range of neurological and behavioral symptoms in patients with RTT…"
- Kaufman et al. (2025) include neurobehavioral symptoms as a keyword for the publication - Rett Syndrome Behaviour Questionnaire: Variability of Scores and Related Factors. In their discussion, they state that the RSBQ is the most widely used behavioral instrument and one of the most commonly implemented clinical outcome assessments in RTT.
- Oberman et al. (2023) acknowledge that RTT is associated with multiple neurobehavioral abnormalities and that RSBQ is the first disorders specific instrument for evaluating behavior in RTT.
- Downs et al. (2026) describe RSBQ as a tool developed to describe the behavioral and emotional features of RTT.
- McGraw et al. (2023) describe RSBQ as a measure used to assess changes in Rett syndrome–related manifestations or core symptoms.
- Percy et al. (2023) identify the neurobehavioural manifestations of RTT along with guidelines for their management (Table 71).

Table 71: Summary of Consensus Guidelines for Primary Care Providers Limited to the Management of Neurobehavioural Symptoms in Rett Syndrome

Area of assessment	Assessment details	Frequency of assessment
Respiratory	• Screen for awake-disordered breathing (hyperventilating, breath holding, color change) and air swallowing	Annual wellness visit
Neurology	• Encourage follow-up with neurologist routinely; every 6 months if treated for seizures • Screen for abnormal movements (stereotypies and dystonia) and level of impact on daily activities	Annual wellness visit Primary care every 6 months is recommended to screen for issues that can appear quickly, progress rapidly, and require intervention
Developmental milestones	• Developmental regression (reduced hand use and language) typically stops between 2 and 3 years • Documentation of baseline, gains and losses of milestones • Fine motor: hand use (raking grasp, pincer grasp, rake, holding cup or spoon) • Gross motor: sitting, standing, and walking • Language: coo, babble, laugh, words • Engagement of regular and appropriate educational and specific therapies (physical, occupational, and speech)	Annual wellness visit
Communication	• Screen communication methods used by family and school: eye pointing, vocalizations, switches, iPad, eye gaze device	Annual wellness visit
Psychological/ behavioral	• Screen for symptoms of anxiety and depression, such as withdrawal, screaming, and irritability • These may become more prominent with age or in individuals with milder clinical presentations • Behavioral inconsistency is typical and may be affected by physical factors such as sleep or environment; assess for intolerance of excessive stimuli (i.e., bright lights, loud noises) • Enquire about sensory processing difficulties	Annual wellness visit Primary care every 6 months is recommended to screen for issues that can appear quickly, progress rapidly, and require intervention
Sleep	• Review sleep initiation, staying asleep, snoring or coughing, and frequency of nocturnal interventions by caregivers • Review safety of bed and bedroom • Consider laboratory evaluation for iron deficiency if concerns arise about disrupted sleep or restless leg syndrome: ferritin, serum iron, total iron binding capacity, transferrin	Annual wellness visit Primary care every 6 months is recommended to screen for issues that can appear quickly, progress rapidly, and require intervention

Source: Percy et al (2023) adapted from Fu et al (2020), which provides the full list of consensus guidelines
Note: Consensus guidelines on managing Rett syndrome across the lifespan by Fu C, Armstrong D, Marsh E, et al. is licensed under CCBY-NC4.0, published by BMJ2020

Post-regression considerations

The proposed narrowed indication limits the population to adult and paediatric patients with RTT 5 years of age and older.

Rett syndrome is characterized by an initial period of apparently normal early development followed by a distinct phase of developmental regression, which represents a defining feature of the disorder. Across classic and atypical forms, regression most commonly occurs between 6 and 30 months of age, with peak onset reported during the second year of life, as reported consistently in the literature (Hagberg and Witt-Engerstrom 1986; Gold et al 2024; Neul and Chang 2022; Neul et al. 2014; Tarquinio et al. 2015). In data from 250 patients in the Australian Rett Syndrome Database and the International Rett Syndrome Phenotype Database, the mean age at regression was 19.3 months (95% CI, 18.3–20.4), ranging from 3 months to 4 years (Fehr et al. 2011). If there is no regression by the age of 5, the diagnosis of RTT should be questioned.

The eligibility criteria for study 003 required that patients be post-regression; this was for methodological reasons and was done from an abundance of caution to avoid confounders in interpreting efficacy data. It is of note that there were no screen failures due to the regression criteria, further supporting the idea that patients in this age range are in the plateau phase.

No worsening of Rett symptoms with trofinetide

Within the context of the narrowed proposed indication focusing on neurobehavioral manifestations of RTT, conversely there was no evidence of deterioration of other manifestations of RTT (i.e., non-neurobehavioural manifestations did not get worse while treating the neurobehavioural manifestations). The secondary endpoint CGI-S in Study 003 did not show a treatment difference (Section 5.1.1.7), indicating that no change in the overall severity of RTT was observed in study subjects.

The CGI-I results from the clinical trials, both in the original analysis and when we use response criteria, support the conclusion that overall RTT got better and certainly did not worsen while the RSBQ supports the benefit on neurobehavioral manifestations.

This is supported by data from the pooled datasets described in the Summary of Clinical Safety and additionally by our postmarketing experience in over 2000 patients since becoming commercially available 17 April 2023.

Seizures

Seizures and epilepsy are commonly experienced at some point in patients with RTT but are not always present. The age of onset is variable. In data from the Rett Natural History Study, onset of first seizure ranged from immediately after birth to 46.8 years in classic RTT. Age of onset increased steeply during mid-childhood and then began to plateau: 25% had epilepsy by age 3.0 years, 50% by 5.9 years, and 75% by 13.2 years (Tarquinio et al. 2017). While the estimated cumulative lifetime prevalence in RTT approaches 90%, the point prevalence of seizures ranged from 30% to 44% (Tarquinio et al. 2017).

While seizures are a common comorbidity, they are not present in all patients and are not required for the diagnosis of RTT (Neul et al. 2010). In the trofinetide studies, subjects were not required to have a history of seizures or to have had recent seizures. In Study 003, 67.7% (63/93) of subjects on trofinetide had medical history of seizures or epilepsy versus 70.2% (66/94) of subjects on placebo.

Seizures were one of the domains on which the Rett-specific CGI was developed (Neul et al. 2015). Assessment of the effect of trofinetide on seizures was included as part of the coprimary endpoint, the CGI-I, in the trofinetide clinical studies. The clinical studies did not include a separate efficacy endpoint for seizure, but the potential negative effects of trofinetide were part of the safety evaluation.

A majority of subjects (128 of 187; 68.4% of the Safety Analysis Set) received at least one concomitant anti-seizure drug during Study 003 . However, only 13 subjects (7.0%) (8 [8.6%] on trofinetide, 5 [5.3%] on placebo) received a new concomitant anti-seizure drug after study start. Only 1 subject (on placebo) received their first anti-seizure drug after their first dose of study drug in Study 003, meaning 12 of the 13 subjects who initiated an anti-seizure drug during Study 003 were already receiving at least one other anti-seizure drug. Only 3 subjects on trofinetide (3.2%) received an increased dose of concomitant anti-seizure drug during the study compared with 6 (6.4%) on placebo. There is no observable pattern of new or increased concomitant anti-seizure drug use during Study 003, indicating that there was no worsening of seizures within the study.

The exposure-response analysis for TEAES of seizure also concluded that none of the exposure measures evaluated were found to be a statistically significant predictor of the probably of a TEAE of seizure; therefore, the incidence of seizure was not considered related to trofinetide exposure. In addition, findings from nonclinical safety pharmacology and repeat dose toxicity studies of trofinetide did not demonstrate any relevant CNS-related effects.

In Study 003, 8 (8.6%) subjects on trofinetide had TEAEs of seizure, and 6 subjects (6.4%) on placebo reported TEAEs of seizure or partial seizures (the term partial seizures increases the number of subjects on placebo by 1). Seven of 8 subjects on trofinetide and 5 of 6 subjects on placebo had

history of epilepsy/seizures. While there is a slight numerical imbalance, these data do not support the conclusion that there is a causal relationship between trofinetide and seizures. Nevertheless, seizure is included as a common adverse drug reaction in the SmPC. The selection of adverse drug reactions (ADRs) takes into account not only the incidence of events, but also the clinical relevance and (potential) event severity. Given the clinical relevance of seizures in RTT, and the inclusion of seizure as an ADR in other approved trofinetide global labels, the Applicant has included seizure in the proposed list of ADRs in the SmPC. However, there is no observable evidence of a risk of increased seizure that is plausibly related to trofinetide.

Data regarding the frequency and severity of seizures has been incorporated in the SmPC and the issue was considered resolved in previous assessments.

5.1.5. CHMP position on the Ground for re-examination #2:

As stated in the submitted Re-examination PI, the Applicant amended the indication from

treatment of Rett syndrome in adults and paediatric patients 2 years of age and older

to (changes highlighted in **bold**)

*treatment of **neurobehavioural manifestations of** Rett syndrome in adults and paediatric patients **aged 5** years and older **(see Section 5.1)**.*

Target Population

With the proposed change of including only patients from an age of 5 years, the proposed target population is better aligned with the study population of pivotal study 003, which comprised female patients with Rett syndrome 5-20 years of age, who were required to be in the plateau phase of their disease (defined as having no loss or degradation within 6 months of Screening of ambulation, hand function, speech, or nonverbal communicative or social skills).

It is agreed that in Rett syndrome regression typically occurs up to the age of 5 years. The Applicant also noted that there were no screen failures due to not being in the plateau phase of the disease, supporting this rationale. Furthermore, the SAG-N experts agreed by consensus that the proposed restriction to patients with Rett Syndrome, aged 5 years and older is a well-defined patient population that can be recognised in the clinical practice.

With the inclusion of only patients older than 5 years of age, the target population is in better alignment with the study population, and it is considered highly unlikely that patients will still be in the regression phase. At the initial MAA it was concluded that only very limited data is available in patients **above the age of 20 years**, and that there was no confirmation of efficacy in this age group. It was also discussed that in principle there is no reason to discontinue trofinetide treatment in patients who become > 20 years if they benefit from the treatment. This view is upheld. It was further discussed at the initial MAA whether **male patients** may be treated with trofinetide, as male patients were not enrolled in the clinical programme. While it is known that male patients with Rett syndrome generally have a more severe phenotype, there is also overlap in symptomatology with female patients. Moreover, from a pragmatic perspective, there is no need to restrict the indication to female patients and the same holds also for patients >20 years at the time of treatment initiation. This view is also upheld.

In summary, extrapolation to older (>20 years) and male patients is agreed to.

Neurobehavioural manifestations

The Applicant further acknowledges that in the clinical development program of trofinetide, key clinical symptoms (including seizures) of Rett syndrome were not assessed separately and therefore proposes to narrow the indication to 'treatment of **neurobehavioral manifestations of** Rett syndrome'.

As discussed under GfR#1, the proposed indication was further amended upon request to replace 'neurobehavioural manifestations' with 'neurobehavioural symptoms' for a clear symptomatic claim.

General comments on the SmPC

The purpose of the cross-reference to section 5.1 is unclear. As outlined in the *Guide to Assessors on the Wording of the Therapeutic Indication*, the target population should be defined in section 4.1. The guide further states that any supplementary data provided in section 5.1 is intended solely to offer additional scientific context supporting the indication described in section 4.1; it cannot constitute a new indication, nor can it be interpreted as a restriction of the authorised indication (e.g. in relation to population characteristics from clinical trials or potential use in combination therapy). The cross-reference was removed upon request.

CHMP conclusion

By including only patients from 5 years of age, the target population is better aligned with the study population, and it is considered highly unlikely that patients would still be in the regression phase, which was confirmed by the SAG-N experts. The proposed indication was further amended to replace 'neurobehavioural manifestations' with 'neurobehavioural symptoms' for a clear symptomatic claim. The extrapolation to older (>20 years) and male patients can be agreed. The cross-reference to section 5.1. of the SmPC was removed. The point is considered resolved.

SAG-Neurology

Following a request from the applicant at the time of the re-examination, the CHMP convened a Scientific Advisory Group Neurology (SAG-N) meeting inviting the experts to provide their views on specific questions in relation to the CHMP grounds for refusal.

Report from the SAG-N

On June 17, 2026, a SAG Neurology meeting was convened upon request by the Applicant. The following issues were discussed:

1) **Do the experts regard the proposed restriction to patients with Rett syndrome 5 years and older (i.e. in the plateau phase, according to the Applicant) as a well-defined patient population?**

The SAG-N experts agree by consensus that the proposed restriction to patients with Rett Syndrome, aged 5 years and older is a well-defined patient population that can be recognised in the clinical practice. Although SAG-N experts acknowledge some variability on the timing of the plateau phase onset, the SAG-N experts agreed that most of the patients above 5 years of age are in the plateau phase.

2) **Do the experts consider the co-primary endpoints RSBQ and CGI-I to sufficiently comprise neurobehavioural manifestations of the Rett syndrome in the plateau phase? Which subscores of the primary endpoints and secondary endpoints do (additionally) address neurobehavioural manifestations of Rett syndrome?**

The SAG-N experts agree by consensus that the co-primary endpoints Rett Syndrome Behaviour Questionnaire (RSBQ) and Clinical Global Impression of Improvement (CGI-I) sufficiently comprise neurobehavioural manifestations of the Rett syndrome in the plateau phase. While some experts indicate that CGI-I scale may capture symptoms beyond neurobehavioural manifestations, the majority

of the SAG-N experts acknowledge that there is a close interconnection of the symptoms in Rett syndrome, so it is difficult to isolate neurobehavioural manifestations in this condition from the other symptoms. Following this line of argumentation, SAG-N experts agree by consensus that all subscores of the co-primary endpoints except the one(s) quantifying seizures (even if it was discussed that they could eventually impact neurobehavioural manifestations) and all secondary endpoints, capture - to a certain extent -aspects in connection with neurobehavioural manifestations.

3) **Does the SAG consider the effect sizes observed on the co-primary endpoints RSBQ and CGI-I and key secondary endpoint CSBS-IT-DP at week 12 in the study 003 as clinically meaningful for the treatment of neurobehavioural manifestations in patients with Rett syndrome in the plateau phase?**

Few experts indicated their doubts about the validity of the estimates, and indicated a potential for an overestimation of the effect sizes due to the functional unblinding and the unbalanced treatment discontinuation due to adverse events in particular diarrhea. This is an aspect that challenges the validity of the estimates. Despite this, the issue at hand is about the clinical meaningfulness of the effect size. Overall, the SAG-N experts found it difficult to determine the clinical meaningfulness of the observed changes in the mentioned scales, for this population. Some experts were of the opinion that any change -even if small -could positively impact the patient's daily life. Other experts indicated that observed effect sizes are below the minimally clinically important difference (MCID). In particular, the MCID for RSBQ was estimated, by the Applicant using their own data, to be around 5-7 points depending on the study, with a lower border of the 95%CI around these estimates of 3 (based on study 002) or 4 (based on the pivotal study 003). Thus, the observed difference of 3 points is at or below the lower level of this range. The SAG members acknowledged the methodological shortcomings in the approach to determine the MCID within the same study population. Furthermore, the 1-point change observed in the Communication and Symbolic Behavior Scales–Developmental Profile: Infant-Toddler Checklist (CSBS-IT-DP) scale was considered a small change and probably not clinically meaningful. It was discussed that other secondary outcomes, which all address symptoms in the same domains as the RSBQ, did not show nominally statistically significant differences, except for one (out of eight). One patient's representative acknowledged that the absence of impact on the quality-of-life (one of the secondary outcomes) adds uncertainty about whether the differences on the primary outcomes could be truly considered as clinically meaningful.

4) **How would the experts expect untreated patients in the plateau phase to develop over a period of 24 months? Specifically, is the RSBQ total score and the CGI-I score after 24 months precisely predictable following such a period, or are there time-course variables dependent on known or unknown patient-related, environmental or other characteristics?**

Overall, SAG-N experts agree that the RSBQ total score and the CGI-I score after 24 months is on average – i.e. on a group level- more or less stable in the plateau phase, based on available data from an Australian cohort study. However, both experts and patient's representatives noted that individual fluctuations are expected based on different factors including environmental ones. Patient representatives considered the disease particularly unstable and unpredictable, with fluctuations over time, depending also on external factors. One patient representative mentioned that in her experience some children among the higher functioning Rett patients regain skills later in their life.

Overall conclusion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant and considered the views of the Scientific Advisory Group and the input from third party

interventions.

The CHMP consider the grounds as resolved and agrees that the benefit risk is positive for the treatment of neurobehavioural symptoms of Rett syndrome in adults and paediatric patients aged 5 years and older.

5.2. Pharmacovigilance

5.2.1. Risk Management Plan

5.2.1.1. Safety concerns

Summary of Safety Concerns	
Important identified risks	None
Important potential risks	None
Missing information	None

5.2.1.2. Pharmacovigilance plan

No pharmacovigilance activities beyond signal management and reporting of adverse reactions are proposed, which is endorsed.

5.2.1.3. Risk minimisation measures

No additional risk minimisation measures are proposed, which is endorsed.

5.2.1.4. Conclusion

The CHMP considered that the risk management plan version 1.0 is acceptable.

The Applicant is reminded that in case of a Positive Opinion, the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of Personal Data (PD) and identification of Commercially Confidential Information (CCI) in any updated RMP submitted throughout this procedure.

5.2.2. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

5.2.3. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 10.03.2023. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

5.3. Product information

5.3.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the Guideline on the readability of the label and package leaflet of medicinal products for human use.

5.3.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Daybu (trofinetide) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

6. Benefit-risk balance following re-examination

6.1. Therapeutic Context

6.1.1. Disease or condition

Rett syndrome (RTT) is a debilitating neurodevelopmental disorder. While the great majority of patients with RTT are females, males who meet the criteria for RTT have also been identified (Neul et al., 2019).

The incidence of RTT is reported as approximately 1 in 10,000 female live births worldwide (Petriti 2023).

In approximately 96% to 98% of patients diagnosed with classic RTT, the disease is caused by mutations in the X-linked methyl-CpG-binding protein 2 gene (MECP2) (Percy et al., 2018). MECP2 encodes human methyl-CpG-binding protein 2 (MeCP2), which modulates gene expression by binding to methylated CpG dinucleotides, primarily by activating but also by repressing transcription (Hite et al., 2009; Ip et al., 2018; Kriaucionis et al., 2003; Neul et al., 2008). The activity of the MeCP2 protein is diminished in RTT in both neurons and astrocytes (Yasui et al., 2013).

In patients with typical RTT, there is seemingly normal psychomotor development for the first 6 months of life but thereafter, the failure to reach normal developmental milestones can be observed, followed by a period of developmental regression in which there is a loss of normal use of the hands and of spoken language (Neul et al., 2014; Samaco et al., 2011).

Rett syndrome is defined by characteristic clinical features, which typically include loss of verbal and nonverbal communication and voluntary motor function, characteristic repetitive hand stereotypies, and gait problems. Loss of purposeful hand use is a defining aspect of RTT during the onset of regression, and at least 30% of individuals remain without any type of purposeful hand use (Downs et al., 2010). Other commonly observed symptoms include seizures, awake breathing disruptions, scoliosis, and intense eye communication (Neul et al., 2010). Seizures have a lifetime prevalence in

RTT of around 90%. The course of seizure occurrence and remission is highly variable, with the most common pattern being waxing and waning over the lifespan. Gastrointestinal dysfunction, including constipation, gastroesophageal reflux disease, and chewing and swallowing difficulties, are observed in most patients with RTT (Baikie et al., 2014; Motil et al., 2012). Aspiration, aspiration pneumonia and co-occurring morbidities are not uncommon as are other infectious complications that can arise from a relatively immobile dependent state (Ramirez et al., 2020). Autonomic manifestations, which include abnormalities in cardiac and respiratory function, as well as in peripheral circulation, are considered to be predominantly of central nervous system (CNS) origin. Rett syndrome is also characterized by impaired growth affecting the brain and other organ systems.

6.1.2. Available therapies and unmet medical need

There are no therapies in the European Union approved specifically for the treatment of RTT. Treatment focuses on the management of each patient's symptoms and comorbidities.

The incidence of RTT is reported as approximately 1 in 10,000 female live births worldwide (Petriti 2023). There is an unmet medical need for treatments that target multiple aspects of the syndrome and improve overall systemic function.

The claimed indication - as amended during the re-examination is: *Daybu is indicated for the treatment of neurobehavioural symptoms of Rett syndrome in adults and paediatric patients aged 5 years and older.*

6.1.3. Main clinical studies

The main efficacy and safety data are from pivotal **study 003**, a randomised, double-blind, placebo-controlled study in girls and women with RTT aged 5 to 20 years post regression with a confirmed MECP2 mutation. Post regression was defined as having no loss or degradation within 6 months of Screening of ambulation, hand function, speech, or nonverbal communicative or social skills.

The double-blind treatment period was 12 weeks and subjects were randomized 1:1 to trofinetide (n = 93) and placebo (n = 94). Treatments were administered BID based on weight-banded dosing (6g [12 – 20 kg], 8g [>20 – 35 kg], 10g [>35 – 50 kg] and 12g [>50 kg]).

Two uncontrolled open-label extension studies provided longer follow-up data: Study 004 (n=154) up to 40 weeks, and Study 005 (n=77) up to an additional 32 months. Additional supportive studies were RETT-001, RETT-002 and study 009.

Safety data from studies 003, 004, 005 were pooled in the RTTLT Pool (n=178), for evaluation of long-term safety.

6.2. Favourable effects

After 12 weeks of trofinetide treatment a statistically significant difference was observed on the co-primary endpoints RSBQ total score and CGI-I. The least square mean (LSM) treatment difference was -3.1 for the RSBQ (95% CI [-5.7, -0.6], p= 0.0175) and -0.3 (95% CI [-0.5, -0.1], p= 0.0030) for the CGI-I.

On the key secondary endpoint CSBS-DP-IT Social Composite Score a statistically significant difference was observed in favour of trofinetide. The LSM difference was 1.0 (95% CI [0.3, 1.7], p=0.0064).

The percentage of RSBQ responders in post hoc analyses, where a response is defined by a >=3-point, >=6-point, >=9-point or >=12-point reduction from baseline at week 12, respectively, was 45.1% (41/91), 35.2% (32/91), 27.5% (25/91), and 20.9% (19/91) in the trofinetide group compared to 39.8% (37/93), 20.4% (19/93), 11.8% (11/93), and 9.7% (9/93) in the placebo group. The difference was nominally statistically significant for >=6-point, >=9-point and >=12-point reductions.

The percentage of CGI-I responders in a post hoc analysis, where a response is defined by having any improvement on the CGI-I (i.e., score 1, 2, or 3 at week 12), was 14% (13/93) in the placebo group compared to 32% (29/91) in the trofinetide group (odds ratio [95% CI]: 2.9 [1.4, 6.1]; p=0.0042).

6.3. Uncertainties and limitations about favourable effects

There is no well-established MCID in this condition for RSBQ. The proposed anchor-based derivation of an MCID for RSBQ using CGI-I as anchor suffers from an unclear relation of the score to the anchor, and the use of data from the same study to derive and apply the MCID is a further limitation.

The observed reduction in RSBQ score of 3.1 indicates a borderline clinically relevant result, and while any change on the CGI-I is intrinsically meaningful, a mean difference of -0.3 points is of limited magnitude.

Aside from the key secondary endpoint CSBS-DP-IT, none of the other secondary or exploratory endpoints have been evaluated in a confirmatory manner as the Type-I error strategy had not foreseen testing of other endpoints following the key secondary endpoint. Secondary outcomes addressing symptoms in the same domains as the RSBQ, did not show nominally statistically significant differences, except for the RTT-COMC (Rett Syndrome Clinician Rating of Ability to Communicate Choices). For other endpoints small numerical differences were observed (without nominal statistical significance) at best, and the clinical interpretation of these remains limited.

The double-blind phase of 12 weeks is too short to draw a conclusion on a long-term treatment effect. In addition, maintenance of effect of trofinetide has not been established in controlled studies of adequate duration. The open-label extension study 004 (40 weeks) and subsequent study 005 (until FDA approval) cannot be used to substantiate trofinetide long-term efficacy/maintenance of effect. Both studies lack a control arm for comparison. Subjects for whom a benefit is perceived are more likely to continue treatment, thus selection bias cannot be excluded. In study 004, almost half of the subjects prematurely discontinued due to the occurrence of adverse events, hampering interpretation of long-term effect. However, short-term efficacy data are considered sufficient to conclude benefit in the treatment of neurobehavioural symptoms of Rett syndrome. Therefore, continued benefit of treatment should be regularly re-assessed (SmPC 4.2).

Patients in the pivotal study had to have a confirmed *MECP2* mutation, and there is heterogeneity in phenotype between different *MECP2* mutations, so there might be in principle uncertainty about the effect in specific genetically-defined subgroups. However, considering the symptomatic mechanism of action of trofinetide, no concerns regarding efficacy across genetic variants within Rett syndrome (including atypical Rett syndrome) are expected.

The target population in the proposed indication are RTT patients from 5 years of age and older. Study 003 evaluated trofinetide in girls and females with RTT aged 5 – 20 years post regression (i.e. plateau phase). It is agreed that in Rett syndrome regression typically occurs up to the age of 5 years. Thus, by including patients from 5 years of age, it is considered highly unlikely that patients in the regression phase would be treated. The SAG-N experts confirmed this view.

Proof of concept study RETT-001 was the only study which evaluated trofinetide in older adult women with RTT (16 – 45 years old). The study provides only limited support for efficacy in this age group, as

the doses of trofinetide evaluated were substantially lower than the final proposed posology and the total treatment duration was only 4 weeks. Results suggested efficacy of trofinetide. RSBQ was not included in this study, but there is no reason to assume that treatment of behavioural symptoms in patients > 20 years is less efficacious compared to starting treatment in patients < 20 years. Hence, there is no reason to discontinue trofinetide treatment in patients who become > 20 years if they were benefiting from treatment already, and patients can also be initiated after 20 years.

Finally, none of the clinical studies evaluated trofinetide in male patients with RTT. There are very limited data of male RTT patients who initiated trofinetide treatment in the ongoing RWE Lotus study, precluding any conclusions with respect to efficacy. However, from a pragmatic perspective, given the rarity of RTT in males, overlap in symptomatology and application of the same diagnostic criteria, CHMP considers that restriction of the indication to exclude male patients is not necessary.

6.4. Unfavourable effects

Study 003: In total 93% of patients with trofinetide versus 54% with placebo experienced any TEAE; 3% in both groups experienced any serious TEAE; AEs were a reason for discontinuation in 17% with trofinetide and 2% with placebo.

RTTLT: In total 97% of patients experienced any TEAE; and 22% experienced any serious TEAE; AEs were a reason for discontinuation in 43% of patients.

The most common TEAEs (trofinetide vs placebo) in Study 003 included: diarrhoea (81% vs 19%), vomiting (27% vs 10%), seizures (9% vs 5%), pyrexia (9% vs 4%), decreased appetite (5% vs 2%), and irritability (7% vs 0%).

Diarrhoea and vomiting

In total 41% of patients with trofinetide had a TEAE of mild diarrhoea, 45% moderate diarrhoea, and 2% severe diarrhoea. In 13% of patients with trofinetide, diarrhoea led to treatment discontinuation.

In total 19% of patients with trofinetide had a TEAE of mild vomiting, 7% moderate vomiting, and 1% severe vomiting. In 1% of patients with trofinetide, vomiting led to treatment discontinuation.

Diarrhoea and vomiting were also the most common TEAEs in the RTTLT Pool (occurring in 85% and 37% of patients), and led to discontinuation in 26% and 7%, respectively. Diarrhoea was persistent/recurrent in 53% of patients.

Weight loss

A decrease in bodyweight (≥7% from baseline) was observed in 13% of patients in the trofinetide group vs 5% in placebo; 3% of patients in the trofinetide group had a concurrent AE of weight loss vs none of the patients in the placebo group. In the RTTLT Pool 22% of patients had a decrease in bodyweight; 8% had an AE of weight decreased.

AEs secondary to gastrointestinal AEs

Other AEs that may have occurred as a consequence of diarrhoea and/or vomiting were in Study 003 (incidence in trofinetide vs placebo): dermatitis diaper (4% vs 1%), urinary tract infection (3% vs 3%), aspiration pneumonia (2% vs 0%) and dehydration (1% vs 1%).

In the RTTLT Pool (incidence): urinary tract infection (11%), dehydration and dermatitis diaper (5% each), aspiration pneumonia (3%), aspiration (1%). Also serious cases of UTI (2%), dehydration (2%), aspiration and aspiration pneumonia (1% each) were reported, including 1 fatal case of vomiting leading to aspiration (considered not related by the investigator).

Seizures

In total, 8 (9%) patients with trofinetide reported 11 TEAEs of seizure, and 6 patients (6%) with placebo reported 6 TEAEs of seizure or partial seizures. The majority of these patients had a history of seizures/epilepsy (7 in the trofinetide group vs 5 in the placebo group). Mild seizures occurred in 3 patients in each group, and moderate seizures occurred in 5 patients with trofinetide and 2 patients with placebo. No severe seizures were reported. One patient with trofinetide reported an SAE of seizure. Two patients in the trofinetide group discontinued due to seizures (vs none with placebo).

In the RTTLT Pool 15% of patients had an AE of seizure (24/28 had a history of seizures/epilepsy); 4% of patients discontinued due to seizure or seizure cluster. There was 1 fatal case of sudden unexplained death in epilepsy.

6.5. Uncertainties and limitations about unfavourable effects

The main uncertainty is the tolerability of trofinetide and the impact of common gastrointestinal AEs (diarrhoea and vomiting) on the overall health and quality of life of patients with RTT. Diarrhoea and vomiting were generally not severe or serious, and vomiting led to discontinuation in a limited number of patients. Yet, there are potential (serious) consequences for the overall health of patients, including weight loss, and other AEs that may develop secondary to diarrhoea or vomiting (e.g., aspiration, dehydration, UTI, dermatitis diaper). A recommendation to interrupt, modify, or discontinue treatment in case of tolerability issues was included in the proposed SmPC (sections 4.2 and 4.4). Further, a warning for the risk of aspiration following vomiting, and a recommendation to monitor patients' weight were included (proposed SmPC section 4.4).

Discontinuations due to AEs were high as well as the number of patients needing dose modifications. The impact of dose modifications on the efficacy of trofinetide remains uncertain, and the Applicant addressed this in the proposed SmPC section 4.2, together with the recommendation to consider reinstatement of the recommended dose based on clinical judgement. In addition seizures were reported as an AE, however considering that seizures are a feature of the disease, there is uncertainty about the potential effect of trofinetide on aggravating seizures in some patients with RTT.

6.6. Effects Table

Table 72: Effects Table for Daybu in the treatment of neurobehavioural symptoms of Rett Syndrome

Effect	Short Description	Unit	Trofinetide 200-500 mg/kg	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
RSBQ (co-primary)	Change from baseline in Rett Syndrome Behaviour Questionnaire at week 12	LSM (SE)	-4.9 (0.94)	-1.7 (0.90)	**SoE**: LSM difference -3.1 (p= 0.0175) **Unc**: - No MCID established - Study duration too short to establish long-term efficacy / Maintenance of effect not substantiated	ACP-2566-003
CGI-I (co-primary)	Clinical Global Impression of Improvement score at week 12	LSM (SE)	3.5 (0.07)	3.8 (0.07)	**SoE**: LSM difference -0.3 (p= 0.0030) **Unc**: - Less suitable to measure benefit in individual symptoms - Study duration too short to establish long-term efficacy / Maintenance of effect not substantiated	ACP-2566-003
CSBS-DP-IT Social Composite score (key secondary)	Change from baseline in Social Composite Score at week 12	LSM (SE)	-0.1 (0.26)	-1.1 (0.25)	**SoE**: LSM difference 1.0 (p= 0.0064) **Unc**: - Clinical relevance of effect not substantiated - No support from secondary endpoints that contextualise results - Study duration too short to establish long-term efficacy / Maintenance of effect not substantiated	ACP-2566-003
Unfavourable Effects						
Diarrhoea	Incidence	%	81	19	**SoE**: high frequency; most common reason for discontinuation (13%); reason for dose modifications (exact % not reported); Consistent effect in RTTLT Pool; persistent/recurrent (53% in RTTLT Pool)	ACP-2566-003
Vomiting	Incidence	%	27	10	**SoE:** high frequency; consistent effect in RTTLT with second most common reason for discontinuation (7%). **Unc:** low discontinuation rate (1%) in Study 003.	ACP-2566-003

Effect	Short Description	Unit	Trofinetide 200-500 mg/kg	Placebo	Uncertainties/ Strength of evidence	References
Weight loss	Overall postbaseline decrease in weight ≥7%	%	13	5	**SoE:** Consistent effect in RTTLT Pool (22%); potential temporal relationship with diarrhoea **Unc:** low number of TEAEs weight loss (n=3 considered underreported); potential for simultaneous increase in growth/weight related to MoA and/or impact on growth factors	ACP-2566-003
Seizures (incl. partial seizures)	Incidence	%	9	6	**SoC:** occurred mainly in patients with history of seizures. **Unc:** manifestation of RTT	ACP-2566-003

Abbreviations: CGI-I: Clinical Global Impression – Improvement, CSBS-DP-IT: Communication and Symbolic Behavior Scales, RSBQ: Rett Syndrome Behaviour Questionnaire, SoE: strength of evidence, Unc: uncertainty
Notes: % of incidence of unfavourable effects rounded.

6.7. Benefit-risk assessment and discussion

6.7.1. Importance of favourable and unfavourable effects

Favourable effects

There are currently no treatments approved specifically for Rett Syndrome in the EU. Available treatment options focus on the management of each patient's symptoms and comorbidities.

The single randomized, double-blind, placebo-controlled pivotal trial (Study 003) met its primary objective. Both co-primary endpoints RSBQ and CGI-I showed a statistically significant benefit of trofinetide over placebo at week 12. While the RSBQ specifically aims to assess behavioural aspects of the disease, the CGI-I also measures benefit in aspects not included in the proposed indication *treatment of neurobehavioural symptoms of Rett syndrome*. No MCID is established in Rett Syndrome for the co-primary endpoint RSBQ, but the effect size of RSBQ (-3.1 points) is at the lower end of a range of clinical relevance proposed by the Applicant (range: 3-7 points), where the derivation of the range itself is associated with uncertainties. Since all RSBQ domains favoured trofinetide, with nominal statistical significance in 2 of 8 individual domains, the observed treatment difference in RSBQ indicates small but consistent improvements in treating behavioural symptoms of Rett syndrome. Efficacy of trofinetide is supported by the co-primary endpoint CGI-I and key secondary endpoint CSBS-DP-IT Social composite score, which both showed a statistically significant difference in favour of trofinetide, but again with minimal clinical relevance. With the exception of one (RTT-COMC), none of the other secondary endpoints were nominally statistically significant in favour of trofinetide to support the co-primary endpoints although directional consistency with the co-primary outcomes is noted in the secondary outcomes (nominally significant improvement in one secondary endpoint, non-significant improvement in five secondary endpoints and no difference up to rounding in two secondary endpoints). Overall, findings on secondary endpoints are numerically small, but though the relevance of these differences has not been substantiated, their results are seen in line with co-primary and key secondary endpoints, rather than contradicting the outcome of small improvements in treating behavioural symptoms of Rett syndrome.

A SAG-N meeting convened on 17 June 2026 confirmed by consensus that all subscores of the co-primary endpoints except the one(s) quantifying seizures (even if they could eventually impact neurobehavioural manifestations) and all secondary endpoints, capture – to a certain extent – aspects in connection with neurobehavioural manifestations or symptoms. Overall, the SAG-N experts found it difficult to determine the clinical meaningfulness of the observed changes in the mentioned scales. Some experts were of the opinion that any change – even if small – can positively impact the patient's daily life, while other experts indicated that observed effect sizes are at or below the MCID. Some of the third-party interventions also presented patients' positive view on the subjective meaningfulness of the changes observed.

Unfavourable effects

The most important unfavourable effects to consider are diarrhoea and vomiting. Although these AEs were generally not severe or serious, their very high frequency makes them relevant for the B/R weighing. Diarrhoea frequently led to dose reductions and/or permanent discontinuation, which questions whether patients received the optimal dose of trofinetide, and whether the efficacy and safety results are representative for the recommended dose. Vomiting may put patients at increased risk of aspiration, and diarrhoea may increase the risk of (serious) urinary tract infections. Especially if persistent/recurrent, diarrhoea and vomiting may have a significant impact on the overall health and

daily functioning of patients (and their caregivers). Diarrhoea and vomiting are described as ADR in Section 4.8 and warnings (Section 4.4) are included in the proposed SmPC.

An important unfavourable effect is weight loss, which may have consequences for the overall growth and development with long-term treatment, and is a concern especially in a paediatric population, already at risk of growth failure. More patients with trofinetide lost weight compared to placebo, and weight loss was a reason for discontinuation. It remains uncertain whether this is solely due to gastrointestinal AEs. Weight loss is included as an ADR in Section 4.8 of the proposed SmPC. A warning (Section 4.4) to monitor bodyweight was also included in the proposed SmPC.

Another consequence of gastrointestinal AEs may include dehydration and aspiration. To minimize the risk for serious consequence (incl. potentially life-threatening), a warning (Section 4.4) for the risk of aspiration in the context of vomiting was included in the proposed SmPC.

Seizures led to discontinuation and were severe in some cases (incl. 1 fatal case). Although there was a slight numerical imbalance of patients with TEAE of seizures or partial seizures in study 003 (8 [8.6%] trofinetide vs 6 [6.4%] placebo), all except one patient in each treatment arm experiencing TEAE of seizure had a history of epilepsy/seizures, indicating no worsening of seizures with trofinetide treatment, moreover a potential mechanism how trofinetide may aggravate the risk of seizures is not known. Furthermore, with trofinetide treatment there is no indication of worsening of CGI-I, which included seizures as part of the efficacy evaluation. Seizures are described as ADR in Section 4.8 of the proposed SmPC.

6.7.2. Balance of benefits and risks

Support for the sought indication is primarily based on the pivotal study 003. Statistically significant improvements in the co-primary and key secondary endpoints were shown, and although effect sizes were small, clinical relevance of these is considered appropriately demonstrated, based on the totality of data and partial support from the SAG-N experts. Indeed, numerical improvements have been consistently observed for most tested behavioural symptoms of Rett syndrome. Efficacy is then considered demonstrated for the proposed indication that emphasizes that trofinetide is a *treatment of neurobehavioural symptoms* of Rett syndrome.

The main unfavourable effects of trofinetide (diarrhoea, vomiting and weight loss) are reversible after dose adjustment or discontinuation. Diarrhoea and vomiting occurred in the vast majority of patients and frequently led to dose modification and/or discontinuation. Persistent gastrointestinal AEs may have (serious) consequences for the overall health (incl. weight loss, aspiration), thriving and quality of life of patients and caregivers. Warnings and recommendations for dose adjustment were included in the proposed SmPC as appropriate. Thus, the safety profile of trofinetide is acceptable, as it is likely manageable with dose adjustment and/or discontinuation.

In the context of a rare, complex, devastating, lifelong disorder such as Rett syndrome, even minimal behavioural improvements can be clinically meaningful. The successful randomized controlled trial and supportive data together are sufficient to conclude that efficacy in the treatment of behavioural symptoms has been demonstrated. Given the manageable safety profile, this supports a positive B/R balance.

The **benefit/risk** of trofinetide is considered **positive**.

6.7.3. Additional considerations on the benefit-risk balance

Third Party Interventions

In relation to this re-examination procedure, the CHMP received several interventions from third parties. The set of documents received comprises letters representing views from learned societies, clinicians and feedback from families, testimonials from families participating in a compassionate use programme, and a list of scientific publications.

The input from the third party interventions was taken into consideration.

SAG-Neurology

On June 17, 2026, a SAG Neurology meeting was convened upon request by the Applicant. See Section 5 for the Final Report.

6.8. Conclusions

The overall benefit/risk balance of Daybu is positive.

Divergent position is appended to this report.

7. Recommendations following re-examination

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the benefit-risk balance of Daybu is favourable in the following indication(s):

Daybu is indicated for the treatment of neurobehavioural symptoms of Rett syndrome in adults and paediatric patients aged 5 years and older.lThe CHMP, therefore, recommends the granting of the marketing authorisation subject to the conditions described in the following sections.

Divergent position(s)

Divergent position to the majority recommendation on Benefit/risk is appended to this report.

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

Periodic safety update reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

Risk management plan (RMP)

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

At the request of the European Medicines Agency;

Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

7.1.1. Considerations related to paediatrics

The requirements for the submitted dossier in relation to paediatrics are described in section 1.3 of this report.

In this procedure, The CHMP reviewed the available data from the studies conducted in accordance with the agreed PIP P/0188/2024, namely: study Study 1 (0621-16031); Study 2 (0621-16032); Study 3 (Neu-2566-RETT-001); Study 4 (Neu-2566-RETT-002); Study 5 (ACP-2566-003); Study 6 (ACP-2566-004); Study 7 (ACP-2566-005); Study 8 (ACP-2566-009); Study 9 (ACP-2566-MS-007, ACP-2566-MS-008, ACP-2566-MS-010, ACP-2566-MS-009). The results of these studies are reflected in section(s) 4.8., 4.9. and 5.1. of the Summary of Product Characteristics (SmPC) and, as appropriate, the Package Leaflet. Further, these results were pivotal in supporting this application.

8. Appendices

8.1. Divergent position to the majority recommendation

The undersigned members of the CHMP did not agree with the CHMP's positive opinion recommending the granting of the marketing authorisation of Daybu indicated for the treatment of neurobehavioural symptoms of Rett syndrome in adults and paediatric patients aged 5 years and older.

The reasons for the divergent opinion are as follows:

Support for the sought indication primarily is based on the single pivotal study 003. While statistically significant improvements in the co-primary and key secondary endpoints were shown, the clinical meaningfulness of the observed effects and consistency across endpoints has not been demonstrated. Most domains explored with the additional secondary endpoints only showed a marginal numerical difference between trofinetide and placebo, despite some of these domains being part of the RSBQ scale. This illustrates the difficulty of interpretation of the RSBQ scale results in particularly in a disease phase (plateau) where also the natural history of the disease is characterized by slight behaviour fluctuations, e.g., crying and irritability and some variability in hand use and communication. The double-blind treatment phase of 12 weeks is considered too short to establish efficacy. Based on the provided data, it is uncertain which symptoms are expected to improve under trofinetide treatment, further questioning the clinical relevance of observed effects in a patient population with a heterogeneous presentation of symptoms within and between individuals.

Moreover, trofinetide is not well tolerated. Gastrointestinal symptoms, such as diarrhoea, vomiting and related weight loss, are the most important unfavourable effects. Diarrhoea and/or vomiting occurred in the vast majority of patients (respectively. 85% and 37% in long-term data), appeared difficult to manage, and frequently led to repeated dose modification and/or discontinuation. These persistent gastrointestinal unfavourable effects may have (serious) consequences for overall health (incl. weight loss, aspiration), thriving,

and quality of life of patients and their caregivers.

Therefore, the undersigning members are of the opinion that the benefit-risk balance for the targeted patients with Rett syndrome is negative.

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Hrefna Guðmundsdóttir