Clinical Study Protocol

NCT Number: NCT04759833

Title: Phase 3, Multicenter, Randomized Study with 2 Different Doses of Prucalopride Administered to Male and Female Pediatric Subjects Aged 6 Months to 17 years with Functional Constipation, Consisting of a 12-week Double-blind, Placebo-controlled Part (Part A) to Evaluate Efficacy and Safety Followed by a 36-week Double-blind Extension Part (Part B) to Document Long-term Safety up to Week 48

Study Number: TAK-555-3010

Document Version and Date: Amendment 3, 27 October 2022

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

TAKEDA PHARMACEUTICALS

PROTOCOL: TAK-555-3010

TITLE: Phase 3, Multicenter, Randomized Study with 2 Different Doses of Prucalopride Administered to Male and Female Pediatric Subjects Aged 6 Months to 17 years with Functional Constipation, Consisting of a 12-week Double-blind, Placebo-controlled Part (Part A) to Evaluate Efficacy and Safety Followed by a 36-week Double-blind Extension Part (Part B) to Document Long-term Safety up to Week 48

SHORT TITLE: Double-blind, Placebo-controlled and Long-term Study Evaluating the Efficacy and Safety of Prucalopride in Pediatric Subjects with Functional Constipation

STUDY PHASE: 3

DRUG: TAK-555, prucalopride succinate

IND NUMBER: 55078

EUDRACT NUMBER: 2022-003221-22

NCT NUMBER: NCT04759833

SPONSOR: Takeda Development Center Americas, Inc. (TDC Americas)
95 Hayden Avenue
Lexington, MA 02421
617-349-0200

PRINCIPAL/ COORDINATING INVESTIGATOR: [redacted], MD
Children's National
Washington, District of Columbia 20010
United States (US)

AMENDMENT HISTORY:

Date	Amendment Number	Type	Region
27 October 2022	Amendment 3	Substantial	Global
20 October 2021	Amendment 2	Non-Substantial	Global
18 December 2020	Amendment 1	Substantial	Global
21 October 2020	Original Protocol	Not applicable	Global

PROTOCOL SIGNATURE PAGE

Sponsor's (Takeda) Approval

Signature: DocuSigned by: [redacted]	**Date:** 28-Oct-2022 | 09:51 EDT
[redacted], MD, MS	

Investigator's Acknowledgement

I have read this protocol for Study TAK-555-3010.

Title: Phase 3, Multicenter, Randomized Study with 2 Different Doses of Prucalopride Administered to Male and Female Pediatric Subjects Aged 6 Months to 17 Years with Functional Constipation, Consisting of a 12-week Double-blind, Placebo-controlled Part (Part A) to Evaluate Efficacy and Safety Followed by a 36-week Double-blind Extension Part (Part B) to Document Long-term Safety up to Week 48

I have fully discussed the objective(s) of this study and the contents of this protocol with the sponsor's representative.

I understand that the information in this protocol is confidential and should not be disclosed, other than to those directly involved in the execution or the scientific/ethical review of the study, without written authorization from the sponsor. It is, however, permissible to provide the information contained herein to a subject in order to obtain their consent to participate.

I agree to conduct this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines on Good Clinical Practice and with the applicable regulatory requirements.

I understand that failure to comply with the requirements of the protocol may lead to the termination of my participation as an investigator for this study.

I understand that the sponsor may decide to suspend or prematurely terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the sponsor.

Investigator Name and Address: (please handprint or type)	

Signature: _____ **Date:** _____

SUMMARY OF CHANGES FROM PREVIOUS PROTOCOL VERSION

Protocol Amendment 3 Summary and Rationale:

This section describes the changes in reference to the protocol incorporating amendment 3. The primary reason for this amendment is to:

- Incorporate changes based on the Food and Drug Administration Advice Letter dated 22 Jan 2022.

In this amendment, minor grammatical, editorial, formatting, and administrative changes not affecting the conduct of the study are included for clarification and administrative purposes only.

Protocol Amendment 3			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
1	Protocol title page	The EudraCT number was added to the protocol title page	Not applicable
2	Protocol Signature Page	Correction of the study title to match the title page of the protocol	Correction
3	Emergency Contact Information	Update of the medical monitor's name and contact details and addition of SAE reporting contact information (as well as removal of language that contact information is located on the forms)	Update after another medical monitor was appointed to the study; addition of safety contact information
4	Section 1.1 Synopsis Section 4.5 Sites and Regions	Update of the number of US sites estimated to participate from 50-60 to 40-45	Update based on more recent projection
5	Section 1.1 Synopsis Section 3.2.4 Exploratory Endpoints Section 9.6.5 Exploratory Efficacy Endpoints	Update of the exploratory endpoints on "proportion of subjects with an average of ≥3 (S)BMs per week and increase of ≥1 (S)BM compared to baseline during a 12-week double-blind, placebo-controlled treatment phase" and "proportion of subjects with an average of ≤1 (S)BMs per week during the 12-week double-blind, placebo-controlled treatment phase" to only evaluate SBMs, not BMs.	As spontaneous bowel movement only accounts for bowel movements without use of rescue medications within the past 24 hours, updating this language in the exploratory endpoints will provide more clinically meaningful results while controlling for the variable "use of rescue medications" as a confounding factor in these analyses.
6	Section 1.1 Synopsis Section 4.4 Duration of Subject Participation and Study Completion Definition	Update of the duration of the study from approximately 60 to approximately 52 months (or from 3-4 to 4-4.5 years)	Update based on more recent projection

Protocol Amendment 3			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
7	Section 1.1 Synopsis Section 1.2 Schema Section 4.1 Overall Design Section 4.2 Scientific Rationale for Study Design Section 4.4 Duration of Subject Participation and Study Completion Definition Section 8.1.1.1 Screening Visit (Visit 1)	Correction of the duration of the screening period from approximately 21 to 33 days (and maximum duration of participation, where mentioned, from 55 to 56 weeks)	Correction to be in line with the current Schedule of Activities
8	Section 1.1 Synopsis Section 5.2 Exclusion Criteria	Clarification of the definition of "current use" to be within 5 days rather than within 5 days prior to the screening visit	Clarification to give subjects the ability to washout during screening following consent
9	Section 1.2 Schema	Addition of information on the dose groups to the study schema	Clarification
10	Section 1.3 Schedule of Activities	Addition of a reminder to check whether the subject has experienced any psychiatric changes throughout the study	Addition reflecting agreement with the FDA on increased SI/SIB monitoring throughout the study.
11	Section 1.3 Schedule of Activities	Removal of the X for targeted physical examination at screening	Correction
12	Section 1.3 Schedule of Activities	Addition of "CBT" (ie, cognitive behavioral therapy) to the line including concomitant surgery and procedures	Addition of a therapy check this is added to the eCRF to capture cognitive behavioral therapy.
13	Section 1.3 Schedule of Activities (schedule and footnote "r")	Addition of the word "optional" for sparse PK sampling	Clarification
14	Section 1.3 Schedule of Activities (footnotes "h" and "i") Section 8.1.1.2 Baseline Visit (Visit 2) Section 8.2.3.1 Physical Examination	The digital rectal, anal and cremasteric reflex, and perianal examinations were made optional	Increasing site flexibility to make these examinations optional at the discretion of the investigator

Protocol Amendment 3			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
15	Section 1.3 Schedule of Activities (footnote "r")	Addition of footnote "c" and text to footnote "t" (Table 1) and footnote "a" (Table 2) to make it clear that all subjects should be instructed to withhold dosing on in-clinic visit days (Part A and Part B) until after their visit. If a subject does take their daily dose of IP prior to the on-site visit, this will not result in a protocol deviation unless the subject is participating in PK sampling at Week 4 (Visit 6), Week 8 (Visit 10), and Week 12 (Visit 14).	Clarification
16	Section 1.3 Schedule of Activities Section 8.1.3.1 Visit 15a, 16, 18, 20, 22 (Weeks 14, 20, 28, 26, and 44) Section 8.1.3.2 Visit 15b, 17, 19, and 21 (Weeks 16, 24, 32, and 40)	Addition of a telephone visit at Week 14. This visit is referred to as Visit 15a; the Week 16 Visit is referred to as Visit 15b	Addition reflecting agreement with the FDA on increased SI/SIB monitoring throughout the study.
17	Section 2.2 Product Background and Clinical Information	Update of the number of countries where prucalopride is authorized and the estimated worldwide patient exposure	Update with the most recent information
18	Section 2.4 Benefit/Risk Assessment	Replacement of the referral to the US package insert was by referral to the prescribing information in general	Update for consistency
19	Section 4.1 Overall Design Section 6.2.3 Dosing	Addition of a reference to the Pharmacy Manual for a table of weight-adjusted dosing	clarification
20	Section 4.1 Overall Design Section 6.2.3 Dosing	Correction of the word "re-randomization" to "randomization" as dose calculation based on body weight is at randomization	Correction
21	Section 5.2 Exclusion Criteria	Addition of a reference to Appendix 7 (Bedside Schwartz Equation) in criterion 8, which mentions the Schwartz equation	Addition for completeness

Protocol Amendment 3			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
22	Section 5.3 Restrictions	Correction of Criterion 2 to indicate that restrictions related to lifestyle modifications (including dietary changes) are applicable from randomization onwards and to include the specification that study-allowed rescue medication is not restricted	Clarification and correction to reflect study practices
23	Section 6.2.3 Dosing	Addition of a sentence to make it clear that on in-clinic visit days (Part A and Part B), all subjects should be instructed to withhold dosing until after the visit.	Clarification
24	Section 6.2.3 Dosing	Addition of a sentence to make it clear that on the day of Visit 14 (Week 12 / End of Part A), IP dose should be withheld until after the visit so the subject will take their first Part B dose on the day of their Week 12 visit	Clarification
25	Section 6.3.1 Labeling	Addition of flexible language related to additional labels (Sites can add information that identifies the study subject by name if allowed per local regulations.)	Increase of flexibility
26	Section 6.3.3 Storage	Clarification of storage information (addition of temperature range for storage)	Clarification
27	Section 6.7.3.1 Rescue Medication	Clarification that rescue medication for children aged 6 months to ≤4 years was at the discretion of the investigator	Clarification
28	Section 6.7.3.1 Rescue Medication Section 5.3 Restrictions Appendix 5 Rescue Medication Algorithm	Addition of a note to clarify that the rescue medication guidelines remain subject to clinical discretion; If the subject has a BM but does not experience relief, additional rescue medication may be warranted.	Clarification
29	Section 6.7.3.1 Rescue Medication	Clarification that rescue medication will be made available throughout the study, but after Baseline Visit 2	Clarification

Protocol Amendment 3			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
30	Section 6.7.3.2 Disimpaction Procedure During Screening Period	Clarification that instructions follow the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition Recommendation 2014; reference to prescribing information for the respective medications was removed	Clarification
31	Section 6.7.3.3 Standard of Care: Behavioral Therapy	Clarification that children are allowed to (re)start toilet training during Part B and that when a child becomes toilet-trained, the site should document the change	Clarification
32	Section 7.6.4 Guidelines for Monitoring/Interruption for Suicidal Ideation/ Suicidal Behavior	Addition of a new section describing what actions should be taken in case of an SI/SIB.	Addition reflecting agreement with the FDA on increased SI/SIB monitoring throughout the study.
33	Section 8.2.2.1 E-Diary	Addition of a statement to indicate that subjects should start completing evening diaries once the 5-day washout period for prohibited therapies has been completed.	Clarification of the start of diary completion
34	Section 8.2.2.2 Impression of Severity Assessment	Addition of a recommendation to complete the PGI-S and CGI-S prior to any other clinical assessments	Clarification of preferred order of assessments
35	Section 9.2.1 DMC	Addition of a reference to Section 7.6.4 Guidelines for Monitoring/Interruption for Suicidal Ideation/ Suicidal Behavior was added as information on referral of SI/SIB to the DMC	Addition reflecting agreement with the FDA on increased SI/SIB monitoring throughout the study.
36	Appendix 3.1 AESI	Clarification that all AESIs, regardless of if they are serious or non-serious are to be reported to Takeda safety	Update to reflect correct practices
37	Appendix 3.2 Collection of Adverse Events	Clarification of the AE follow-up period	Clarification
38	Appendix 3.4 Safety Reporting	Update of report form name; addition of language to refer to the emergency contact information	Update to reflect correct practices and nomenclature
39	Appendix 3.5 SAE Collection Time Frame	Update of report form name; addition of language to refer to the emergency contact information	Update to reflect correct practices and nomenclature

Protocol Amendment 3			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
40	Appendix 3.8 Pregnancy	Update of pregnancy report form name; addition of language to refer to the emergency contact information	Update to reflect correct practices and nomenclature
41	Appendix 3.9 Abuse, Misuse, Overdose and Medication Error	Addition of requirement to submit an SSR form; addition of language to refer to the emergency contact information	Update to reflect correct practices and nomenclature
42	Appendix 8 Site Workflow in Case of SI/SIB Assessment		Addition reflecting agreement with the FDA on increased SI/SIB monitoring throughout the study.

AE=adverse event; AESI=adverse event of special interest; BM=bowel movement; CBT=cognitive behavioral therapy; CGI-S=caregiver global impression of severity; DMC=data monitoring committee; eCRF=electronic case report form; EudraCT=European Union Drug Regulating Authorities Clinical Trials Database; FDA=Food and Drug Administration; IP=investigational product; PGI-S=patient global impression of severity (PGI-S); PK=pharmacokinetic(s); SAE=serious adverse event; SI=suicidal ideation; SIB=suicidal behavior; SSR=Special Situation Report; US=United States

See Appendix 19 for protocol history.

EMERGENCY CONTACT INFORMATION

In the event of a serious adverse event (SAE), the investigator must fax or e-mail the provided SAE form within 24 hours to the Takeda Global Patient Safety Evaluation (GPSE) Department. A copy of this form must also be sent to the contract research organization (CRO)/Takeda medical monitor using the details below.

Email: GPSE@takeda.com
Fax: +1-484-595-8155

For protocol- or safety-related questions or concerns during normal business hours, the investigator must contact the CRO/Takeda medical monitor:

[redacted]
E-mail: [redacted]
Mobile: [redacted]
US toll free: [redacted]

For protocol- or safety-related questions or concerns outside of normal business hours, the investigator must contact the central out-of-hours group:

24 Hour Answering Service for Urgent Medical Issues:
+1 973 659 6677 US 1st number
+1 512 652 0191 US back up number
+33 186 990 019 European Union (EU) number

PRODUCT QUALITY COMPLAINTS

Investigators are required to report investigational product (IP) quality complaints or non-medical complaints to Takeda within 24 hours. If requested, defective product(s) will be returned to the sponsor for inspection and analysis.

A product quality complaint includes any instances where there is an allegation or report relating to Takeda licensed or IPs, received in writing, electronically, or orally, which indicates an impact to a product's strength, identity, safety, purity, or quality, or which suggests that a product did not meet the criteria defined in the regulatory applications, licenses, or marketing authorizations for the product. Examples of IP quality complaints include, but are not limited to, the following:

Unit issues	Capsule fill empty or overage Bottle/vial fill shortage or overage Capsule/tablet damaged/broken Syringe/vial cracked/broken	Syringe leakage Missing components Product discoloration Device malfunction
Labeling	Label missing Leaflet or Instructions For Use (IFU) missing Label illegible	Incomplete, inaccurate, or misleading labeling Lot number or serial number missing
Packaging	Damaged packaging (eg, secondary, primary, bag/pouch) Tampered seals Inadequate or faulty closure	Missing components within package
Foreign material	Contaminated product Particulate in bottle/vial Particulate in packaging	

Please report the product quality complaint via email:

ctmcomplaint@takeda.com

Telephone number (provided for reference if needed): 1-800-828-2088 (US Toll Free)

For instructions on reporting adverse events (AEs) related to product complaints, see Appendix 3.4.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

1. PROTOCOL SUMMARY

1.1 Synopsis

Protocol number: TAK-555-3010	**Drug:** Prucalopride succinate
Title of the study: Phase 3, Multicenter, Randomized Study with 2 Different Doses of Prucalopride Administered to Male and Female Pediatric Subjects Aged 6 Months to 17 Years with Functional Constipation, Consisting of a 12 week Double-blind, Placebo-controlled Part (Part A) to Evaluate Efficacy and Safety Followed by a 36-week Double-blind Extension Part (Part B) to Document Long-term Safety up to Week 48	
Short title: Double-blind, Placebo-controlled and Long-term Study Evaluating the Efficacy and Safety of Prucalopride in Pediatric Subjects with Functional Constipation	
Study phase: 3	
Number of subjects (total and per treatment arm): Approximately 240 toilet-trained subjects (~80 subjects per treatment arm) are planned to be included. In addition, for exploratory purposes, up to 15 non-toilet-trained subjects (~5 subjects per treatment arm) are planned to be included.	
Investigator(s): to be determined	
Site(s) and Region(s): It is anticipated that the study will be conducted at approximately 40-45 sites in the United States (US). Other countries/regions may be added if needed.	
Study period (planned): Approximately 52 months	**Clinical phase:** 3

Objectives:

Primary:

- To evaluate the efficacy of prucalopride during the 12-week double-blind, placebo-controlled treatment phase in toilet-trained subjects with functional constipation who are at least 3 years of age.
- To evaluate the long-term (48 weeks) safety and tolerability of prucalopride in toilet-trained subjects with functional constipation who are at least 3 years of age.

Key Secondary:

- To evaluate the efficacy of prucalopride on signs and symptoms of functional constipation (stool consistency, straining, and stool frequency responder index), during the 12-week double-blind, placebo-controlled treatment phase in toilet-trained subjects with functional constipation who are at least 3 years of age.

Other Secondary:

- To evaluate the effect of prucalopride on the frequency of fecal incontinence.
- To evaluate safety and tolerability of prucalopride over 12 weeks of treatment in toilet-trained subjects who are at least 3 years of age.
- To evaluate the pharmacokinetics (PK) of prucalopride by combining sparse PK blood sampling that will be conducted during the 12-week double-blind, placebo-controlled phase together with the data from previous pediatric studies for further population PK analysis (separate reporting).

Exploratory:

- To further explore the efficacy of prucalopride on signs and symptoms of functional constipation during the 12-week double-blind, placebo-controlled treatment phase in toilet-trained subjects with functional constipation who are at least 3 years of age.
- To explore the efficacy of prucalopride during the 12-week double-blind, placebo-controlled treatment phase in non-toilet-trained subjects with functional constipation who are at least 6 months of age.

- To evaluate the long-term (48 weeks) safety and tolerability of prucalopride in non-toilet-trained subjects with functional constipation who are at least 6 months of age.
- To evaluate safety and tolerability of prucalopride over 12 weeks of treatment in non-toilet-trained subjects with functional constipation who are at least 6 months of age.
- To explore the relationship between PK data and efficacy/safety endpoints of interest.

Rationale:

This study is designed to satisfy the 2 post marketing requirements (PMR 3529-1 and PMR 3529-2/6) as mandated in the new drug application (NDA) approval letter of December 2018 for prucalopride (indicated for the treatment of chronic idiopathic constipation in adults) and to fulfill the Pediatric Research Equity Act (PREA). The FDA agreed to the combination of these 2 PMRs in a post-approval interaction.

Investigational product, dose, and mode of administration:

Dose of prucalopride (oral solution or tablet) or placebo (oral solution or tablet) is dependent on the subject's body weight (BW) at the randomization visit. The investigational product will be provided as oral solution (prucalopride 0.4 mg/mL or placebo) or as tablets (prucalopride 2 mg or placebo).

- Low Dose Group: each subject weighing <50 kg will receive a daily dose of 0.04 mg/kg with the volume of the oral solution based on their individual BW at the randomization visit. These subjects will draw equal volumes from one bottle of 0.4 mg/mL prucalopride oral solution and one bottle of placebo oral solution to account for the daily dose assigned. Subjects weighing ≥50 kg will be dosed with one 2-mg tablet of prucalopride and 1 placebo tablet.
- High Dose Group: each subject weighing <50 kg will receive a daily dose of 0.08 mg/kg with the volume of the oral solution based on their individual BW at the randomization visit. These subjects will draw equal volumes from 2 bottles of 0.4 mg/mL prucalopride oral solution to account for the daily dose assigned. Subjects weighing ≥50 kg will be dosed daily with two 2-mg tablets of prucalopride.
- Matching placebo: Subjects weighing <50 kg will draw equal volumes from 2 bottles of placebo oral solution to account for the daily dose assigned. Subjects weighing ≥50 kg will be dosed daily with 2 tablets of placebo.

Each subject weighing <50 kg at baseline can undergo a dose adjustment for oral solution based on weight at Week 24. In case the subject has crossed the 50-kg threshold, he/she will be switched from oral solution to tablet, provided he/she can swallow the tablet. If the subject cannot swallow the tablet, he/she can receive the tablet dose as oral solution. Depending on the treatment group, subjects cannot exceed the maximum dose of 2 or 4 mg. More detailed information will be provided in study materials.

The color, size, and shape of the prucalopride and placebo tablets are identical, as are the color and taste of prucalopride and placebo bottles and oral solution.

Methodology:

This is a Phase 3, multicenter, randomized study consisting of a 12-week double-blind, placebo-controlled part (Part A) followed by a 36-week double-blind safety extension part (Part B) to document safety and tolerability up to 48 weeks in male and female pediatric and adolescent subjects with functional constipation as defined by the modified Rome IV criteria for child/adolescent Functional Gastrointestinal Disorders (FGID).

The study will start with a 10- to 33-day screening period, including a disimpaction for all subjects, a 12-week double-blind Placebo-controlled Part (Part A) followed by a 36-week long-term double-blind (for dose) Safety Extension Part (Part B), and a follow-up call approximately 4 weeks after the last administration of the IP.

Approximately 240 toilet-trained pediatric/adolescent subjects, ages 3 to 17 years with functional constipation are planned for randomization in a 1:1:1 ratio to the Low Dose Group, High Dose Group, or matching placebo (placebo-controlled part [Part A]). After completion of the 12-week placebo-controlled Part A, subjects in the placebo group will be re-randomized in a 1:1 ratio to the Low Dose Group or the High Dose Group (safety extension part [Part B]). For exploratory purposes, in addition to the 240 toilet-trained pediatric/adolescent subjects who are at least 3 years of age, the study is targeted to enroll a maximum of 15 non-toilet-trained subjects who are at least 6 months of age. These subjects will not be included in the primary analyses; they will be part of the exploratory analyses for descriptive purposes.

The Enrolled Analysis Set will include both toilet-trained and non-toilet-trained subjects. Study enrollment of toilet-trained subjects will be stopped as soon as 240 subjects who are at least 3 years of age are included. If, at that point, less than 15 non-toilet trained subjects who are at least 6 months of age have been included, enrollment in that group will continue.

Randomization at study entry will be stratified by toilet-trained status. Toilet-trained subjects will be additionally stratified by age group (<12 years, 12 to 17 years) and average number of spontaneous bowel movements (SBM)/week (≤1; >1) during the screening period. Non-toilet-trained subjects will be additionally stratified by SBM/week (≤1; >1) during the screening period.

The study completion date is defined as the date the last subject, across all sites, completes his/her final protocol-defined assessment (ie, the follow-up call or end of study visit). The study completion date is used to ascertain timing for study results posting and reporting.

Inclusion and Exclusion Criteria:

Inclusion Criteria:

The subject will be eligible for the study when meeting all of the criteria below:

To be verified at screening:

1. Subjects and/or their parent(s)/caregiver(s)/legally authorized representative(s) have an understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via parent[s]/caregiver[s]/legally authorized representative[s]) informed consent/assent as applicable to participate in the study.

   Note: Subjects and/or parent(s)/caregiver(s)/legally authorized representative(s) (where appropriate depending on age and local regulation) can also provide consent/assent to the PK sparse sampling in this study.
3. Toilet-trained subjects 3 years to 17 years of age, inclusive, or non-toilet-trained subjects 6 months to 17 years of age, inclusive.
4. Subject weighs ≥5.5 kg (12 lbs).
5. Male, or non-pregnant, non-lactating female subjects who are sexually active and agree to comply with the applicable contraceptive requirements of the protocol or females of non-childbearing potential.

   Note: All female subjects ≥12 years and/or female subjects <12 years who have started menarche must have a negative serum pregnancy test at screening.
6. Subject meets modified Rome IV criteria:
   - For child/adolescent (aged >4 years) functional constipation (H3a):

     Subjects must have ≤2 defecations per week and 1 or more of the following occurring at least once per week for a minimum of 1 month:

     a. ≥1 episode of fecal incontinence per week (only for subjects after the acquisition of toileting skills).
     b. History of retentive posturing* or excessive volitional stool retention.
     c. History of painful or hard bowel movements (BMs).
     d. Presence of large fecal mass in rectum.
     e. History of large diameter stools which can obstruct the toilet.

     In addition, the subject does not satisfy sufficient criteria for a diagnosis of irritable bowel syndrome (IBS) and, after appropriate evaluation, the subject's symptoms cannot be fully explained by another medical condition.
   - For infants/toddler (aged 6 months to ≤4 years) functional constipation (G7):

     Subjects must have ≤2 defecations per week and ≥1 month of at least 1 of the following:

     a. History of excessive stool retention
     b. History of painful or hard BMs
     c. History of large-diameter stools (in the diaper)
     d. Presence of a large fecal mass in the rectum

In toilet-trained children, the following additional criteria may be used:

*e. At least 1 episode/week of incontinence** after the acquisition of toileting skills*

f. History of large-diameter stools which may obstruct the toilet

7. Subject and/or parent(s)/caregiver(s)/legally authorized representative(s) is willing to discontinue any laxatives during the screening period up to disimpaction and agrees to adhere to the protocol-specified disimpaction and rescue medication rules, if applicable.

To be evaluated prior to randomization:

8. Subject has an average of <3 SBMs (defecations) per week during the screening period and prior to the disimpaction.
9. Subject or legally authorized representative (dependent on subject age) is compliant with completing the electronic diary for at least 7 consecutive days preceding the disimpaction.

Exclusion Criteria:

The subject will not be eligible for participation in the study if any of the following exclusion criteria are met.

To be evaluated during the screening period:

1. Current or recurrent disease that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessments.
2. Any clinically significant abnormal findings on the electrocardiogram (ECG) that indicates a dysrhythmia or conduction abnormalities (such as abnormal heart rate, PR, QRS, or QT).
3. Major cardiovascular disease such as: cardiomyopathy, cardiac insufficiency, uncorrected congenital heart disease, symptomatic valve disorders, or septal defects.
4. Current or relevant history of physical or psychiatric illness (eg, severe autism, depression, etc), any medical disorder that may require treatment or make the subject unlikely to fully complete the study, or any condition that presents undue risk from the IP or procedures.
5. Non-retentive fecal incontinence*.
6. Intestinal perforation or obstruction due to structural or functional disorder of the gut wall, obstructive ileus, severe inflammatory conditions of the intestinal tract such as Crohn's disease, ulcerative colitis, and toxic megacolon/megarectum.
7. Current use of any medication (including over the counter, herbal, or homeopathic preparations) that could affect (improve or worsen) the condition being studied (eg, opioids), or could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within 5 days.)
8. Subjects with renal impairment:
   a. Subjects ≤2 years of age with serum creatinine greater than normal (screening sample results using central laboratory pediatric reference ranges).
   b. Subjects >2 years of age with severe renal impairment or end stage renal disease (estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m²)
9. Known or suspected intolerance or hypersensitivity to the IP(s), closely related compounds, or any of the stated ingredients.
10. Known history of alcohol or other substance abuse within the last year.
11. Within 30 days prior to the first dose of the IP in the current study:
    - Have used any IP.
    - Have been enrolled in a clinical study (including vaccine studies) that may or may not include the administration of an IP that, in the investigator's opinion, may impact this study.
12. Subject used prucalopride within 10 days prior to the first dose of the IP or has been unsuccessfully treated with prucalopride before.
13. Subject meets Rome IV criteria for other Child/Adolescent FGID (H1 – H2 and H3b).
14. Subject with secondary causes of constipation, eg:

- Endocrine disorders (eg, hypopituitarism, hypothyroidism, hypercalcemia, pheochromocytoma, glucagon-producing tumors) unless these are controlled by appropriate medical therapy. Subject with uncontrolled diabetes mellitus is to be excluded
- Metabolic disorders (eg, porphyria, uremia, hypokalemia, hypothyroidism, amyloid neuropathy), unless controlled by appropriate medical therapy
- Neurological disorders (eg, cerebral tumors, cerebrovascular accidents, multiple sclerosis, meningocele, aganglionosis, hypoganglionosis, hyperganglionosis, autonomic neuropathy, spinal cord injury, Chagas disease)
- Organic disorders (known or suspected) of the large bowel (eg, obstruction from any cause including biliary obstruction, malignancy, intestinal perforation, obstructive ileus, pseudo-obstruction, history of or current anorectal malformations, severe inflammation of the intestinal tract, such as Crohn's disease, ulcerative colitis or toxic megacolon/megarectum, Hirschsprung's disease)
- Celiac disease, cowmilk allergy
- Surgery: history of gastrointestinal surgery related or possibly related to the presence of constipation
- Lactose intolerance

15. Any of the following clinically significant abnormalities of serum biochemistry:
    - Serum aspartate aminotransferase (AST) >1.5 times upper limit of normal (ULN) at screening.
    - Serum alanine aminotransferase (ALT) >1.5 times ULN at screening.
    - Total bilirubin outside the age-adjusted normal range, except for subjects with Gilbert's syndrome.
16. Any significant underlying liver disease.
17. Subject is not able to swallow the IP (liquid or tablet).
18. Subject is pregnant or planning to get pregnant during the study period.

<u>To be evaluated prior to randomization:</u>

19. Subject has used other disimpaction medication in lieu of the protocol-provided medication.
20. Subject has used non-protocol approved medications to induce BMs during the screening period or disimpaction.
21. The subject has failed the disimpaction based on the investigator's assessment.
22. Worsening of depression and emergence of suicidal thoughts.

** Fecal incontinence is defined as unintentional smear or liquid stool in the underwear that is not due to poor wiping. Fecal incontinence can only occur in toilet-trained subjects. Non-retentive fecal incontinence is diagnosed (must include at least a 1-month history in a child with a developmental age older than 4 years for all the following): (i) defecation in places inappropriate to the sociocultural context, (ii) no evidence of fecal retention, and (iii) after appropriate evaluation, the fecal incontinence cannot be explained by another medical condition.*

*** Retentive posturing is defined as the attempt to preserve continence by vigorous contraction of the gluteal muscles. Children with retentive posturing will be typically tight legged, tiptoed, and/or will have a back-arching posture.*

Maximum duration of subject participation in the study:

- Planned duration of screening period: 10 to 33 days
- Planned duration of Placebo-controlled Part (Part A): 12 weeks
- Planned duration of Safety Extension Part (Part B): 36 weeks
- Planned duration of follow-up: 4 weeks

Statistical analysis:

Analysis Sets

- **<u>Toilet-trained subjects who are at least 3 years of age</u>**

- Screened Set: The screened set will consist of all toilet-trained subjects who are at least 3 years of age and who have signed an informed consent form.
- Enrolled Set: The enrolled set will consist of all toilet-trained subjects who are at least 3 years of age and who have signed informed consent and also passed inclusion/exclusion criteria.
- Intent-to-treat (ITT) Analysis Set: The ITT analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age.
- Modified Intent-to-treat (mITT) Analysis Set: The mITT analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age and who receive at least 1 dose of investigational product. The treatment arm will be the arm to which they were randomized, regardless of what treatment they received.
- Safety Analysis Set: The safety analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age and who receive at least 1 dose of investigational product. Subjects will be analyzed according to treatment received.
- Per-protocol (PP) Analysis Set: The PP analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age and who do not have major protocol deviations that may affect the primary efficacy endpoint.
- Completers Analysis Set: The Completers Analysis Set will consist of all toilet-trained subjects who are at least 3 years of age and in the mITT Analysis Set who have completed the daily diary for at least 12 weeks during Part A of the study.

– **Non-toilet-trained subjects**

- Non-toilet-trained Enrolled Set: The enrolled set will consist of all non-toilet-trained subjects who have signed informed consent and also passed inclusion/exclusion criteria.
- Non-toilet-trained Intent-to-treat (NTTITT) Analysis Set: The ITT analysis set will consist of all non-toilet-trained randomized subjects.
- Non-toilet-trained Modified Intent-to-treat (NTTmITT) Analysis Set: The mITT analysis set will consist of all non-toilet-trained randomized subjects who receive at least 1 dose of IP. The treatment arm will be the arm to which they were randomized, regardless of what treatment they received.
- Non-toilet-trained Safety Analysis Set (NTTSAF): The safety analysis set will consist of all non-toilet-trained randomized subjects who receive at least 1 dose of IP. Subjects will be analyzed according to treatment received.

– **PK analysis Set**

- Pharmacokinetic Analysis Set: The Pharmacokinetic Analysis Set is defined as all subjects regardless of age in the Safety Analysis Set and for whom at least 1 PK sample is evaluable.

Study endpoints:

– **Primary Endpoint (toilet-trained subjects who are at least 3 years of age):**

- The average change from baseline in number of SBMs per week derived from the (diary) data over 12 weeks, collected during the placebo-controlled part (Part A).

– **Key Secondary Endpoint (toilet-trained subjects who are at least 3 years of age):**

- The average change from baseline in stool consistency (based on BSFS score), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
- The average change from baseline in straining (based on a 3-point Likert scale, assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
- The proportion of responders with a responder defined as a subject having an increase of ≥1 SBM/week compared to baseline and ≥3 SBMs/week for at least 9 out of the 12 weeks of placebo-controlled part (Part A), including 3 of the last 4 weeks.

– **Other Secondary Endpoints (toilet-trained subjects who are at least 3 years of age):**

- Proportion of subjects with fecal incontinence per week during the 12-week treatment Period.

- **Exploratory Efficacy Endpoints:**
  - Exploratory efficacy endpoints in toilet-trained subjects who are at least 3 years of age:
    - The average change in worst abdominal pain score over the past 24 hours (based on a Wong-Baker faces scale in subjects <8 years and the 11-point NRS in subjects ≥8 years), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
    - Proportion of subjects with an average of ≥3 SBMs per week and increase of ≥1 SBM compared to baseline during the 12-week double-blind, placebo-controlled treatment phase.
    - Proportion of subjects with an average of ≤1 SBMs per week during the 12-week double-blind, placebo-controlled treatment phase.
    - Proportion of subjects with an average of ≥1 day(s) with rescue medication intakes per week.
    - Proportion of subjects assessed with retentive posturing at monthly visits during the 12-week treatment Period.
    - Global evaluation and QoL Health economics and outcomes research endpoints: patient global impression of severity (PGI-S), caregiver global impression of severity (CGI-S), and Pediatric Quality of Life Inventory (PedsQL) Gas and Bloating Domain Gastrointestinal Symptoms Scale (Placebo-controlled Part [Part A]):

    Absolute values and change from baseline.

    For PedQL Gas and Bloating Domain GI Symptoms Scale-SRM: classified as no, small, moderate, and large effect.
    - Proportion of subjects with ≤2 signs/symptoms from the Rome IV Criteria following the 12-week double-blind, placebo-controlled treatment phase.
  - Efficacy endpoints in non-toilet-trained subjects who are at least 6 months of age:
    - Proportion of subjects with an average of ≥3 SBMs per week and increase of ≥1 SBM compared to baseline during the 12-week double-blind, placebo-controlled treatment phase.
    - Number of SBMs per week in categories ≤1 and >1 during the 12-week double-blind, placebo-controlled treatment phase.
    - The average change from baseline in stool consistency (based on BSFS score), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
- **Safety Endpoint:** The proportion of subjects with treatment Emergent Adverse Events (TEAEs) (serious, non-serious, related, non-related) and the proportion of subjects with clinically relevant laboratory test abnormalities, ECG findings, vital signs findings, or new findings in physical examination over 12 and 48 weeks of treatment.

  Note: An AE (classified by preferred term) that occurs during this study will be considered a TEAE if it has a start date on or after the first dose of double-blind investigational product or if it has a start date before the date of the first dose of double-blind investigational product, but increases in severity on or after the date of the first dose of double-blind investigational product. An AE that occurs more than 5 days (5x half-life of 24 hours) after the date of the last dose of double-blind investigational product will not be counted as a TEAE.
- **Pharmacokinetic Endpoint:** For all subjects who consent, regardless of toilet-training and age, sparse PK blood sampling will be conducted during the 12-week double-blind, placebo-controlled phase. The obtained plasma concentrations, together with the data from previous pediatric studies (PRU-USA-12, PRU-USA-24 and SHP555-303), will be used for further population PK analysis and the refined model will be used to characterize prucalopride's PK properties and the relationship between PK and efficacy/safety endpoints of interest may be explored (separate reporting). Individual plasma concentrations with corresponding sampling times post-dose will be tabulated per visit. A graphical presentation will be used to visualize the plasma concentration-time relationship. Descriptive statistics will be calculated per visit and at specific post dose time intervals.

Statistical methodology for primary efficacy endpoint(s):

The primary endpoint will be derived as follows:

- For each subject, the number of SBMs will be calculated (using the formula below) for each of the 12 weeks in the Placebo-controlled Part (Part A) of the study and for baseline:

  The (average) number of SBMs during the screening period (baseline) will be calculated as follows:

$$Number\ of\ SBMs\ (baseline) = \frac{7 * (total\ frequency\ of\ SBMs\ during\ the\ screening\ period)}{(number\ of\ days\ with\ observations\ during\ the\ screening\ period)}$$

$$Note: excluding\ the\ days\ of\ the\ disimpaction\ procedure\ (3\ to\ 12\ days)$$

- For the calculation of weekly number of SBM, it is required that there are at least 4 days with completed diary data (ie, evaluable days). A day is considered evaluable (day with diary information) when at least some information on BM was recorded (ie, "no BM today" or the time of at least 1 BM was recorded). A minimum of 4 days each week are necessary to perform a weekly evaluation.
- If there is no completed diary data available for at least 4 days, then weekly number of SBM will be set to missing and the missing weekly number of SBMs per week up to Week 12 will be imputed using hybrid imputation approach.
- The (average) number of SBM per week post-baseline will be calculated as:

$$Number\ of\ SBMs\ (Week\ i) = \frac{7 * (total\ frequency\ of\ SBMs\ in\ Week\ i)}{(number\ of\ days\ with\ observation\ in\ Week\ i)}$$

- The change from baseline in number of SBM/week can be derived at each week during the Placebo-controlled Part (Part A) of the study. The average change from baseline in number of SBM/week over the 12-week placebo-controlled Part A will be estimated through statistical modeling.

Note that BMs during the disimpaction period(s) which can take 3 to 12 days during the screening period and BMs within 24 hours after rescue medication intake during Part A, will not be counted.

Missing data will be imputed using a hybrid imputation approach prior to analysis of primary efficacy endpoint. The primary efficacy endpoint will be analyzed using a mixed model for repeated measures (MMRM). The MMRM will include treatment group, age groups, study week, treatment-group-by-study-week interaction as fixed effects, baseline number of SBM/week as a covariate and subject as a random effect. An unstructured variance-covariance matrix will be used to model the within-subject errors for both treatment groups. The average change from baseline over 12 weeks will be estimated (LS means) by the above MMRM. The treatment difference in LS means between active treatment group versus placebo will be estimated. P-value, treatment difference in LS means and associated 95% confidence interval from the multiple imputed datasets will be combined using Rubin's rules, as implemented in the PROC MIANALYZE procedure of SASÒ Version 9.4 or higher (SAS Institute, Cary, NC 27513). The restricted maximum likelihood method will be used, with an unstructured covariance structure (UN). If the UN covariance structure fails to converge, as an alternative, the Compound symmetry (CS) covariance matrix will be applied. For purpose of convergence, pooling of smaller centers might be needed, decisions on pooling will be made before unblinding.

Statistical methodology for key secondary efficacy endpoints

Missing data will be imputed using a hybrid imputation approach prior to analyses of key secondary efficacy endpoints.

The continuous endpoints using the MMRM with the same covariates and factors as for the primary endpoint.

The binary endpoints using Cochran Mantel Haenszel test, controlling for the stratification variables age and baseline number of SBMs/week. An overall combined p-value, response rates and difference in response rates plus 95% confidence interval will be derived using Rubin's rules, as implemented in the PROC MIANALYZE procedure. The CMH test statistic will be normalized using the Wilson-Hilferty transformation before combining.

Statistical methodology for other and exploratory efficacy endpoints

For the other and exploratory efficacy endpoints based on diary data, the weekly number of SBMs scores will be derived in a same way as for the primary endpoint.

The other and exploratory efficacy endpoints will be summarized descriptively by treatment group and evaluated over the mITT analysis set. Where possible data will be presented graphically (over time) to support the interpretation of the results.

For binary endpoints, treatment arms will be compared using the Cochran Mantel Haenszel test controlling for the stratification variables age and baseline number of SBMs/week. The difference in proportions will be presented with a 95% confidence interval.

For continuous endpoints, treatment arms will be compared using the same MMRM as used for primary endpoint analysis. No multiplicity adjustment will be performed for the analysis of other and exploratory efficacy endpoints.

Additional details are described in the Statistical Analysis Plan.

Safety Analysis

The safety analysis will be performed using the Safety Analysis Set, according to the investigational product/treatment the subject received.

All safety data will be summarized for the whole study (Part A and Part B combined), and also separately for the double-blind treatment part A.

Safety variables include AEs, clinical laboratory variables, vital signs, and ECG variables. For each safety variable, the last value collected before the first dose of investigational product will be used as baseline for all analyses of that safety variable. Last Value on Treatment will be defined as the last valid assessment obtained after Baseline and whilst on investigational product. Last Observed Value will be defined as the last valid assessment obtained after Baseline.

Pharmacokinetic and Pharmacokinetic/Pharmacodynamic Analyses

For subjects who consent to the sparse PK sampling, individual plasma concentrations with corresponding sampling times post-dose will be tabulated per visit. A graphical presentation will be used to visualize the plasma concentration-time relationship. Descriptive statistics will be calculated per visit and at specific post-dose time intervals. Plasma levels below the limit of quantification will be flagged.

Plasma concentrations measured in this study will be combined with the results from previous trials in pediatric subjects and a population PK analysis will be performed on the pooled data. This analysis will be subject of a separate report.

Interim Analysis

Two interim analyses (IA) will be conducted during the study.

The primary objective of the first IA is to evaluate stopping the study for futility.

The first IA will be conducted when 50% the target number of toilet-trained subjects (ie, 120 subjects) are randomized into the study and have either completed or had withdrawn from the Placebo-controlled Part (Part A). Futility evaluation will be based on the conditional power using a stopping threshold of 20% for each dose arm.

A second IA will be performed when all randomized subjects have completed or had withdrawn from the Placebo-controlled Part (Part A). At this IA, the primary endpoint will be analyzed as planned. The data monitoring committee (DMC) will then make a recommendation to the sponsor to stop or continue the remainder of the study (part B) based on the statistical comparisons of the primary endpoint between both the low and high dose prucalopride arms with placebo.

If one of the doses shows a statistically significant difference (based on the Hochberg step-up procedure adjusted p-values) with placebo the study will be continued.

To maintain data integrity, the results from the IAs based on unblinded data will be accessible only to an external DMC to make a recommendation to the sponsor. At all points, the study team directly involved in the conduct of the study will remain blinded to maintain the double-blind nature of the study.

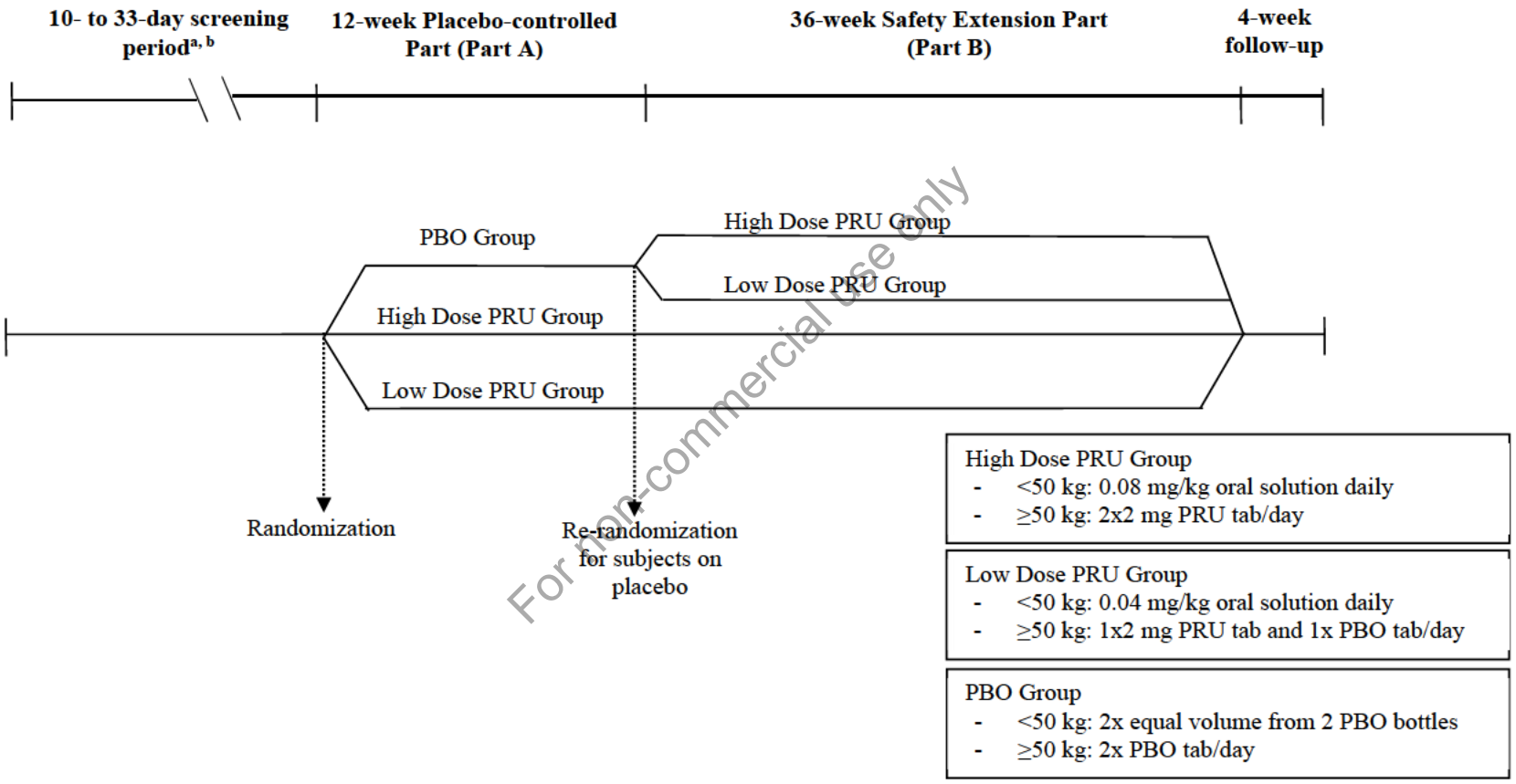
Sample size and power considerations:

With 80 toilet-trained subjects who are at least 3 years of age per treatment arm, Part A of the study will have at least 90% power to detect a treatment difference of 1.40 SBMs/week in primary efficacy endpoint between at least one active dose versus placebo assuming a pooled standard deviation [SD] of 2.5, using a two-sided two-sample t-test at a significance level of 5% based on the Hochberg step-up procedure to control the type I error rate for primary efficacy endpoint.

In addition, for exploratory purposes, a maximum of 15 non-toilet-trained subjects is targeted to be enrolled.

1.2 Schema

Figure 1 Study Schematic Diagram



PBO: placebo; PRU: prucalopride; tab: tablet

[a] Including disimpaction for all subjects.
[b] The screening period can be as short as 10 days, as long as 7 consecutive days of diary data have been recorded.

1.3 Schedule of Activities

Table 1 Schedule of Activities – Placebo-controlled Part (Part A)

Period	Screening[a]	Placebo-controlled Part												
Visit	**1**	**2 Base-line**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Week	**Approx. -4**	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Study Day	**-33 to –1[b]**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**
Assessment window (in days)	NA	0	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3
On-site visit[c]	X	X				X				X				X
Telephone contact[d]	X[e]		X	X	X		X	X	X		X	X	X	
Informed consent/assent	X													
Eligibility assessment	X													
Randomization		X												
Re-randomization (placebo-subjects only)														X
Demography, medical and medication history[f]	X													
Assessment of Rome IV criteria[g]	X													X
Physical examination (complete)	X[h]													
Physical examination (targeted)		X[i]				X				X				X
Height	X	X				X				X				X
Weight	X	X				X				X				X
Vital signs	X	X				X				X				X
12-lead ECG	X[j]	X												X
Dispense investigational product		X				X				X				X[k]
Collect investigational product						X				X				X
Provide rescue medication[l,m]		X								X				
Provide disimpaction medication[m]	X													
Removal of fecal impaction[n]	X													
Dispense e-Diary	X													
Check e-Diary[o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Table 1 Schedule of Activities – Placebo-controlled Part (Part A)

Period	**Screening[a]**	**Placebo-controlled Part**												
Visit	**1**	**2 Base-line**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Week	**Approx. -4**	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Study Day	**-33 to –1[b]**	**0**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**
Assessment window (in days)	NA	0	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3
Confirm visit on eCOA device[p]	X	X				X				X				X
Behavioral therapy reminder	X	X				X				X				X
Concomitant medications check	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant surgeries/ procedures/psychiatric changes/CBT check	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Prohibited medications check	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE check[q]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Fecal incontinence clinical assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Retentive posturing clinical assessment[r]	X	X				X				X				X
Schedule date for next visit	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Laboratory Assessments														
Hematology	X					X								X
Serum chemistry	X					X								X
Urinalysis	X					X								X
Serum pregnancy test[s]	X													
Urine pregnancy test[s]		X				X				X				X
Sparse PK sampling (optional)[t]		X				X				X				X
Clinical Outcome Assessments														
e-Diary (including Wong-Baker or NRS, BSFS)	X	◄———												———►
PGI-S and CGI-S	X	X				X				X				X
PedsQL™ GI (gas and bloating module)	X	X				X				X				X

Table 1 Schedule of Activities – Placebo-controlled Part (Part A)

Period	Screening[a]	Placebo-controlled Part												
Visit	1	2 Base-line	3	4	5	6	7	8	9	10	11	12	13	14
Week	Approx. -4	0	1	2	3	4	5	6	7	8	9	10	11	12
Study Day	-33 to –1[b]	0	7	14	21	28	35	42	49	56	63	70	77	84
Assessment window (in days)	NA	0	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3

AE=adverse event; BSFS=Bristol Stool Form Scale; CBT=cognitive behavioral therapy; CGI-S=Caregiver Global Impression of Severity; ECG=electrocardiogram; eCOA=electronic clinical outcome assessment; eCRF=electronic case report form; e-Diary=electronic diary; ET=early termination; ICF=informed consent form; IP=investigational product; NRS=numerical response scale; PedsQL™ GI=Pediatric Quality of Life Inventory (PedsQL™) Gastrointestinal Symptoms; PEG=polyethylene glycol; PGI-S=Patient Global Impression of Severity; PK=pharmacokinetic(s)

[a] Since, in the screening period, the subject's diagnosis is checked and since all subjects need to be disimpacted during the screening period, the screening period includes at least 3 telephone contacts and can include an unscheduled visit.

[b] The screening period can be as short as 10 days, as long as 7 consecutive days of diary data have been recorded.

[c] All subjects should be instructed to withhold dosing on in-clinic visit days (Part A and Part B) until after their visit. This includes Baseline (Week 0), Week 4 (Visit 6), Week 8 (Visit 10), Week 12 (Visit 14), Week 16 (Visit 15b), Week 24 (Visit 17), Week 32 (Visit 19), Week 40 (Visit 21), and Week 48 (Visit 23/ET). Note that if a subject does take their daily dose of IP prior to the on-site visit, this will not result in a protocol deviation unless the subject is participating in PK sampling at Week 4 (Visit 6), Week 8 (Visit 10), and Week 12 (Visit 14). Subjects will be dosed on site on Baseline (Visit 0).

[d] Any telephone visit can be changed to an onsite visit at the investigator's discretion.

[e] Regular telephone contacts will be made with the subject/parent(s)/caregiver(s) to decide on the continuation of the screening period, the initiation of the disimpaction procedure, and the success or failure of the procedure.

[f] Information related to disease history will also be collected.

[g] Rome IV criteria will be assessed at the time of screening and at the Week 12 visit. If a subject discontinues from the study for any reason prior to completing Part A, Rome IV will be assessed at the time of the early termination visit.

[h] The screening visit (Visit 1) physical examination may include an optional rectal examination to confirm the presence or absence of fecal impaction. The anal and cremasteric reflex examination is also optional.

[i] The Visit 2 physical examination may also include an optional rectal examination to confirm the absence of fecal impaction.

[j] An ECG recording up to 6 months prior to the screening visit is also acceptable. The eligibility of the subject is based on the assessment of the ECG by the investigator at screening (Visit 1).

[k] Subjects will be rerandomized to Part B IP.

[l] Current laxatives will be discontinued and replaced by the protocol-specified rescue medication.

[m] Subjects will get a prescription for the rescue and/or disimpaction medication as well as instructions on how to obtain these medications. Rescue medications can be provided more or less frequently at the discretion of the investigator.

[n] The disimpaction can take 3 days if the PEG 3350 is effective the first time, and it can take as long as 12 days if a 3-6 day cycle of PEG needs to be repeated (see Section 6.7.3.2).

[o] The investigator or study staff will remind the subject and/or parent(s)/caregiver(s)/legally authorized representative(s) to complete the e-Diary every day, to transmit the data regularly, and to bring the device at the next visit.

[p] Visit confirmation can be done on eCOA portal for all visits other than screening.

Table 1 Schedule of Activities – Placebo-controlled Part (Part A)

Period	Screening[a]	Placebo-controlled Part												
Visit	1	2 Base-line	3	4	5	6	7	8	9	10	11	12	13	14
Week	Approx. -4	0	1	2	3	4	5	6	7	8	9	10	11	12
Study Day	-33 to –1[b]	0	7	14	21	28	35	42	49	56	63	70	77	84
Assessment window (in days)	NA	0	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3	±3

[q] Adverse events will be reported from signing consent/assent onwards until the last study-related contact.
[r] The presence of any of these symptoms in the past 4 weeks will be recorded in the eCRF.
[s] A serum (screening)/urine (all other time points) pregnancy test will be performed for female subjects aged ≥12 years and/or subjects <12 years who have started menarche.
[t] This is an optional assessment and requires specific consent on the ICF. Sampling times between 1 to 3 hours post-dose after the first dose (on Day 0) and between 14 to 26 hours post-dose from the previous day's dosing at Week 4 (Visit 6), Week 8 (Visit 10), and Week 12 (Visit 14). At Week 4 (Visit 6), Week 8 (Visit 10), and Week 12 (Visit 14), dosing should be withheld until after the PK sample has been drawn.

Table 2 Schedule of Activities – Safety Extension Part (Part B)

Period	Safety Extension Part										Follow-up
Visit	**15a**	**15b**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23/ET**	**24**
Week	**14**	**16**	**20**	**24**	**28**	**32**	**36**	**40**	**44**	**48**	**52**
Study Day	**98**	**112**	**140**	**168**	**196**	**224**	**252**	**280**	**308**	**336**	**364**
Assessment Window (in days)	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**
Onsite Visit[a]		X		X		X		X		X	
Telephone Visit[b]	X		X		X		X		X		X
Targeted Physical examination		X		X		X		X		X	
Weight		X		X		X		X		X	
Vital signs		X		X		X		X		X	
12-lead ECG		X				X				X	
Dose increase based on weight				X [c]							
Dispense investigational product		X		X		X		X			
Collect investigational product		X		X		X		X		X	
Provide rescue medication [d]		X		X		X		X			
Check e-Diary[e]		X		X		X		X		X	
Return e-Diary										X	
Confirm visit on eCOA device[f]										X[g]	
Behavioral therapy reminder		X		X				X			
Check toilet-training status	X	X	X	X	X	X	X	X	X	X	
Concomitant medications check	X	X	X	X	X	X	X	X	X	X	X
Concomitant surgeries/procedures check/psychiatric changes/CBT check	X	X	X	X	X	X	X	X	X	X	X
Prohibited medications check	X	X	X	X	X	X	X	X	X	X	X
AE check[h]	X	X	X	X	X	X	X	X	X	X	X
Schedule/confirm date for next visit	X	X	X	X	X	X	X	X	X		

Table 2 Schedule of Activities – Safety Extension Part (Part B)

Period	Safety Extension Part										Follow-up
Visit	**15a**	**15b**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23/ET**	**24**
Week	**14**	**16**	**20**	**24**	**28**	**32**	**36**	**40**	**44**	**48**	**52**
Study Day	**98**	**112**	**140**	**168**	**196**	**224**	**252**	**280**	**308**	**336**	**364**
Assessment Window (in days)	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**	**±3**
Clinical Laboratory Assessments											
Hematology		X				X				X	
Serum chemistry		X				X				X	
Urinalysis		X				X				X	
Urine pregnancy test[i]		X		X		X		X		X	
Clinical Outcomes Assessments											
e-Diary	◄									►	

AE=adverse event; CBT= cognitive behavioral therapy; ECG=electrocardiogram; eCOA=electronic clinical outcome assessment; e-Diary=electronic diary; ET=early termination; IP=investigational product; PedsQL GI=Pediatric Quality of Life Inventory (PedsQL Gastrointestinal Symptoms)

[a] All subjects should be instructed to withhold dosing on in-clinic visit days (Part A and Part B) until after their visit. This includes Baseline (Week 0), Week 4 (Visit 6), Week 8 (Visit 10), Week 12 (Visit 14), Week 16 (Visit 15b), Week 24 (Visit 17), Week 32 (Visit 19), Week 40 (Visit 21), and Week 48 (Visit 23/ET). Note that if a subject does take their daily dose of IP prior to the on-site visit, this will not result in a protocol deviation unless the subject is participating in PK sampling at Week 4 (Visit 6), Week 8 (Visit 10), and Week 12 (Visit 14). Subjects will be dosed on site on Baseline (Visit 0).

[b] Any telephone visit can be changed to an onsite visit at the investigator's discretion.

[c] At Week 24 (Visit 17), each subject weighing <50 kg at baseline can undergo a dose adjustment for oral solution based on weight. In case the subject has crossed the 50-kg threshold, he/she will be switched from oral solution to tablet, provided he/she can swallow the tablet. If the subject cannot swallow the tablet, he/she can receive the tablet dose as oral solution. Depending on the treatment group, subjects cannot exceed the maximum dose of 2 or 4 mg.

[d] Subjects will get a prescription for the rescue medication as well as instructions on how to obtain these medications. Rescue medication can be provided more or less frequently at the discretion of the investigator.

[e] The investigator or study staff will remind the subject and/or parent(s)/caregiver(s)/legally authorized representative(s) to complete the e-Diary every day, to transmit the data regularly, and to bring the device at the next visit.

[f] Visit confirmation can be done on eCOA portal.

[g] For ET, not Visit 23.

[h] Adverse events will be reported from signing the TAK-555-3010 consent/assent onwards until the last study-related contact.

[i] A urine pregnancy test will be performed for female subjects aged ≥12 years and/or subjects <12 years who have started menarche.

2. INTRODUCTION

2.1 Indication and Current Treatment Options

Functional defecation disorders are among the most common complaints that bring a child to the attention of a pediatric gastroenterologist. It is rare to find an underlying organic disease as the cause, and most children with these symptoms are eventually diagnosed with functional constipation (Drossman et al. 2016). A systematic review reported a mean and median prevalence of 14% and 12%, respectively (Mugie et al. 2011).

In the majority of children, constipation is successfully treated with osmotic laxatives and intake of a diet rich in fiber (Tabbers et al. 2014); however, in approximately one third of children, symptoms persist and cannot be fully resolved with regular enemas and maximum laxative intake (Bongers et al. 2009). The constipation may even be severe enough to result in complete cessation of spontaneous bowel movements (SBMs) (Wong and Lubowski 2007).

2.2 Product Background and Clinical Information

Prucalopride (also referenced as TAK-555, SPD555, SHP555, M0001, R093877, and R108512 in countries where the drug is approved) is a drug that stimulates gastrointestinal motility.

Prucalopride was first approved in the EU in October 2009 (centralized procedure) for use in women only, and approval for use in both adult men and women was received in June 2015. The drug was approved in the US (2018) for adult patients with chronic idiopathic constipation. Overall, as of 06 Sep 2022, prucalopride has been approved in 74 countries, has active marketing authorizations in 65 countries, and is marketed in 54 of these countries as MOTEGRITY®, RESOLOR®, RESOTRAN®, RESOTRANS® and RESOLOR™. The estimated worldwide patient exposure to prucalopride as of 31 Aug 2022 is over 707,113 person-years of treatment since launch.

Prucalopride belongs to a chemical class of dihydrobenzofuran-carboxamide derivatives with strong enterokinetic activity. In fasted awake dogs, the compound induces giant migrating contractions, stimulates proximal colonic motility, enhances gastro-pyloric-duodenal motility, and accelerates gastric emptying (Briejer et al. 1997b; Wellens and Schuurkes 1996).

It is the first selective high affinity 5-hydroxytryptamine type 4 (5-HT_4) receptor agonist, which is likely to explain its enterokinetic effects in dogs as well as in humans (Briejer et al. 1997a; Briejer et al. 1997b; Briejer et al. 1995). Prucalopride is highly selective when compared to other drugs with 5-HT_4 receptor agonistic properties: tegaserod and cisapride are non-selective 5-HT_4 receptor agonists that interact with other receptors in a concentration range relevant for their interaction with 5-HT_4 receptors.

The most accurate and current information regarding the drug metabolism, pharmacokinetics (PK), efficacy, and safety of prucalopride as well as the overall benefit/risk assessment in adults can be found in the investigator's brochure (IB) and in the prescribing information (MOTEGRITY [prucalopride] tablets, for oral use; initial US approval: 2018).

2.3 Study Rationale

The safety (both adults and pediatrics) and efficacy (adults only) of prucalopride in gastrointestinal motility disorders such as chronic constipation was confirmed in an extensive clinical development program consisting of 93 studies (ie, 52 Phase 1, 25 Phase 2, 14 Phase 3, and 2 Phase 4 studies). To date, 1 Phase 3 study in pediatric subjects with functional constipation has been completed (Study SPD555-303; part of the European Pediatric Investigational Plan for prucalopride). Prucalopride was well tolerated in this study; however, the primary efficacy endpoint did not reach statistical significance.

2.4 Benefit/Risk Assessment

The benefit of prucalopride was established in multiple clinical studies with the following outcomes: improvement in the frequency of bowel movements (BMs), bowel symptoms (eg, stool symptoms, abdominal symptoms, rectal symptoms), and quality of life (QoL) in adults with chronic idiopathic constipation. The post marketing safety experience is consistent with the safety data from clinical studies. The safety data collected so far from worldwide sources are in accordance with the safety profile described in the IB. The most frequently reported adverse drug reactions (ADRs) associated with administration of prucalopride are headache and gastrointestinal symptoms (nausea, diarrhea, and abdominal pain). These ADRs occur predominantly in the first few days after initiation of treatment and wane with continued treatment. They are generally mild to moderate in severity and the proportion of patients who discontinue treatment due to these events is low (ie, 13.9%).

Prucalopride benefits/risk profile remains favorable and provides a new treatment option for patients with chronic constipation.

Always refer to the latest version of the prucalopride IB and the prescribing information for the overall benefit/risk assessment and the most accurate and current information regarding drug metabolism, PK, efficacy, and safety of prucalopride.

The safety measures in this study are considered standard for a study of an IP in pediatric subjects. These include an evaluation of AEs, physical examinations, vital signs measurements, clinical laboratory tests (eg, biochemistry, hematology, and urinalysis), and electrocardiograms (ECG).

2.5 Compliance Statement

This study will be conducted in accordance with this protocol, the International Council for Harmonisation Guideline for Good Clinical Practice E6 (International Council for Harmonisation [ICH] Good Clinical Practice [GCP], 1996; E6 R2, 2017), Title 21 of the US Code of Federal Regulations (CFR), and applicable national and local regulatory requirements.

The responsibilities of the study sponsor and investigator(s) are described fully in Appendix 1.

3. OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Objectives

- To evaluate the efficacy of prucalopride during the 12-week double-blind, placebo-controlled treatment phase in toilet-trained subjects with functional constipation who are at least 3 years of age.
- To evaluate the long-term (48 weeks) safety and tolerability of prucalopride in toilet-trained subjects with functional constipation who are at least 3 years of age.

3.1.2 Key Secondary Objectives

- To evaluate the efficacy of prucalopride on signs and symptoms of functional constipation (stool consistency, straining, and stool frequency responder index), during the 12-week double-blind, placebo-controlled treatment phase in toilet-trained subjects with functional constipation who are at least 3 years of age.

3.1.3 Other Secondary Objectives

- To evaluate the effect of prucalopride on the frequency of fecal incontinence.
- To evaluate safety and tolerability of prucalopride over 12 weeks of treatment in toilet-trained subjects who are at least 3 years of age.
- To evaluate the PK of prucalopride by combining sparse PK blood sampling that will be conducted during the 12-week double-blind, placebo-controlled phase together with the data from previous pediatric studies for further population PK analysis (separate reporting).

3.1.4 Exploratory Objectives

- To further explore the efficacy of prucalopride on signs and symptoms of functional constipation during the 12-week double-blind, placebo-controlled treatment phase in toilet-trained subjects with functional constipation who are at least 3 years of age.
- To explore the efficacy of prucalopride during the 12-week double-blind, placebo-controlled treatment phase in non-toilet-trained subjects with functional constipation who are at least 6 months of age.
- To evaluate the long-term (48 weeks) safety and tolerability of prucalopride in non-toilet-trained subjects with functional constipation who are at least 6 months of age.
- To evaluate safety and tolerability of prucalopride over 12 and 48 weeks of treatment in non-toilet-trained subjects with functional constipation who are at least 6 months of age.
- To explore the relationship between PK and efficacy/safety endpoints of interest.

3.2 Study Endpoints

3.2.1 Primary Endpoint

The primary endpoint (in toilet-trained subjects who are at least 3 years of age) is as follows:

- The average change from baseline in number of SBMs[1] per week derived from the (diary) data over 12 weeks, collected during the placebo-controlled part (Part A).

3.2.2 Key Secondary Endpoints

- Efficacy endpoints in toilet-trained subjects who are at least 3 years of age:
  - The average change from baseline in stool consistency (based on Bristol Stool Form Scale [BSFS] score), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
  - The average change from baseline in straining (based on a 3-point Likert scale), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
  - The proportion of responders with a responder defined as a subject having an increase of ≥1 SBM/week compared to baseline and ≥3 SBMs/week for at least 9 out of the 12 weeks of placebo-controlled part (Part A), including 3 of the last 4 weeks.

3.2.3 Other Secondary Endpoint

- Efficacy endpoints in toilet-trained subjects who are at least 3 years of age:
  - Proportion of subjects with fecal incontinence per week during the 12-week treatment Period.

3.2.4 Exploratory Endpoints

- Efficacy endpoints in toilet-trained subjects who are at least 3 years of age:
  - The average change in worst abdominal pain score over the past 24 hours (based on a Wong-Baker faces scale in subjects <8 years and the 11-point numeric rating scale [NRS] in subjects ≥8 years), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
  - Proportion of subjects with an average of ≥3 SBMs per week and increase of ≥1 SBM compared to baseline during a 12-week double-blind, placebo-controlled treatment phase.
  - Proportion of subjects with an average of ≤1 SBMs per week during the 12-week double-blind, placebo-controlled treatment phase.

[1] A BM is defined as spontaneous (SBM) if not preceded within a period of 24 hours by the intake of rescue medication.

- Proportion of subjects with an average of ≥1 day(s) with rescue medication intakes per week.
- Proportion of subjects assessed with retentive posturing at monthly visits during the 12-week treatment Period.

  Retentive posturing is defined as the attempt to preserve continence by vigorous contraction of the gluteal muscles. Children with retentive posturing will be typically tight legged, tiptoed, and/or will have a back-arching posture.
- Global evaluation and QoL Health economics and outcomes research endpoints: Patient Global Impression of Severity (PGI-S), Caregiver Global Impression of Severity (CGI-S), and Pediatric Quality of Life Inventory™ (PedsQL) Gas and Bloating Domain Gastrointestinal Symptoms Scale (Placebo-controlled Part [Part A]):
  - Absolute values and change from baseline.
  - For PedsQL Gas and Bloating Domain Gastrointestinal Symptoms Scale-standardized response mean (SRM): classified as no, small, moderate, and large effect.
- Proportion of subjects with ≤2 signs/symptoms from the Rome IV Criteria following the 12-week double-blind, placebo-controlled treatment phase

– Efficacy endpoints in non-toilet-trained subjects who are at least 6 months of age:

- Proportion of subjects with an average of ≥3 SBMs per week and increase of ≥1 SBM compared to baseline during the 12-week double-blind, placebo-controlled treatment phase.
- Number of SBMs per week in categories ≤1 and >1 during the 12-week double-blind, placebo-controlled treatment phase.
- The average change from baseline in stool consistency (based on BSFS score), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.

3.2.5 Safety Endpoint

– The proportion of subjects with treatment-emergent adverse events (TEAEs) (serious, non-serious, related, non-related) and the proportion of subjects with clinically relevant laboratory test abnormalities, ECG findings, vital signs findings, or new findings in physical examination over 12 and 48 weeks of treatment.

3.2.6 PK Endpoint

– For all subjects who consent, regardless of toilet-training and age, sparse PK blood sampling will be conducted during the 12-week double-blind, placebo-controlled phase. The obtained plasma concentrations, together with the data from previous pediatric studies (PRU-USA-12, PRU-USA-24, and SHP555-303), will be used for further population PK analysis (see Section 9.8 PK Analyses) and the refined model will be used to characterize prucalopride's PK properties and the relationship between PK and efficacy/safety endpoints of interest may be explored (separate reporting). Individual plasma concentrations with corresponding sampling times post-dose will be tabulated per visit. A graphical presentation will be used to visualize the plasma concentration-time relationship. Descriptive statistics will be calculated per visit and at specific post dose time intervals.

4. STUDY DESIGN

4.1 Overall Design

This is a Phase 3, multicenter, randomized study consisting of a 12-week double-blind, placebo-controlled part (Part A) followed by a 36-week double-blind safety extension part (Part B) to document safety and tolerability up to 48 weeks in male and female pediatric and adolescent subjects with functional constipation as defined by the modified Rome IV criteria for child/adolescent Functional Gastrointestinal Disorders (FGID).

The study will start with a 10- to 33-day screening period, including a disimpaction for all subjects, a 12-week double-blind placebo-controlled Part (Part A) followed by a 36-week long-term double-blind (for dose) safety extension part (Part B), and a follow-up call approximately 4 weeks after the last administration of the IP.

Approximately 240 toilet-trained pediatric/adolescent subjects, ages 3 to 17 years with functional constipation are planned for randomization in a 1:1:1 ratio to the Low Dose Group, High Dose Group, or matching placebo (placebo-controlled part [Part A]). After completion of the 12-week placebo-controlled Part A, subjects in the placebo group will be re-randomized in a 1:1 ratio to the Low Dose Group or the High Dose Group (safety extension part [Part B]). For exploratory purposes, in addition to the 240 toilet-trained pediatric/adolescent subjects, the study is targeted to enroll a maximum of 15 subjects who are not toilet-trained and at least 6 months of age. These subjects will not be included in the primary analyses; they will be part of the exploratory analyses for descriptive purposes.

The Enrolled Analysis Set will include both toilet-trained and non-toilet-trained subjects. Study enrollment of toilet-trained subjects will be stopped as soon as 240 subjects who are at least 3 years of age are included. If, at that point, less than 15 non-toilet trained subjects who are at least 6 months of age have been included, enrollment in that group will continue.

Prucalopride once daily (QD) (0.4 mg/mL oral solution or 2-mg tablet) or placebo QD (oral solution or tablet):

- Low Dose Group:
  - Placebo-controlled Part A:
    - Subjects weighing <50 kg will receive a daily dose of 0.04 mg/kg and will draw equal volumes from one bottle of 0.4 mg/mL prucalopride oral solution and one bottle of placebo oral solution to account for the daily dose assigned.

      The volume of the oral solution will be based on the subject's body weight (BW) at the randomization visit.
    - Subjects weighing ≥50 kg will be dosed with one 2-mg tablet of prucalopride and one placebo tablet.

- Safety Extension Part B:
  - Subjects weighing <50 kg will receive a daily dose of 0.04 mg/kg and will draw the required volume from one bottle of 0.4 mg/mL prucalopride oral solution and one bottle of placebo oral solution to account for the daily dose assigned.

    The volume of the oral solution will be based on the subject's BW at the randomization visit.
  - Subjects weighing ≥50 kg will be dosed with one 2-mg tablet of prucalopride and 1 placebo tablet.

– High Dose Group:

- Placebo-controlled Part A:
  - Subjects weighing <50 kg will receive a daily dose of 0.08 mg/kg and will draw equal volumes from two bottles of 0.4 mg/mL prucalopride oral solution to account for the daily dose assigned.

    The volume of the oral solution will be based on the subject's BW at the randomization visit.
  - Subjects weighing ≥50 kg will be dosed with two 2-mg tablets of prucalopride.
- Safety Extension Part B:
  - Subjects weighing <50 kg will receive a daily dose of 0.08 mg/kg and will draw the required volume from two bottles of 0.4 mg/mL prucalopride oral solution to account for the daily dose assigned.

    The volume of the oral solution will be based on the subject's BW at the randomization visit.
  - Subjects weighing ≥50 kg will be dosed with two 2-mg tablets of prucalopride.

– Matching placebo (Placebo-controlled Part A only):

- Subjects weighing <50 kg will draw equal volumes from two bottles of placebo oral solution to account for the daily dose assigned.
- Subjects weighing ≥50 kg will be dosed daily with 2 tablets of placebo.

Each subject weighing <50 kg at baseline can undergo a dose adjustment for oral solution based on weight at Week 24. In case the subject has crossed the 50-kg threshold, he/she will be switched from oral solution to tablet, provided he/she can swallow the tablet. If the subject cannot swallow the tablet, he/she can receive the tablet dose as oral solution. Depending on the treatment group, subjects cannot exceed the maximum dose of 2 or 4 mg. More detailed information will be provided in study materials.

A table of weight adjusted dosing is provided in the Pharmacy Manual.

Randomization at study entry will be stratified by toilet-trained status. Toilet-trained subjects will be additionally stratified by age group (<12 years, 12 to 17 years) and average number of

SBMs/week (≤1; >1) during the screening period. Non-toilet-trained subjects will be additionally stratified by SBM/week (≤1; >1) during the screening period.

The study completion date is defined as the date the last subject, across all sites, completes his/her final protocol-defined assessment (ie, the follow-up call or end of study visit). The study completion date is used to ascertain timing for study results posting and reporting.

When approximately 50% of toilet-trained subjects (ie, 120 subjects) are randomized into the study and have completed the 12-week placebo-controlled part (Part A), an interim analysis (IA) will be performed to compare the efficacy of both prucalopride doses with placebo. The main purpose of the IA is to decide to continue or stop the study for futility, ie, to stop the study when none of the two prucalopride treatment arms show a meaningful difference in the change from baseline of the number of SBM/week (see to Section 9.2 for additional details).

4.2 Scientific Rationale for Study Design

This study is designed to satisfy the 2 post marketing requirements (PMR 3529-1 and PMR 3529-2/6) as mandated in the new drug application (NDA) approval letter of December 2018, for prucalopride (indicated for the treatment of chronic idiopathic constipation in adults) and to fulfill the Pediatric Research Equity Act (PREA). Agreement has been reached with the US Food and Drug Agency (FDA) that both PMRs can be combined in one study protocol.

Prucalopride has been developed for the treatment of chronic idiopathic constipation in adults. To date, 1 Phase 3 study in pediatric subjects has been completed (SPD555-C303); however, no efficacy was demonstrated. The study was a multicenter, randomized, placebo-controlled, double-blind, study to evaluate the efficacy and safety of prucalopride in 213 children (6 months to 18 years old) with functional constipation. Children with functional constipation, based on the Rome III criteria, were given prucalopride (N=106) or placebo (N=107) QD for 8 weeks. Subjects received either prucalopride or placebo at dose of 0.04 mg/kg QD using a liquid formulation or at a dose of 2 mg QD using a tablet formulation. After 4 weeks of treatment, a dose adjustment to 0.06 mg/kg or 0.02 mg/kg could occur for subjects on the liquid formulation only. The primary efficacy endpoint was the proportion of children with toileting skills who had a mean of ≥3 SBMs/week and ≤1 episode of fecal incontinence/2 weeks (responders). The results showed that the proportion of responders was similar between treatment groups (prucalopride, 17.0% and placebo, 17.8%) and the incidence of TEAEs was similar in the prucalopride (69.8%) and placebo (60.7%) groups (Mugie et al. 2014).

The explanations offered for the results (Nurko and Saps 2014) were (i) functional constipation in children is considered a heterogeneous disorder with a complex pathophysiology that is not necessarily related to an abnormal transit, (ii) common pathophysiologic feature in functional constipation in children is withholding behavior. Therefore, the influence of behavioral factors may also explain some of the results, (iii) the primary endpoints differ from the endpoints used in studies in the adult population in which fecal incontinence is not included, and (iv) whether the short length of the trial might have contributed to the results.

The current study will address several of the possible issues of the previous study: the study duration will be 12 weeks instead of 8 weeks, the study population will have the same starting point for their colon content with all subjects undergoing a fecal disimpaction procedure prior to randomization, the primary efficacy endpoint will be different from the endpoint used in studies with adults, and there will be a standardized continuous behavior therapy procedure applied to all subjects.

The current study will evaluate the efficacy and safety of prucalopride in pediatric subjects aged 6 months to 17 years. Non-toilet-trained subjects who are at least 6 months of age will be included in an exploratory group since the instruments used to evaluate the different efficacy parameters have not been validated in subjects <3 years. The study will consist of a 10- to 33-day screening period, including a disimpaction for all subjects, a 12-week double-blind placebo-controlled part (Part A) followed by a 36-week long-term safety extension part (Part B), and a follow-up call approximately 4 weeks after the last administration of the IP.

The primary efficacy will be evaluated by comparing the average change from baseline in number of SBMs in each prucalopride (high dose and low dose) versus (vs) the placebo arm over 12 weeks of double-blind, placebo-controlled treatment in toilet-trained subjects who are at least 3 years of age.

Safety will be evaluated over the combined 48-week treatment period.

Further, though prucalopride exhibits a linear PK profile across a broad dose range in adult and pediatric subjects, the sponsor will incorporate PK sparse sampling in this pediatric study to provide additional plasma concentration data from higher exposures in pediatric subjects.

Subjects with a history of large fecal mass will be included in the study. Disimpaction will be performed for all subjects within the screening period.

Non-toilet-trained subjects are analyzed separately from the toilet-trained group as the assessment of BMs from the content of a diaper is considered difficult. Therefore, this study will primarily evaluate the effect of the IP in toilet-trained subjects.

4.3 Justification for Dose

Prucalopride exhibited linear PK in adults receiving daily doses up to 20 mg (Study PRU-GBR-10) and pediatric subjects receiving daily doses up to 0.06 mg/kg (to a maximum of 2 mg, SPD555-303). Exposure was similar between adults receiving 2 mg and children and adolescents receiving 0.04 mg/kg up to a maximum of 2 mg and is projected to be similar between a dose of 0.08 mg/kg in children and adolescents and a 4-mg dose in adults.

A population PK analysis was conducted based on pooled data from Study PRU-USA-12 (0.03 mg/kg single-dose with robust PK sampling in 38 children), Study PRU-USA-24 (extension study to PRU-USA-12; 0.03 mg/kg QD, with sparse PK sampling in children), and Study SPD555-303 (0.04 mg/kg up to a maximum of 2 mg vs placebo QD; one-step titration after 4 weeks when subjects could decrease to 0.02 mg/kg or increase to 0.06 mg/kg up to a maximum of 2 mg with sparse PK sampling in children and adolescents).

In summary, the final updated model was used to simulate concentration-time profiles following single and q.d. multiple doses of 0.02 mg/kg, 0.04 mg/kg and 0.06 mg/kg with a maximal daily dose of 2 mg. The simulated concentrations and 90% prediction intervals in pediatrics were compared to simulations in adults following a 2-mg dose and suggested the following:

- QD dosing of 0.02 mg/kg would result in concentrations at the low end of the adult prediction interval in all pediatric age groups and can potentially impact on efficacy.
- A dose of 0.04 mg/kg can be considered an acceptable but conservative dose to treat children, as it is predicted to result in maximal concentrations that remain within the range predicted for adults. However, the predicted area under the plasma concentration-time curve (AUC) will be lower than the predicted AUC for a 2-mg dose in adults, and well under the values seen with a 4-mg dose.
- The dose of 0.06 mg/kg results in a predicted higher maximum observed plasma concentration (C_{max}) than those observed at 2-mg in adults. Simulations with this dose, however, suggest that trough concentrations will be similar to those observed in adults at the 2-mg dose. By extrapolation the C_{max} for a 4-mg dose in children will be similar to or slightly higher than that seen in upon multiple dosing in adults and with similar total exposure (AUC) over the dosing interval.

Four mg is the highest dose tested in adult safety and efficacy studies of greater than 4 weeks dosing. The incidence of AEs in adult subjects receiving the 4-mg dose daily for 12 weeks was only slightly greater than in those adult subjects receiving the 2-mg daily dose, and the AE profile was similar between the two treatment regimens. Pediatric subjects receiving the oral solution had a lower percentage of TEAE incidence than those pediatric subjects receiving the tablet dosage form.

No efficacy was observed in the pediatric safety and efficacy Study SPD555-303 with a starting dose of 0.04 mg/kg, although most subjects (52 of 79) increased the dose to 0.06 mg/kg at 4 weeks (range from 0.02 to 0.06 mg/kg).

Therefore, the sponsor plans to study the efficacy and safety of a prucalopride therapy using two dose regimens that approximate the adult 2-mg and 4-mg doses in comparison to placebo therapy.

4.4 Duration of Subject Participation and Study Completion Definition

The subject's maximum duration of participation is expected to be 56 weeks (consisting of a 10- to 33-day screening period, a 12-week placebo-controlled part A, a 36-week safety extension part B, and a follow-up call 4 weeks after the last intake of IP.

The study is expected to be completed in approximately 4 to 4.5 years.

The study completion date is defined as the date the last subject in the study completes the final protocol-defined assessment(s). This includes the follow-up visit or contact, whichever is later (refer to Section 4.1 for the defined follow-up period for this protocol).

4.5 Sites and Regions

It is anticipated that the study will be conducted at approximately 40-45 sites in the US.

Other countries/regions may be added if needed.

5. STUDY POPULATION

Approximately 240 toilet-trained subjects (~80 subjects per treatment arm) who are at least 3 years of age and up to 15 non-toilet-trained subjects (~5 per treatment arm) who are at least 6 months of age are planned to be randomized.

Each subject and/or parent(s)/caregiver(s)/legally authorized representative(s) (where appropriate depending on age and local regulation) must participate in the informed consent process and provide written informed consent/assent before any procedures specified in the protocol are performed. Subjects and/or parent(s)/caregiver(s)/legally authorized representative(s) (where appropriate depending on age and local regulation) can also provide consent/assent to the sparse PK sampling in this study.

5.1 Inclusion Criteria

The subject will be eligible for the study when meeting all of the criteria below:

<u>To be verified at screening:</u>

1. Subjects and/or their parent(s)/caregiver(s)/legally authorized representative(s) have an understanding, ability, and willingness to fully comply with study procedures and restrictions.
2. Ability to voluntarily provide written, signed, and dated (personally or via parent[s]/caregiver[s]/legally authorized representative[s]) informed consent/assent as applicable to participate in the study.

   Note: Subjects and/or parent(s)/caregiver(s)/legally authorized representative(s) (where appropriate depending on age and local regulation) can also provide consent/assent to the sparse PK sampling in this study.
3. Toilet-trained subjects 3 years to 17 years of age, inclusive, or non-toilet-trained subjects 6 months to 17 years of age, inclusive.
4. Subject weighs ≥5.5 kg (12 lbs).
5. Male, or non-pregnant, non-lactating female subjects who are sexually active and agree to comply with the applicable contraceptive requirements of the protocol or females of non-childbearing potential.

   Note: All female subjects ≥12 years and/or female subjects <12 years who have started menarche must have a negative serum pregnancy test at screening.
6. Subject meets modified Rome IV criteria:
   - For <u>child/adolescent (aged >4 years)</u> functional constipation (H3a):

     Subjects must have ≤2 defecations per week and 1 or more of the following occurring at least once per week for a minimum of 1 month:

     - ≥1 episode of fecal incontinence per week (only for subjects after the acquisition of toileting skills).

- History of retentive posturing or excessive volitional stool retention.
- History of painful or hard BMs.
- Presence of large fecal mass in rectum.
- History of large diameter stools which can obstruct the toilet.

In addition, the subject does not satisfy sufficient criteria for a diagnosis of irritable bowel syndrome (IBS) and, after appropriate evaluation, the subject's symptoms cannot be fully explained by another medical condition.

Retentive posturing is defined as the attempt to preserve continence by vigorous contraction of the gluteal muscles. Children with retentive posturing will be typically tight legged, tiptoed, and/or will have a back-arching posture.

– For <u>infant/toddler (aged 6 months to ≤4 years)</u> functional constipation (G7):

Subjects must have ≤2 defecations per week and ≥1 month of at least 1 of the following:

- History of excessive stool retention.
- History of painful or hard BMs.
- History of large-diameter stools (in the diaper).
- Presence of a large fecal mass in the rectum.

In toilet-trained children, the following additional criteria may be used:

- *At least 1 episode/week of incontinence after the acquisition of toileting skills.*
- *History of large-diameter stools which may obstruct the toilet.*

7. Subject and/or parent(s)/caregiver(s)/legally authorized representative(s) is willing to discontinue any laxatives during the screening period up to disimpaction and agrees to adhere to the protocol-specified disimpaction and rescue medication rules, if applicable.

<u>To be evaluated prior to randomization:</u>

8. Subject has an average of <3 SBMs (defecations) per week during the screening period and prior to the disimpaction.
9. Subject or legally authorized representative (dependent on subject age) is compliant with completing the electronic diary for at least 7 consecutive days preceding the disimpaction.

5.2 Exclusion Criteria

The subject will not be eligible for participation in the study if any of the following exclusion criteria are met:

<u>To be evaluated during the screening period:</u>

1. Current or recurrent disease that could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessments.

2. Any clinically significant abnormal findings on the ECG that indicates a dysrhythmia or conduction abnormalities (such as abnormal heart rate, PR, QRS, or QT).
3. Major cardiovascular disease such as: cardiomyopathy, cardiac insufficiency, uncorrected congenital heart disease, symptomatic valve disorders, or septal defects.
4. Current or relevant history of physical or psychiatric illness (eg, severe autism, depression, etc.), any medical disorder that may require treatment or make the subject unlikely to fully complete the study, or any condition that presents undue risk from the IP or procedures.
5. Non-retentive fecal incontinence.

   Fecal incontinence is defined as unintentional smear or liquid stool in the underwear that is not due to poor wiping. Fecal incontinence can only occur in toilet-trained subjects. Non-retentive fecal incontinence is diagnosed (must include at least a 1-month history in a child with a developmental age older than 4 years for all the following): (i) defecation in places inappropriate to the sociocultural context, (ii) no evidence of fecal retention, and (iii) after appropriate evaluation, the fecal incontinence cannot be explained by another medical condition.
6. Intestinal perforation or obstruction due to structural or functional disorder of the gut wall, obstructive ileus, severe inflammatory conditions of the intestinal tract such as Crohn's disease, ulcerative colitis, and toxic megacolon/megarectum.
7. Current use of any medication (including over-the-counter, herbal, or homeopathic preparations) that could affect (improve or worsen) the condition being studied (eg, opioids), or could affect the action, absorption, or disposition of the IP, or clinical or laboratory assessment. (Current use is defined as use within the past 5 days.) See Section 6.7 (Prior and Concomitant Treatment) for a list of prohibited and restricted medications.
8. Subjects with renal impairment:
   - Subjects ≤2 years of age with serum creatinine greater than normal (screening sample results using central laboratory pediatric reference ranges).
   - Subjects >2 years of age with severe renal impairment or end stage renal disease (estimated glomerular filtration rate [eGFR] <30 mL/min/1.73 m²)

     The Bedside Schwartz equation should be used if the serum creatinine is being assayed using enzymatic methodology. [Bedside Schwartz equation: eGFR=0.413 x height (cm)/ standardized serum creatinine (SCr) (mg/dL)]
     The original Schwartz equation should be used if the serum creatinine is being assayed using the Jaffe methodology. (Original Schwartz equation: creatinine clearance [CrCl]=[k*height/serum creatinine]; see Appendix 7)
9. Known or suspected intolerance or hypersensitivity to the IP(s), closely-related compounds, or any of the stated ingredients.
10. Known history of alcohol or other substance abuse within the last year.
11. Within 30 days prior to the first dose of the IP in the current study:
    - Have used any IP.

- Have been enrolled in a clinical study (including vaccine studies) that may or may not include the administration of an IP that, in the investigator's opinion, may impact this study.

12. Subject used prucalopride within 10 days prior to the first dose of the IP or has been unsuccessfully treated with prucalopride before.
13. Subject meets Rome IV criteria for other Child/Adolescent FGID (H1 – H2 and H3b).
14. Subject with secondary causes of constipation, eg:
    - Endocrine disorders (eg, hypopituitarism, hypothyroidism, hypercalcemia, pheochromocytoma, glucagon-producing tumors) unless these are controlled by appropriate medical therapy. Subject with uncontrolled diabetes mellitus is to be excluded
    - Metabolic disorders (eg, porphyria, uremia, hypokalemia, hypothyroidism, amyloid neuropathy), unless controlled by appropriate medical therapy
    - Neurological disorders (eg, cerebral tumors, cerebrovascular accidents, multiple sclerosis, meningocele, aganglionosis, hypoganglionosis, hyperganglionosis, autonomic neuropathy, spinal cord injury, Chagas disease
    - Organic disorders (known or suspected) of the large bowel (eg, obstruction from any cause including biliary obstruction, malignancy, intestinal perforation, obstructive ileus, pseudo-obstruction, history of or current anorectal malformations, severe inflammation of the intestinal tract, such as Crohn's disease, ulcerative colitis or toxic megacolon/megarectum, Hirschsprung's disease)
    - Celiac disease, cow milk allergy
    - Surgery: history of gastrointestinal surgery related or possibly related to the presence of constipation
    - Lactose intolerance
15. Any of the following clinically significant abnormalities of serum biochemistry:
    - Serum aspartate aminotransferase (AST) >1.5 times upper limit of normal (ULN) at screening.
    - Serum alanine aminotransferase (ALT) >1.5 times ULN at screening.
    - Total bilirubin outside the age-adjusted normal range, except for subjects with Gilbert's syndrome.
16. Any significant underlying liver disease.
17. Subject is not able to swallow the IP (liquid or tablet).
18. Subject is pregnant or planning to get pregnant during study period.

To be evaluated prior to randomization:

19. Subject has used other disimpaction medication in lieu of the protocol-provided medication.
20. Subject has used non-protocol approved medications to induce BMs during the screening period or disimpaction.
21. The subject has failed the disimpaction based on the investigator's assessment.
22. Worsening of depression and emergence of suicidal thoughts.

5.3 Restrictions

All subjects must be willing to adhere to the following prohibitions and restrictions during the course of the study:

1. Prohibited medications (as described in Section 6.7.4) are not allowed during the entire period of the study.
2. The subject and/or parent(s)/caregiver(s)/legally authorized representative(s) have to be instructed not to change the lifestyle or diet of the subject, including exercise, and fiber intake from randomization onwards. No other over-the-counter medications for constipation are permitted other than the study-allowed rescue medications (see Section 6.7.3).
3. The subject should refrain from donating blood for the entire period during the study until 30 days after the last intake of IP.
4. Female subject and/or parent(s)/caregiver(s)/legally authorized representative(s) should be instructed that in case of severe diarrhea, the efficacy of oral contraceptives may be reduced and the use of an additional contraceptive method is recommended to prevent possible failure of oral contraceptives (see the prescribing information of the oral contraceptive).
5. All subjects will be disimpacted within the screening period.
6. Subjects who are being toilet-trained at the start of the study, should suspend toilet-training for the duration of the placebo-controlled part (Part A) of the study. Subjects who are not toilet-trained, should not start toilet-training during the placebo-controlled part (Part A) of the study.

   Note: A child is considered toilet-trained if, during the day, he/she uses the toilet to defecate (even if the child continues to have episodes of fecal incontinence). Children who use the toilet during the day to defecate, but wear a diaper at night for nocturnal bedwetting, are considered toilet-trained.

   Note: Children are allowed to (re)start toilet training during the safety extension part (Part B). When a child becomes toilet-trained, the site should document the change.
7. Subject or legally authorized representative(s) (dependent on subject age) is compliant with completing the e-Diary for at least 5 out of the 7 days during each week of the double-blind treatment phase.
8. If after one dose increase of treatment with rescue medication the treatment is still ineffective, the investigator may consider other protocol-approved treatment options allowed

per Section 6.7.3.1 of the protocol. Contact the study monitor if use of rescue medications, dose escalations and other alternative treatments remain ineffective to assess the subject's continued participation in the study.

5.4 Reproductive Potential

5.4.1 Female Contraception

Sexually active females of childbearing potential should use an acceptable form of contraception. Females of childbearing potential must be advised to use acceptable contraceptives throughout the study period and for 30 days following the last dose of IP. If used, hormonal contraceptives should be administered according to the package insert. Any female of childbearing potential who is not currently sexually active must agree to use acceptable contraception, as defined below, if she becomes sexually active during the study and for 30 days following the last dose of IP.

Female children and adolescent subjects should be either:

- Premenarchal, or
- Have a negative urine pregnancy test at the time points indicated in the study Schedule of Activities (Table 1; for subjects aged ≥12 years and/or subjects <12 years who have started menarche). Female subjects of childbearing potential must agree to abstain from sexual activity that could result in pregnancy or agree to use acceptable methods of contraception.

Acceptable methods of contraception include the following:

- Intrauterine devices plus condoms.
- Double-barrier methods (eg, condoms and diaphragms with spermicidal gel or foam).
- Hormonal contraceptives (oral, depot, patch, injectable, or vaginal ring), stabilized for at least 30 days prior to the screening visit (Visit 1), plus condoms.

Note: If a subject becomes sexually active during the study, they should use one of the other acceptable methods noted above in addition to the hormonal contraceptive until it has been stabilized for 30 days.

In case of severe diarrhea, use of additional contraceptive methods should be considered.

5.4.2 Male contraception

Male participants with female partners of childbearing potential are eligible to participate if they agree to ONE of the following throughout the study:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent

- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year as described when having penile-vaginal intercourse with a partner of childbearing potential who is not currently pregnant

Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration throughout the study.

6. STUDY INTERVENTION

6.1 IP

6.1.1 Identity of IP

The IP is prucalopride (TAK-555), which will be provided as an oral solution (for subjects weighing <50 kg) or as a tablet (for subjects weighing ≥50 kg). Additional information is provided in the current IB.

The reference product is placebo which will be provided in a matching oral solution or tablet form.

6.1.2 Blinding the Treatment Assignment

Matching placebo solution/tablets will be available such that the blinding can be assured in identical packaging.

The subject, investigator, study coordinator, sponsor, and the entire study processing team will remain blinded to the treatment assignment. The set-up of the randomization system will ensure that the blind is maintained and will not reveal treatment allocation to any unauthorized personnel.

Note that the IA will be conducted by an independent data monitoring committee (DMC) (see Section 9.2) and the unblinded data will be visible to the DMC only to ensure the study remains blinded to the sponsor, the investigator, and the study subjects.

6.2 Administration of IP

6.2.1 Interactive Response Technology for Investigational Product Management

Randomization will be implemented by interactive response technology (IRT) and individual subject treatment is automatically assigned by the IRT.

– Placebo-controlled Part (Part A):

The IRT system will apply a minimization algorithm to ensure an appropriate 1:1:1 balance between the 3 treatment groups (prucalopride low dose, prucalopride high dose, and placebo) in each stratum.

Randomization will be performed with the permuted blocks and will be stratified by toilet-trained status, age group (<12 years, 12 to 17 years) for those toilet-trained, and average number of SBM/week (≤1; >1) during the screening period. The block size in randomization will be kept unknown to the investigator sites as well as the study team.

– Safety Extension Part (Part B):

The IRT system will apply a minimization algorithm to ensure an appropriate 1:1 balance between the 2 treatment groups (prucalopride low dose and prucalopride high dose.

Re-randomization of the placebo treatment group at the end of the Placebo-controlled Part will be performed with the permuted blocks and will not be stratified. The block size in randomization will be kept unknown to the investigator sites as well as the study team.

Drug accountability will be collected using IRT (see Section 6.5).

6.2.2 Allocation of Subjects to Treatment

This study consists of a 12-week double-blind randomized treatment part (placebo-controlled Part A) followed by a 36-week double-blind long-term safety extension (safety extension Part B).

At the start of the safety extension Part B, subjects on placebo will all be re-randomized to high or low dose prucalopride. This re-randomization will ensure that subject who were treated with prucalopride will remain on their double-blind dose and subjects who were treated with placebo will be equally split (ratio 1:1) to the high or low dose of prucalopride.

Subject numbers are assigned to all subjects as they consent to take part in the study. Within each site (numbered uniquely within a protocol), the subject number is assigned to subjects according to the sequence of presentation for study participation. Screen failure subject numbers cannot be reassigned.

The randomization number represents a unique number corresponding to IP allocated to the subject, once eligibility has been determined.

Individual subject treatment and corresponding medication kit numbers will be assigned by the IRT.

6.2.3 Dosing

Following initial randomization, subjects will enter the 12-week Placebo-controlled Part (Part A) where they will receive either prucalopride at a low dose, prucalopride at a high dose, or placebo. Upon completion of the Placebo-controlled Part (Part A), all subject who were on placebo in the Placebo-controlled Part (Part A) will be re-randomized into the Safety Extension Part (Part B) where they will receive prucalopride at either a low dose or a high dose.

Prucalopride (oral solution or tablet) or placebo (oral solution or tablet) will be dosed depending on the subject's BW at the randomization visit. The IP will be provided as oral solution (prucalopride 0.4 mg/mL or placebo) or as tablets (prucalopride 2 mg or placebo).

- Low Dose Group:
  - Subjects weighing <50 kg will receive a daily dose of 0.04 mg/kg and will draw the required volume from one bottle of 0.4 mg/mL prucalopride oral solution and one bottle of placebo oral solution to account for the daily dose assigned.

    The volume of the oral solution will be based on the subject's BW at the randomization visit.

- Subjects weighing ≥50 kg will be dosed with one 2-mg tablet of prucalopride and one placebo tablet.

– High Dose Group:

- Subjects weighing <50 kg will receive a daily dose of 0.08 mg/kg and will draw the required volume from 2 bottles of 0.4 mg/mL prucalopride oral solution to account for the daily dose assigned.

  The volume of the oral solution will be based on the subject's BW at the randomization visit.

- Subjects weighing ≥50 kg will be dosed with two 2-mg tablets of prucalopride.

– Matching placebo (Part A only):

- Subjects weighing <50 kg will draw equal volumes from 2 bottles of placebo oral solution to account for the daily dose assigned.
- Subjects weighing ≥50 kg will be dosed daily with 2 tablets of placebo.

Each subject weighing <50 kg at baseline can undergo a dose adjustment for oral solution based on weight at Week 24. In case the subject has crossed the 50-kg threshold, he/she will be switched from oral solution to tablet, provided he/she can swallow the tablet. If the subject cannot swallow the tablet, he/she can receive the tablet dose as oral solution.

At any point following the baseline visit, if a subject weighing ≥50 kg cannot tolerate the tablets, they can be switched to oral solution for the duration of study participation. Switching from oral solution to tablets is not allowed unless the subject crosses the 50-kg weight barrier at Week 24. Depending on the treatment group, subjects cannot exceed the maximum dose of 2 or 4 mg. More detailed information will be provided in study materials.

A table of weight adjusted dosing is provided in the Pharmacy Manual.

The color, size, and shape of the prucalopride and placebo tablets are identical as are the color and taste of prucalopride and placebo bottles and oral solution.

The tablet has to be taken as a whole, ie, cannot be chewed or crushed.

The prucalopride oral solution will be administered in an at-home setting by lay caregivers for subjects less than 13 years old or by self-administration for subjects 13-17 years old using an oral syringe. The proposed devices to be used for the dosing are all off-the-shelf devices and are intended for a single use. The required number of syringes will be provided to the subjects. The size of the dosing syringe will be selected based on the subject's prescribed dose based on the BW. Pediatric subjects should be supervised by the parent(s)/legally authorized representative(s)/caregiver(s). Subjects/caregivers will be trained to use the oral syringe using the Oral Syringe Training Guide and Instructions for Use included in the Pharmacy Manual. Oral solution should not be mixed with orange juice or any other beverage and must be administered through an oral syringe as instructed.

Subjects should take the IP at the same time each day, 1 to 3 hours before either the morning or evening meal. Subjects should not take more than one dose in a day if they miss a dose. If a dose is missed at the normally scheduled time, the subject can make up the dose within 12 hours after the normally scheduled dosing time.

Subjects will be instructed to administer the first dose of IP in the study center on the day of randomization. The last dosing will occur on the day prior to Visit 23.

All subjects should be instructed to withhold dosing on in-clinic visit days (Part A and Part B) until after their visit. This includes Baseline (Week 0), Week 4 (Visit 6), Week 8 (Visit 10), Week 12 (Visit 14), Week 16 (Visit 15), Week 24 (Visit 17), Week 32 (Visit 19), Week 40 (Visit 21), and Week 48 (Visit 23/ET). Note that if a subject does take their daily dose of IP prior to the on-site visit, this will not result in a protocol deviation unless the subject is participating in PK sampling at Week 4 (Visit 6), Week 8 (Visit 10), and Week 12 (Visit 14). Subjects will be dosed on site on Baseline (Visit 0).

The exact timing of dosing has to be documented in the e-Diary (see Section 8.2.2.1).

6.2.4 Unblinding the Treatment Assignment

The treatment assignment must not be unblinded during the study except in emergency situations where the identification of the IP is required for medical management of the subject. The investigator should contact the study medical monitor immediately after the treatment code has been broken and the investigator is unblinded.

In the event that the treatment assignment code is broken, the date and the signature of the person who broke the code are recorded on the IRT and the source documents, as applicable. Upon breaking the blind, the subject is withdrawn from the study, but should be followed up for safety purposes. Any code breaks that occur must be reported to the sponsor immediately.

Note that the IAs will be conducted by an independent DMC (see Section 9.2) and the unblinded data will be visible to the DMC only to ensure the study remains blinded to the sponsor, the investigator, and the study subjects.

6.2.5 Dose Modification

Each subject weighing <50 kg at baseline can undergo a dose adjustment for oral solution based on weight at Week 24. In case the subject has crossed the 50-kg threshold, he/she will be switched from oral solution to tablet, provided he/she can swallow the tablet. If the subject cannot swallow the tablet, he/she can receive the tablet dose as oral solution. Depending on the treatment group, subjects cannot exceed the maximum dose of 2 or 4 mg. More detailed information will be provided in study materials.

6.3 Labeling, Packaging, Storage, and Handling of Investigational Product

6.3.1 Labeling

Labels containing study information and pack identification are applied to the IP container.

All IP is labeled at a minimum with the following: protocol number, product identification number, dosage form, directions for use, storage conditions, expiry date (if applicable), batch number and/or packaging reference, the statements "For clinical trial use only" and/or "CAUTION: New Drug – Limited by Federal (or US) Law to Investigational Use". Any additional requirements for participating countries will also be included on the label.

Space is allocated on the label so that the site representative can record a unique subject identifier.

Additional labels (eg, those used when dispensing marketed product) may, on a case-by-case basis, be applied to the IP in order to satisfy local or institutional requirements, but must not:

- Contradict the clinical study label.
- Obscure the clinical study label.
- Identify the study subject by name if not allowed per local regulations.

Additional labels may not be added without the sponsor's prior full agreement.

6.3.2 Packaging

IP is packaged in labeled containers. Prucalopride 2-mg and placebo tablets will be primary packaged in blisters and these blisters will be contained within a 64-tablet Low-dose, High-dose, or Placebo wallet. All IP will be packaged with a unique medication ID number.

For the liquid formulation, the active prucalopride and matching placebo will be packaged in bottles with child-resistant closures.

Changes to sponsor-supplied packaging prior to dosing may not occur without full agreement in advance by the sponsor.

6.3.3 Storage

The investigator has overall responsibility for ensuring that IP is stored in a secure, limited-access location. Limited responsibility may be delegated to the pharmacy or member of the study team, but this delegation must be documented. IP is distributed by the pharmacy or nominated member of the study team. The pharmacist/nominated team member will enter the unique subject identifier on the IP bottle/carton labels as they are distributed.

Prior to dispensing at the site, IP must be stored in accordance with labeled storage conditions.

The study IP oral solution is to be stored at 20° to 25°C (68° to 77°F)

The study IP tablets are to be stored at 20° to 25°C (68° to 77°F).

NOTE: excursions are permitted between 15° to 30°C (59°F to 86°F) (see US Pharmacopeia Controlled Room Temperature) but must be reported via Temperature Excursion process defined in the pharmacy manual.

The investigator is responsible for ensuring that the storage temperature is monitored throughout the duration of the study and that records are maintained; the temperature should be monitored continuously by using either an in-house system, a mechanical recording device such as a calibrated chart recorder, or by manual means, such that both minimum and maximum thermometric values over a specific period can be recorded and retrieved as required. Such a device (ie, certified min/max thermometer) would require manual resetting upon each recording. The sponsor must be notified immediately upon discovery of any excursion from the established range. Temperature excursions will require site investigation as to cause and remediation. The sponsor will determine the ultimate impact of excursions on the IP and will provide supportive documentation as necessary. Under no circumstances should the IP be dispensed to subjects until the impact has been determined and the product is deemed appropriate for use by the sponsor.

The sponsor should be notified immediately if there are any changes to the storage area of the IP that could affect the integrity of the product(s), eg, fumigation of a storage room.

6.4 Labeling, Packaging, Storage, and Handling of Rescue Medication

Rescue medication will be provided to the subject or parent (caregiver). All medication should be stored as per the labeled instructions.

6.5 Drug Accountability

Investigators will be provided with sufficient IP to carry out this protocol for the agreed number of subjects. The investigator or designee will acknowledge receipt of the IP, documenting shipment content and condition. Accurate records of all IP dispensed, used, returned, and/or destroyed must be maintained as detailed further in this section.

The investigator has overall responsibility for dispensing IP. Where permissible, tasks may be delegated to a qualified designee (eg, a pharmacist) who is adequately trained in the protocol and who works under the direct supervision of the investigator. This delegation must be documented in the applicable study delegation of authority form.

The investigator or his/her designee (as documented by the investigator in the applicable study delegation of authority form) will dispense the IP only to subjects included in this study following the procedures set out in the study protocol or applicable study manual. Each subject will be given only the IP carrying his/her treatment assignment. All dispensed medication will be documented in the subject's source and the IRT system. The investigator is responsible for ensuring the retrieval of all study supplies from subjects including empty vials or bottles that contained IP (see Section 6.6). Due to the health/safety concerns with returning the IP container, the investigator must request that subjects keep the empty IP packaging after use and return it to the site for drug accountability purposes.

No IP stock or returned inventory from this study may be removed from the site where originally shipped without prior knowledge and consent by the sponsor. If such transfer is authorized by the sponsor, all applicable local, state, and national laws must be adhered to for the transfer.

The sponsor or its representatives must be permitted access to review the supplies storage and distribution procedures and records provided that the blind of the study is not compromised.

With the written agreement of the sponsor, at the end of the study all unused stock, subject-returned IP, and empty/used IP packaging may be destroyed at the site or a local facility. In this case, destruction records identifying what was destroyed, when and how, must be documented in the IRT. Destruction of IP must be in accordance with local, state, and national laws.

Based on entries in the site drug accountability module in IRT, it must be possible to reconcile IPs delivered with those used and returned. All IP must be accounted for and all discrepancies investigated and documented to the sponsor's satisfaction.

6.6 Subject Compliance

Subjects must be instructed to bring unused IP and empty/used IP packaging as well as rescue medication to every visit. Drug accountability must be assessed at the container/packaging level for unused IP that is contained within the original tamper-evident sealed container (eg, bottles, trays, vials) or at the individual count level for opened containers/packaging. The pharmacist/nominated person will record details in the site drug accountability module in IRT.

6.7 Prior and Concomitant Treatment

All non-study treatment (ie, all medications besides the IP including but not limited to herbal treatments, vitamins, etc.) received within 30 days prior to the screening visit (Visit 1) (or PK equivalent of 5 half-lives, whichever is longer) and through the final study contact (including protocol-defined follow-up period) must be recorded in the subject's source document.

All behavioral and non-pharmacological treatment (such as psychotherapy, as appropriate) received within 30 days prior to the screening visit (Visit 1) and through the final study contact (including protocol defined follow-up period) must be recorded in the subject's source document and on the eCRF.

6.7.1 Prior Treatment

Prior treatment is defined as any treatment with the start date prior to the date of the first dose of IP and includes all treatment including but not limited to: herbal treatments, vitamins, received as well as all behavioral and non-pharmacological treatment (such as psychotherapy, as appropriate).

Prior treatment information must be recorded in the subject's source documents.

6.7.2 Concomitant Treatment

Concomitant treatment refers to all treatment with a start date prior to the date of the first dose of IP and continuing after the first dose of IP or with a start date between the dates of the first and last doses of IP, inclusive. Concomitant treatment information must be recorded in the subject's source document.

6.7.3 Permitted Treatment

6.7.3.1 Rescue Medication

If necessary, rescue medication may be used to help induce (a) BM(s). In the event that no BM has occurred within a 72-hour (3-day) period, the use of rescue medications is permitted per the investigator's instructions as described below. The instructions follow the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) Recommendation 2014 (Tabbers et al. 2014).

The investigator will write a prescription (including clear instructions for use) for the rescue medication. Note that only use of the rescue medications listed below are allowed to be prescribed by the investigator during the study, with the exception of children aged 6 months to <4 years where investigator discretion can be exercised.

The following rescue medication will be provided to the subject:

- bisacodyl tablets (5 mg) or suppositories (10 mg)
- polyethylene glycol (PEG) 3350 (oral) for the safety extension part (Part B) only

Dosing instructions for the double-blind placebo-controlled part (Part A):

- Subjects aged 6 months to <4 years at screening: at the discretion of the investigator to acknowledge different treatment protocols in each clinic. However, the investigator is encouraged using bisacodyl as first choice.
- Subjects 4-10 years at screening: 1 tablet or ½ suppository (=5 mg bisacodyl) per day. If ineffective within 12 hours, the dose can be increased once to 2 tablets or 1 suppository (=10 mg bisacodyl).
- Subjects >10 years at screening: 2 tablets or 1 suppository (=10 mg bisacodyl). If ineffective within 12 hours, the dose can be increased once to 3 tablets or 1½ suppository (=15 mg bisacodyl).
- The use of PEG 3350 as rescue medication is not allowed in the placebo-controlled Part (Part A).

If after one dose increase, treatment with rescue medication is still ineffective within an additional 12 hours, the study medical monitor should be contacted to assess continued study participation.

The dosing recommendations for the double-blind part (Part B) in the rescue medication algorithm are as follows:

- Subjects aged 6 months to <4 years at screening: at the discretion of the investigator.
- Subjects aged 4 to 10 years at screening: 1 tablet or ½ suppository (=5 mg bisacodyl) per day or PEG 3350 (oral) dosed at 0.2-0.8 g/kg/day.
- Subjects aged >10 years at screening: 2 tablets or 1 suppository (=10 mg bisacodyl) or PEG 3350 (oral) dosed at 0.2-0.8 g/kg/day.

If rescue medication is ineffective after a period of 12 hours, the rescue medication dose may be increased at the discretion of the investigator. The recommended dose increases are as follows:

- Subjects aged 6 months to <4 years at screening: at the discretion of the investigator.
- Subjects aged 4 to 10 years at screening: 2 tablets or 1 suppository (=10 mg bisacodyl) or a higher dose of PEG 3350 (oral) at 1-1.5 g/kg/day if already on PEG.
- Subjects aged >10 years at screening: 3 tablets or 1½ suppositories (=15 mg bisacodyl) or a higher dose of PEG 3350 (oral) at 1-1.5 g/kg/day if already on PEG.

If after 12 hours following a dose increase treatment with bisacodyl (oral or suppository) is still ineffective, the investigator may consider shifting to PEG 3350 (oral) dosed at 0.2-0.8 g/kg/day.

If after 12 hours following initiation with PEG 3350 treatment remains ineffective, the investigator, at his discretion, may consider a higher dose of PEG:

- Subjects aged 6 months to <4 years at screening: at the discretion of the investigator.
- Subjects aged 4 to 10 years at screening: Consider a higher dose of PEG 3350 at 1-1.5 g/kg/day.
- Subjects aged >10 years at screening: Consider a higher dose of PEG 3350 at 1-1.5g/kg/day.

If after a dose increase with PEG 3350 and after 6 consecutive days of use treatment is still ineffective, the investigator should contact the study monitor to assess if the subject should continue with the study.

A rescue medication algorithm is provided in Appendix 5. If the subject has a BM after taking rescue medication but does not experience relief of symptoms, an additional dose(s)/escalated dose(s) of rescue medication may be recommended based on investigator discretion.

Rescue medication will be made available throughout the study (after Baseline Visit 2).

If use of rescue medication is required, the exact timing of the dosing has to be documented in the e-Diary (see Section 8.2.2.1).

6.7.3.2 Disimpaction Procedure During Screening Period

The disimpaction will be performed prior to the baseline visit (Visit 2). All subjects will be supplied with the appropriate dose of PEG.

Staff at the study site will call the subject/parent(s)/caregiver(s)/legally authorized representative(s) to decide on the initiation of the disimpaction procedure, remind him/her when and how to perform the disimpaction and to check on the success or failure of the procedure 1 to 2 days thereafter. The call will be recorded in the eCRF.

The instructions follow the NASPGHAN Recommendation 2014 (Tabbers et al. 2014) and the Prescribing Information for the respective medications. However, it is allowed to follow the respective hospital or office treatment protocols.

It is recommended to follow the disimpaction protocol below:

- PEG 3350 with or without electrolytes 1-1.5 g/kg/day divided into 2 doses for 3 to 6 consecutive days until watery stool is passed with confirmation that the stool has cleared from the rectum.
- If watery stool is not passed, Step 1 may be repeated once or – for subjects aged ≥2 years – sodium phosphate enemas may be given once per day for up to three days.
- For subjects aged ≥2 years with difficulties to swallow or unsuccessful initial disimpaction therapy - sodium phosphate enemas may be given once per day for up to three days. The recommended dose for subjects up to 18 years is 2.5 mL/kg with a max of 133 mL/dose.

Note: The enema dose for subjects up to 18 years is 2.5 mL/kg with a max of 133 mL/dose.

6.7.3.3 Standard of Care: Behavioral Therapy

Behavioral therapy will be applied throughout the study.

At a minimum, parents and subjects aged ≥3 years at randomization should be educated on the following:

- Parent education.

It is equally important to teach parents to ignore or to neutrally react to the inappropriate behavior of stool-withholding behavior and pant soiling. Positive reinforcement is also a powerful technique in shaping adequate toileting behavior. Parents must stop mainly negatively reinforcing the subject for inappropriate behavior.

- The importance of positive reinforcement:

Successful BMs in the toilet should be rewarded. For example, subjects could be rewarded with a sticker/token (eg, when enough stickers/tokens, they can be exchanged for social or material rewards, ie, helping to prepare dinner, inviting friends for a sleepover, buying a [small] toy,

staying up late, etc). Adequate timing and quality of reinforcement carried out by parents is crucial in contingency management.

– The importance of a toilet routine:

When the urge to defecate occurs, it must become a habit for the subject to use the toilet to evacuate sufficient feces instead of withholding it. Subjects in particular should visit the toilet 3 times per day for 5 minutes after each meal in a silent and relaxed atmosphere.

– The importance of a correct toilet position:

The subject has to sit in a relaxed position, feet should touch the ground and knees should be at the same height or slightly above the hip (a step for the feet should be used if needed). Subjects who are not using active straining should use straining by blowing on their hand.

Note that during the placebo-controlled part (Part A), subjects who are being toilet-trained at the start of the study should discontinue toilet-training for the duration of the study. Subjects who are not toilet-trained should not start toilet-training during Part A of the study. Children are allowed to (re)start toilet training during the safety extension part (Part B). When a child becomes toilet-trained, the site should document the change.

6.7.3.4 Other

Subjects who are on a stable regimen of over-the-counter constipation medicine such as fiber supplements or probiotics may continue to receive the same stable regimen during the study. Subjects not on a stable regimen cannot start using these medicines during the study.

6.7.4 Prohibited Treatment

The following medication is not allowed during the placebo-controlled part (Part A) of the study:

– Agents that influence bowel habits, including, but not limited to:

- Opiates (eg, codeine), antacids containing calcium carbonate or aluminum hydroxide, magnesium containing drugs, beta-blockers, clonidine, diuretics (nonpotassium-sparing), ganglionic blockers, muscle blockers (D-tubocurarine, succinylcholine, botulinum toxin), phenytoin, serotonin (5-HT_3) antagonists, loperamide, sucralfate, antispasmodics (eg, dicyclomine), metoclopramide, and erythromycin are not allowed during the entire study and the use of these medications must be stopped at the start of the screening period.
- Chronic use of nonsteroidal anti-inflammatory agents is not allowed. Occasional use of acetaminophen is allowed.
- Anticholinergics are not allowed, apart from occasional use of diphenhydramine or second generation oral antihistamines such as desloratadine for allergic rhinitis or urticaria.
- Sympathomimetics are not allowed, with the exception of inhaled sympathomimetics.
- For subjects aged >1 year, iron preparations are not allowed.

- Treatment with antidepressants, attention-deficit/hyperactivity disorder (ADHD) medication, inhaled sympathomimetics or Ca^{2+} blockers, taken at start of the study, can be continued if the subject is on a stable dose of antidepressant/ADHD medication/inhaled sympathomimetics/Ca^{2+} blockers for at least 4 weeks prior to Visit 1 (screening) and if this same regimen will be used for the entire study.

The following medication is not allowed during the entire study (placebo-controlled part [Part A] and safety extension part [Part B]):

- Antibiotics (except for erythromycin) are only allowed for as long as appropriate to treat the condition requiring the antibiotics. Care should be taken to avoid dehydration if diarrhea would occur.

Use of probiotics is allowed, if prescribed in addition to the antibiotic treatment.

- Subject should stop their current laxative regiment from Visit 1 (screening) onwards. During the study, subjects and/or parent(s)/caregiver(s)/legally authorized representative(s) have to agree to only use the protocol-allowed rescue medication (see Section 6.7.3).

All medications (prescriptions or over-the-counter medications) taken during the course of the study must be documented on the Concomitant Medication page of the electronic case report form (eCRF).

The investigator must contact the study medical monitor to discuss any changes to concomitant medications that may impact the study; the outcome of such discussions should be documented in detail in the subject's source documentation. Any prohibited treatments taken during the course of the study will be recorded as protocol deviations.

7. DISCONTINUATION OF STUDY INTERVENTION AND SUBJECT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Treatment

Any medically important event that in the opinion of the investigator, study medical monitor, or sponsor compromises a subject's safety may require discontinuation of IP and/or withdrawal of the subject from the study.

7.2 Discontinuation of Study IP

If IP is discontinued, regardless of the reason, the evaluations listed for Visit 23 (early termination [ET]) will be performed as completely as possible. Whenever possible, all discontinued subjects should also undergo the protocol-specified evaluations at Visit 24 (follow-up). Comments (spontaneous or elicited) or complaints made by the subject must be recorded in the source documents. The reason for discontinuation, date of discontinuation of the IP, and the total amount of IP administered must be recorded in the source documents.

Subjects who withdraw from the study are not to be replaced.

7.3 Reasons for Discontinuation

The reason for withdrawal must be determined by the investigator and recorded in the subject's source document. If a subject is discontinued for more than 1 reason, each reason should be documented in the source document and the most clinically relevant reason should be indicated.

Reasons for discontinuation include but are not limited to:

- AE.
- Withdrawal of consent by subject.

Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded (ie, withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category). If a subject chooses to withdraw from study participation due to personal concerns related to the Coronavirus Disease 2019 (COVID-19) pandemic (other than a COVID-19–related AE), this should be specified as the reason for subject withdrawal in the eCRF.

- Lost to follow-up (see Section 7.4).
- Death.
- Screen failure.
- Noncompliance with IP.
- Physician decision.
- Pregnancy.

- Study terminated by sponsor.
- Disease worsening.
- Other.

Note: The specific reasons should be recorded in the "specify" field of the eCRF. The reason for discontinuation should be entered into the eCRF including such unavoidable circumstances as the COVID-19 pandemic. Subjects may withdraw from the study at any time at the discretion of the investigator or sponsor for safety reasons, which should be entered into the eCRF.

7.4 Withdrawal from the Study

A subject may withdraw from the study at any time and for any reason without prejudice to his/her future medical care by the physician or at the institution or may be withdrawn at any time at the discretion of the investigator or sponsor (eg, in the interest of subject safety). The investigator is encouraged to discuss withdrawal of a subject with the study medical monitor when possible.

7.5 Subjects Lost to Follow-up Prior to the Last Scheduled Visit

A minimum of 3 documented attempts must be made to contact any subject who is lost to follow-up at any time point prior to the last scheduled contact (office visit or telephone contact). At least 1 of these documented attempts must include a written communication sent to the subject's last known address via courier or mail (with an acknowledgement of receipt request) asking that they return to the site for final safety evaluations and return any unused IP.

7.6 Safety-related Stopping Rules

If any of the stopping criteria outlined in Section 7.6.1, Section 7.6.2, and Section 7.6.3 and described in detail in the DMC charter are confirmed, the investigator, in consultation with the study medical monitor or appropriately qualified designee, will withhold IP or permanently discontinue the subject from further treatment with IP. Specific stopping rules related to diarrhea are discussed in Section 7.6.3.

Administration of IP must initially be suspended until retest for the results are available and an assessment is made by the investigator and communicated to the study medical monitor. Alternatively, interruption in the administration of IP may be required to assess the effect of and resolution of an intercurrent illness.

Should the investigator and sponsor decide that IP is to be permanently discontinued, the subject is encouraged to complete the study withdrawal procedures and the termination visit. Subjects who do not meet the stopping rules based on retest may continue dosing and the investigator with the study medical monitor should consult if additional close monitoring of the subject is appropriate.

If the administration of IP is suspended on 2 different occasions for the same AE, the subject shall be permanently discontinued from the IP.

Baseline values for safety monitoring are the average between the value obtained during the screening visit and the baseline visit, unless a confounder (eg, intercurrent illness) causes variability outside of what is expected based on the investigator's judgment (in which case a third value may need to be obtained).

7.6.1 Guidelines for Monitoring/Interruption for Clinically Significant Findings in Laboratory Parameters

For the monitoring rules, the ULN and lower limit of normal (LLN) range in children based on the reference range used by the central laboratory will be used.

If at any time during the study, a laboratory result the exceeds the ULN or LLN (for age) or the predefined ULN or LLN or there is a sustained doubling compared with the subject's baseline level, the measurement(s) should be re-confirmed 48 to 72 hours after the availability of the initial finding of potential concern.

Frequency of Repeat Measurements for Safety Monitoring:

Subjects with a confirmed test level that is continuing to rise should have it repeated according to standard of care until levels stabilize or trends towards recovery.

Guidance for Discontinuation of IP for Increased Laboratory Levels:

If a laboratory test result level (eg, prolactin, liver associated enzymes ALT, AST, total bilirubin, and renal function tests) shows a clinically significant increase compared with the baseline or if at any time during the study the ALT and AST exceeds >3x the ULN and the total bilirubin level >2x the ULN, the IP should be discontinued/withheld temporarily. Other criteria resulting in discontinuation of the IP and causality assessment are:

- A single ALT or AST value >8 × ULN.
- ALT or AST >5 × ULN at least twice over a 2-week period.
- AST or ALT >3 × ULN along with the appearance of symptoms of fatigue, nausea, vomiting, right upper quadrant pain, rash, fever, or eosinophilia (>5%).

A new blood draw should be performed 48 to 72 hours after the availability of the initial finding during an unscheduled visit. If the initial finding is confirmed, the IP should be permanently discontinued and a causality assessment should be completed. The subject should then be closely monitored until levels stabilize or trends towards recovery.

Other potential causes should be evaluated where there is a combined increased AST or ALT and total bilirubin, such as viral hepatitis A, B, or C; pre-existing or acute liver disease; or another potential interaction or insult caused by a concomitant medication leading to the observed injury.

Re-introduction of the IP (re-challenge) should be discussed by the investigator with the study medical monitor after consideration of the benefit/risk to the subject and will only be done after the subject signs an informed consent.

7.6.2 Guidelines for Monitoring/Interruption for Clinically Significant Findings on ECG from Baseline, Cardiac-related Findings in Signs and Symptoms

In the event of a confirmed clinically significant change on ECG related to heart rhythm, rate, or any other findings, the investigator and the study medical monitor should consider a temporary interruption of IP. Dosing may resume after the subject's status and ECG as well, as other relevant parameters, stabilize or trend towards recovery (at the discretion of the investigator and in consultation with the study medical monitor).

If the subject reports as having palpitations (an unpleasant sensation of irregular and/or forceful beating of the heart) and/or has syncope (including vasovagal syncope, orthostatic syncope), the subject needs to be carefully examined (ECG, physical examination, etc).
If a coexisting clinically important finding is confirmed, the investigator and the study medical monitor should consider a temporary interruption of IP and decide if re-administration of the product is appropriate.

In the event of any clinically important changes in ECG parameters, IP should be temporarily discontinued, the findings should be investigated, and the investigator should inform the study medical monitor. If the subject (or investigator) wishes to withdraw consent the termination visit should be scheduled and completed.

Re-introduction of the IP (re-challenge) should be discussed by the investigator with the study medical monitor after consideration of the benefit/risk to the subject and will only be done after the subject signs an informed consent. If another clinically important change in ECG parameters is subsequently observed for the same subject, he/she should be permanently withdrawn from the study.

7.6.3 Guidelines for Monitoring/Interruption for Clinically Significant Findings from Gastrointestinal AEs

IP should be discontinued if a subject sustains severe gastrointestinal disorders that require hospitalization and/or other interventions.

Diarrhea:

IP should be discontinued if a subject reports sustained and severe diarrhea that requires hospitalization and/or an intravenous or nutritional supplementation or leads to severe electrolyte disturbances.

7.6.4 Guidelines for Monitoring/Interruption for Suicidal Ideation/Suicidal Behavior

Throughout the study, subjects and caregivers will be reminded to be aware of any emerging psychiatric changes.

In case a suicidal ideation (SI)/suicidal behavior (SIB), defined as an adverse event of special interest (AESI) in this study (see Appendix 3), is identified by the investigator, subjects will temporarily discontinue IP and will be referred to a mental health professional (MHP) and/or sent to the emergency room (ER). The MHP identified by the site will provide initial psychiatric

management and/or continuing cognitive behavioral therapy, per standard of care. These healthcare visits including any emergency room visits for urgent cases will be documented in the eCRF. A detailed site workflow in case of SI/SIB is provided in Appendix 8.

The site will report the event on the AE eCRF page and the Safety Report Form within 24 hours. The MHP will complete his or her evaluation of the subject and will provide an assessment to the site with recommendations that may include continuing psychiatric management per standard of care. The investigator will then assess the AESI for causality (relatedness to the IP) and will update the information on the AE eCRF page and the Safety Report Form. The investigator will also send his or her redacted notes on the case to the sponsor. If the AESI is confirmed as a case of SIB, the subject will be permanently discontinued from the IP and the study. The subject will be followed up for 28 days or until closure of the AE. If the AESI is not confirmed as a case of SIB, the investigator should consult with the sponsor to assess the benefits and risks of re-initiating IP in the subject. If the investigator and sponsor agree to restart the IP, the subject will continue in the study with routine safety assessments as planned in the Schedule of Activities. If the investigator and sponsor think it is not in the best interest for the subject to restart IP, despite assessment of causality being unrelated to IP, the subject will be permanently discontinued from the IP and the study. The subject will be followed up for 28 days or until closure of the AE.

Further, SI/SIB cases will be reported to and reviewed by the DMC.

Note that other psychiatric events should be captured as regular AE/SAE and followed per protocol (see Appendix 3).

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Study Periods

Refer to Table 1 (Placebo-controlled Part [Part A]) and Table 2 (Safety Extension Part [Part B]) for the schedule of study activities. Study assessments are detailed in Section 8.2.

8.1.1 Screening Period

8.1.1.1 Screening Visit (Visit 1)

The screening visit(s) (Visit 1) will be performed between 10 to 33 days before Visit 2. (The screening period can be as short as 10 days, if 7 consecutive days of diary data have been recorded prior to disimpaction and disimpaction takes as little as 3 days.) The assessments and procedures specified in Table 1 will be performed. The screening visit will result in a screen success or a screen failure.

- A screen success is a subject and/or their parent(s)/caregiver(s)/legally authorized representative(s) who has given informed consent/assent and met all inclusion criteria and none of the exclusion criteria.
- A screen failure is a subject who has given informed consent and failed to meet all inclusion criteria and/or met at least 1 of the exclusion criteria and has not been randomized or administered IP. A subject who has been designated as a screen failure may be rescreened – after an appropriate break (7-28 days) – up to 1 time. However, rescreened subjects must begin the screening procedure again, must be re-consented, and will be assigned a new subject number. Additionally, a subject's abnormal screening laboratory results may be repeated once for confirmation before designating a subject as a screen failure if the repeated assessment is conducted during the screening window.

All successfully screened subjects will enter the screening period. During the screening period it will be checked whether the subjects can fulfill the entry criteria for randomization into the study. Subject and/or parent(s)/caregiver(s)/legally authorized representative(s) will discontinue any laxatives and complete the required e-Diary entries.

All subjects need to undergo the fecal disimpaction procedure during the screening period (see Section 6.7.3.2). All subjects will be supplied with the appropriate fecal disimpaction medication at the screening visit (Visit 1).

The screening period includes at least 3 telephone contacts (and can include an unscheduled visit) to check the subject's symptoms and to decide when to start the fecal disimpaction procedure.

- The first telephone contact should occur approximately 7 days after the screening visit (Visit 1) to check the subject's symptoms. If the subject has <3 BMs during the previous 7 days (confirmed by the e-Diary entries), the disimpaction procedure can be planned for the following 7 days. In case the subject has ≥3 BMs during the previous 7 days

(confirmed by the e-Diary entries), the screening will continue. A follow-up call will be planned within 3-7 days. The call will be recorded in the eCRF.

- The second telephone contact should occur at the day of the fecal disimpaction procedure. The call will be recorded in the eCRF.
- The third telephone contact should occur within 2 days after the fecal disimpaction procedure to check if the procedure was successful. Subject/parent(s)/caregiver(s) may be invited for an on-site visit to discuss further disimpaction procedures with the investigator (unscheduled visit). If the disimpaction was successful, the subject can proceed to the baseline visit (Visit 2). If the disimpaction was not successful (or fecal impaction is still detected at the unscheduled visit), the subject is allowed to repeat disimpaction once, as long as within the screening window (up to 33 days), and the subject may receive a second round of disimpaction medication (see Section 6.7.3.2). The call will be recorded in the eCRF.

A successful disimpaction is a prerequisite for randomization.

8.1.1.2 Baseline Visit (Visit 2)

The baseline visit (Visit 2) will be scheduled within 7 days of the successful disimpaction. Note that the physical examination at Visit 2 may also include a rectal examination to confirm the presence or absence of fecal impaction (optional; if clinically warranted). If fecal impaction is still detected and repeating assessment would occur outside of screening window, subject will be allowed to rescreen (starting with the screening visit - Visit 1) with 1 remaining attempt for disimpaction.

After eligibility has been confirmed and all baseline procedures and assessments have been completed, a subject will be randomized to 1 of the 3 treatment arms as described in Section 4.1 and the first dose of IP will be administered.

Results of the baseline laboratory tests are not required for IP administration, but must be reviewed as soon as possible thereafter.

The assessments and procedures specified in Table 1 will be performed.

8.1.2 Placebo-controlled Part (Part A)

8.1.2.1 Visits 3-5, 7-9, and 11-13 (Weeks 1-3, 5-7, and 9-11)

Visits 3-5, 7-9, and 11-13 are telephone contacts (not on-site visits). These visits will be scheduled:

- on Day 7 (targeted to occur ±3 days) (Week 1).
- on Day 14 (targeted to occur ±3 days) (Week 2).
- on Day 21 (targeted to occur ±3 days) (Week 3).
- on Day 35 (targeted to occur ±3 days) (Week 5).

- on Day 42 (targeted to occur ±3 days) (Week 6).
- on Day 49 (targeted to occur ±3 days) (Week 7).
- on Day 63 (targeted to occur ±3 days) (Week 9).
- on Day 70 (targeted to occur ±3 days) (Week 10).
- on Day 77 (targeted to occur ±3 days) (Week 11).

At the investigator's discretion, an on-site visit may be scheduled instead of a telephone contact.

The assessments and procedures specified in Table 1 will be performed.

8.1.2.2 Visits 6, 10, and 14 (Weeks 4, 8, and 12)

Visits 6, 10, and 14 are on-site visits. These visits are scheduled will be scheduled:

- on Day 28 (targeted to occur ±3 days) (Week 4).
- on Day 56 (targeted to occur ±3 days) (Week 8).
- on Day 84 (targeted to occur ±3 days) (Week 12).

8.1.3 Safety Extension Part (Part B)

8.1.3.1 Visits 15a, 16, 18, 20, 22 (Weeks 14, 20, 28, 36, and 44)

Visits 15a, 16, 18, 20 and 22 are telephone contacts (not on-site visits). These visits will be scheduled:

- on Day 98 (targeted to occur ±3 days) (Week 14).
- on Day 140 (targeted to occur ±3 days) (Week 20).
- on Day 196 (targeted to occur ±3 days) (Week 28).
- on Day 252 (targeted to occur ±3 days) (Week 36).
- on Day 308 (targeted to occur ±3 days) (Week 44).

At the investigator's discretion, an on-site visit may be scheduled instead of a telephone contact.

The assessments and procedures specified in Table 2 will be performed.

8.1.3.2 Visits 15b, 17, 19, and 21 (Weeks 16, 24, 32, and 40)

Visits 15b, 17, 19, and 21 are on-site visits. These visits will be scheduled:

- on Day 112 (targeted to occur ±3 days) (Week 16).
- on Day 168 (targeted to occur ±3 days) (Week 24).
- on Day 224 (targeted to occur ±3 days) (Week 32).
- on Day 280 (targeted to occur ±3 days) (Week 40).

8.1.3.3 Visit 23 (Week 48): Final Visit or Early Termination

Visit 23 is scheduled to take place on Day 336 (targeted to occur ±3 days) (Week 48). The assessments and procedures specified in Table 2 will be performed. The Week 48 assessments and procedures will also form the ET assessments for any subject who is withdrawn early or discontinued from the study.

A subject's status for study completion will be collected in the eCRF. The subject is considered to have completed the study if IP was administered without discontinuation of study treatment (see Section 7) prior to Visit 23.

8.1.4 Follow-up Period

The follow-up period for this protocol is 4 weeks (28 days). The follow-up period starts after Visit 23 or the ET visit. No additional data will be collected after subject completes the follow-up period. Subjects can return to standard of care.

At the end of this period there will be a logged telephone call or email (with confirmation receipt) initiated by the site staff to query for SAEs, AEs, and concomitant and prohibited medications. All AEs and SAEs that are not resolved at the time of this contact will be followed to closure (see Appendix 3).

8.1.5 Unscheduled Visits

An unscheduled visit may occur when the subject returns to the clinic at any time during the treatment period for additional evaluation of symptoms or side effects, study medication resupply, or additional laboratory sample redraws. An unscheduled visit may also occur for other reasons (eg, technical issues with the e-Diary), in consultation with the sponsor/CRO. The date and reason for the unscheduled visit will be recorded in the source documents and the eCRF.

If an unscheduled visit occurs, at a minimum, the following procedures are to be performed:

- Concomitant medications.
- AE monitoring.
- Access IRT, if replacement IP is needed.
- e-Diary compliance.

Additional procedures described in Table 1 and Table 2 may be performed at the investigator's discretion. If required per protocol based on subject's laboratory results during the study, additional procedures may be performed at this visit. Repeated laboratory assessments may be performed, as appropriate (refer to separate laboratory manual). If the visit results in premature discontinuation, ET assessments should be performed.

8.1.6 Additional Care of Subjects after the Study

No aftercare is planned for this study.

8.2 Study Assessments

Every effort should be made to ensure that the protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances outside the control of the investigator which may make it unfeasible to perform the tests and procedures. In these cases, the investigator will take all steps necessary to ensure the safety and wellbeing of the subject.

All clinical outcome or patient-reported questionnaires should be completed before completing any other visit assessments.

8.2.1 Demographic and Other Baseline Characteristics

Subject demographic information including sex, age, ethnicity, country/region, and race will be collected prior to the subject receiving the first dose of IP.

8.2.1.1 Height and Weight

A subject should have weight and height or length, as age appropriate, measured while wearing indoor clothing and with shoes off. Care should be taken to ensure accurate height and weight measurements are obtained and the same method of measurement (same measuring device/scale) is used at required visits.

8.2.1.2 Medical and Medication History

Medical and medication history will be collected and recorded in the subject's source documents.

The severity of constipation will be recorded, and baseline characteristics of the functional constipation (current symptoms) will be documented, including a history of retentive posturing or excessive volitional stool retention and other symptoms included in the Rome IV criteria. A repeat assessment of the Rome IV criteria will be completed at the Week 12 visit or at the study visit associated with an early termination in Part A.

In addition, in order to assess transit time, specific questions will be included on night or daytime soiling and absent urge to defecate. To assess IBS with constipation (IBS-C), specific questions will be included on IBS-C diagnosis.

8.2.2 Efficacy

To record efficacy, the subject and/or parent(s)/caregiver(s)/legally authorized representative(s) must be able to read and/or complete the e-Diary and questionnaires.

8.2.2.1 E-Diary

All subjects and/or parent(s)/caregiver(s)/legally authorized representative(s) who are to complete the e-Diary during the course of the study, must be trained on its use and interpretation. The e-Diary will be administered electronically and in the event of technical device failure a web-based back-up system will be used.

There will be 3 versions of the e-diary:

- self-completed for toilet-trained subjects ≥8 years of age
- caregiver completed for toilet-trained subjects ≥3 but <8 years of age, with subject input, as appropriate
- caregiver completed for non-toilet-trained subjects ≥6 months years of age

Each version will address only those signs and symptoms that are essential for daily data capture in each subpopulation. Note that the use of the e-Diary and included questionnaires will be based on age at consent.

The end user completing the e-Diary should preferably remain the same throughout the duration of the study. Should a backup be required, this person must also be trained on the use of the e-Diary.

Efficacy will be assessed based on variables recorded in the e-Diary.

A summary of the items that will be recorded daily during the screening, Part A, and Part B parts of the study is provided below.

- e-Diary items to be recorded during screening for toilet-trained subjects:
  - for each bowel movement: date and time, consistency (using the BSFS), level of straining needed (using a 3-level Likert scale), worst abdominal pain (using Wong-Baker for subjects ≥3 to <8 years of age and the 11-point NRS for subjects ≥8 years of age)
- e-Diary items to be recorded during screening for non-toilet-trained subjects:
  - for each bowel movement: date and time, consistency (using the BSFS), level of straining needed (using a 3-level Likert scale)
- e-Diary items to be recorded during Part A for toilet-trained subjects:
  - date and time of IP intake
  - for each bowel movement: date and time, consistency (using the BSFS), level of straining needed (using a 3-level Likert scale), worst abdominal pain (using Wong-Baker for subjects ≥3 to <8 years of age and the 11-point NRS for subjects ≥8 years of age)
  - rescue medication: date and time
- e-Diary items to be recorded during Part A for non-toilet-trained subjects:
  - date and time of IP intake
  - for each bowel movement: date and time, consistency (using the BSFS), level of straining needed (using a 3-level Likert scale),
  - rescue medication: date and time

- e-Diary items to be recorded during Part B for all subjects:
  - date and time of IP intake
  - rescue medication: date and time

Paper outlines of the e-Dairy including all items (ie, those for Part A) are provided in Appendix 9, Appendix 10, and Appendix 11 for non-toilet-trained subjects who are at least 6 months of age, for toilet-trained subjects ≥3 but <8 years, and for subjects ≥8 years, respectively.

Subjects should be instructed to start completing daily evening diaries once the 5-day washout period for prohibited therapies has been completed at the start or during the screening period.

Additional information on different outcome measurements included in the e-Diary (Wong-Baker faces scale, NRS, and BSFS) are provided in the sections below.

Wong-Baker Faces Scale

In toilet-trained subjects aged ≥3 to <8 years at baseline, worst abdominal pain will be measured using the Wong-Baker self-assessment faces scale within the e-Diary. Although the caregiver should complete other e-Diary assessments for this age group, the Wong-Baker faces Scale should be completed with subject input (ie, subjects need to select the face they feel illustrates the pain they are experiencing).

Assessments performed by caregivers and subjects will be analyzed separately.

The Wong-Baker Faces Scale is provided in Appendix 14.

Numerical Response Scale

In subjects aged ≥8 years, worst abdominal pain will be measured within the e-Diary using an 11-point NRS where 0 presents no pain and 10 presents the worst pain imaginable. Subjects will only be able to select whole numbers on the NRS.

A screenshot of the planned NRS within the e-Diary is provided in Appendix 15.

Bristol Stool Form Scale (BSFS)

Stool consistency will be scored using the BSFS scale.

Subjects aged ≥8 years (at screening) need to complete the e-Diary using the BSFS themselves. For subjects <8 years, who are toilet-trained the parent(s)/caregiver(s)/legally authorized representative(s) should complete the e-Diary using the BSFS based on their interactions and observations. For subjects at least 6 months of age who are not toilet-trained, the parent(s)/caregiver(s)/legally authorized representative(s) should complete the e-Diary using the BSFS.

Assessments performed by caregivers and subjects will be analyzed separately.

The standard BSFS is provided in Appendix 16.

8.2.2.2 Impression of Severity Assessment

The PGI-S and CGI-S will only be used during Part A of the study and should be administered prior to the PedsQL Gas and Bloating questionnaire discussed in Section 8.2.2.3. It is also recommended to be completed prior to any other clinical assessments or procedures.

PGI-S

The PGI-S is a single question that will be administered to toilet-trained subjects aged ≥8 years (at screening) as outlined in the Schedule of Activities (Table 1) and Appendix 6. Subjects will self-complete the questionnaire.

The PGI-S is a global index that is used to capture the patient's overall perception of their constipation severity (a single-state scale) and will be completed electronically.

The PGI-S is provided in Appendix 17.

CGI-S

The CGI-S is a single question that will be administered to caregivers of toilet-trained subjects 3 to <8 years as outlined in the Schedule of Activities (Table 1) and Appendix 6.

The CGI-S is designed for caregivers to evaluate the severity of their child's condition and will be completed electronically.

The CGI-S is provided in Appendix 17.

8.2.2.3 Pediatric Quality of Life Inventory Gastrointestinal Symptom Scales (PedsQL GI; Gas and Bloating Module)

At the times indicated in the Schedule of Activities (Table 1 [Part A only]) the subject and/or parent(s)/caregiver(s)/or legally authorized representative(s) will be asked to electronically complete the PedsQL GI Acute version 3.0 Module on gas and bloating.

The PedsQL GI score is a series of validated independent questionnaire modules to assess the quality of life in subjects with gastrointestinal symptoms. The modules to be used in this study include the parent report form for toilet-trained toddlers (2-4 years) and young children (5-7 years) and the self-report form for children (8-12 years) and for teens (13-18 years). Only the gas and bloating (7 questions) module of the PedsQL GI will be used and symptoms will be assessed over the previous 7 days.

All questioned items are rated on a 5-point scale for "problems with" (0=never, 1=almost never, 2=sometimes, 3=often, 4=almost always).

Subjects aged ≥8 years (at screening) need to complete the questionnaire themselves. For younger toilet-trained subjects (≥3 to <8), parent(s)/caregiver(s)/legally authorized representative(s) should complete the questionnaire based on their interactions and observations.

Assessments performed by caregivers and subjects will be analyzed separately. The assessments will not be completed for non-toilet-trained subjects.

A listing of PedsQL GI Gas and Bloating instrument is provided in Appendix 12. An excerpt of the PedsQL GI including the only the Gas and Bloating Module for each age group is provided in Appendix 13 (Copyright© 1998 JW Varni, Ph.D. All Rights Reserved. Not to be reproduced without permission).

8.2.2.4 Retentive Posturing

Retentive posturing is defined as the attempt to preserve continence by vigorous contraction of the gluteal muscles. Children with retentive posturing will be typically tight legged, tiptoed, and/or will have a back-arching posture.

At the times indicated in the Schedule of Activities (Table 1 [Part A only]) the subject and/or parent(s)/caregiver(s)/or legally authorized representative(s) will be asked whether retentive posturing has occurred during the previous 4 weeks by the clinical investigator. This assessment is applicable only to toilet-trained subjects at least 3 years of age. The investigator will log responses in source notes and eCRF.

8.2.2.5 Fecal Incontinence

Fecal incontinence is defined as unintentional smear or liquid stool in the underwear that is not due to poor wiping. Fecal incontinence can only occur in toilet-trained subjects. Non-retentive fecal incontinence is diagnosed (must include at least a 1-month history in a child with a developmental age older than 4 years for all the following): (i) defecation in places inappropriate to the sociocultural context, (ii) No evidence of fecal retention, and (iii) after appropriate evaluation, the fecal incontinence cannot be explained by another medical condition.

At the times indicated in the Schedule of Activities (Table 1 [Part A only]) the subject and/or parent(s)/caregiver(s)/or legally authorized representative(s) will be asked whether fecal incontinence has occurred during the previous 7 days by the clinical investigator. This assessment is applicable only to toilet-trained subjects at least 3 years of age. The investigator will log responses in source notes and eCRF.

8.2.3 Safety

8.2.3.1 Physical Examination

A physical examination will be performed by the investigator. The initial physical examination (conducted during screening) will include the following:

- Height, weight, and body mass index (BMI).
- Inspection and palpation of thyroid.
- Respiratory and cardiovascular exams.
- Abdominal exam (muscle tone, distension, palpable abdominal mass, fecal mass).

- Exam of perianal region (anal position, stool staining around anus or on undergarments, erythema, skin tags, anal fissure).
- Exam of lumbosacral region (dimple, tuft of hair, gluteal cleft deviation, sacral agenesis, flat buttocks).
- Lower limb neuromuscular examination including tone, strength, and deep tendons reflexes.
- Optional: Digital rectal exam if clinically indicated or at the discretion of the investigator.
- Optional: Anal and cremasteric reflex if clinically indicated or at the discretion of the investigator.

Subsequent physical examinations, conducted during the Placebo-controlled Part (Part A) and the Safety Extension Part (Part B) will include the following:

- Respiratory and cardiovascular exams.
- Abdominal exam (muscle tone, distension, fecal mass).
- Optional: exam of perianal region (anal position, stool staining around anus or on undergarments, erythema, skin tags, anal fissure) if clinically indicated or at the discretion of the investigator.

Abnormalities identified at the screening visit (Visit 1) and at subsequent study visits will be recorded in the subject's source documents.

8.2.3.2 Adverse Events

At each study visit, subjects will be questioned in a general way to ascertain if AEs have occurred since the previous visit (eg, "Have you had any health problems since your last visit?"). Adverse events are collected from the time informed consent is signed. Refer to Appendix 3 for AE definitions, assessment, collection time frame, and reporting procedures.

8.2.3.3 Vital Signs

Vital signs include blood pressure and pulse rate. Blood pressure should be determined by cuff (using the same method, the same arm, and the same position throughout the study).

Vital signs should be taken supine after at least 5 minutes of rest and every attempt should be made to assure that the subject is calm (ie, not crying).

Vital signs measurements will be recorded in the eCRF. Any clinically significant deviations from baseline (Visit 2) vital signs that are deemed clinically significant in the opinion of the investigator are to be recorded as an AE.

The investigator will assess whether a change from baseline (Visit 2) in vital signs may be deemed clinically significant and whether the change should be considered and recorded as an AE.

8.2.3.4 Clinical Laboratory Tests

All clinical laboratory tests will be performed according to the laboratory's standard procedures. Reference ranges will be supplied by the laboratory and used to assess the results for clinical significance and out-of-range changes which may be associated with, or constitute, an AE. The investigator should assess out-of-range clinical laboratory values for clinical significance, indicating if the value(s) is/are not clinically significant or clinically significant.

Abnormal clinical laboratory values which are unexpected or not explained by the subject's clinical condition, may, at the discretion of the investigator or sponsor, be repeated as soon as possible until confirmed, explained, or resolved.

A complete list of the clinical laboratory tests to be performed is provided in Appendix 2.

In addition, in subjects up to the age of approximately 6 years, a venipuncture will occur with a small or standard butterfly needle. For subjects above the age of approximately 6 years, a venipuncture will occur with a standard butterfly needle or a small standard needle.

The use of local anesthetic creams is allowed to minimize the pain during blood draw.

8.2.3.5 Pregnancy Test

A serum (at screening) or urine (at all other time points indicated in Table 1) pregnancy test will be performed on all female subjects aged ≥12 years and/or subjects <12 years who have started menarche at the times indicated in the study Schedule of Activities (Table 1), or if pregnancy is suspected.

A positive urine pregnancy test must be followed with a serum beta-human chorionic gonadotropin (β-hCG) pregnancy test performed by the central laboratory. Additional testing can be performed at the investigator's discretion. Also, refer to Appendix 3.8, Pregnancy.

8.2.3.6 12-Lead Electrocardiogram

The ECG should be performed before laboratory blood collection.

The eligibility of the subject will be based on the assessment of the ECG by the investigator at screening (Visit 1).

If abnormal results are observed, the investigator, in consultation with the sponsor, will reconfirm the subject's eligibility to continue.

8.2.4 Clinical Pharmacology

For subjects who consent to the sparse PK sampling, their plasma prucalopride concentrations will be pooled with existing data and that data will be analyzed using population PK modeling approach.

One blood sample will be collected after the first dose of prucalopride (Baseline/Visit 3) around the time to maximum observed plasma concentration (t_{max}) (between 1 to 3 hours post-dose).

Three additional samples will be taken, once each at Weeks 4, 8, and 12, near trough concentrations (between 14 and 26 hours post-dose) once steady state conditions have been established. Blood samples (1 mL) for drug analysis will be obtained at each visit or at premature discontinuation, simultaneously with the blood sample for clinical laboratory tests. The date, time, and dose of the last two intakes of trial medication and the exact time of blood sampling are to be recorded on the laboratory sample requisition form.

8.2.4.1 Blood Sample Collection and Handling Procedures

Blood samples will be collected at the time specified in study Schedule of Activities (Table 1 and Table 2) to measure plasma concentrations of prucalopride. Details of sample collection (including recording the exact time of blood sampling), handling, shipment, and bioanalysis will be provided in the laboratory manual.

8.2.4.2 Plasma Drug Assay Methodology

Plasma sample analysis will be performed according to GCP and Organization for Economic Co-operation and Development regulations and applicable bioanalytical CRO Standard Operating Procedures.

Plasma concentrations will be measured using a validated bioanalytical method. In addition, selected plasma samples may be used to assess incurred sample reproducibility. The presence of other metabolites or artifacts may be monitored or quantified as appropriate. Raw data will be stored in the archive of the designated bioanalytical contract laboratory.

8.2.5 Volume of Blood to Be Drawn from Each Subject

The volume of blood drawn will be approximately 7.0 mL per visit in subjects >6 years, approximately 4.5 mL per visit in subjects 2-6 years, and approximately 2.8 mL per visit in subjects <2 years.

For subjects who consent to the sparse PK sampling, an additional 4 mL may be added to the total volumes of each subject, regardless of age group.

Efforts will be made to optimize the use of available blood samples (specialized laboratories which are familiar with lowering the amount of blood volume taken will be used). The use of local anesthetic creams is allowed to minimize the pain during blood draw.

Note: The amount of blood to be drawn for each assessment is an estimate. The amount of blood to be drawn may vary according to the instructions provided by the manufacturer or laboratory for an individual assessment. When more than 1 blood assessment is to be done at the time point/period, if they require the same type of tube, the assessments may be combined.

Table 3 Volume of Blood to Be Drawn from Each Subject

Assessment		Sample Volume (mL)	Number of Samples	Total Volume (mL)
Subjects aged >6 years				
Clinical pharmacology (optional [a])		*1*	*4*	*4*
Safety [b]	Biochemistry	5.0	6	30
	Hematology	2	6	12
Total mL				**42-46**
Subjects aged 2-6 years				
Clinical pharmacology (optional)		*1*	*4*	*4*
Safety	Biochemistry	3.3	6	19.8
	Hematology	1.2	6	7.2
Total mL				**27-31**
Subjects aged <2 years				
Clinical pharmacology (optional)		*1*	*4*	*4*
Safety	Biochemistry	2.2	6	13.2
	Hematology	0.6	6	3.6
Total mL				**16.8-20.8**

[a] For subjects and/or their parent(s)/caregiver(s)/legally authorized representative(s) who consent to PK sampling.
[b] Screening serum pregnancy is included in these volumes, as required for all female subjects aged ≥12 years and/or subjects <12 years who have started menarche

8.3 Changes to Study Procedures Due to COVID-19 or Similar Pandemic

The following information provides guidance regarding changes to the study procedures that could be implemented for study participants or study sites affected by the COVID-19 Public Health Emergency. This guidance takes references from the FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Public Health Emergency - Guidance for Industry, Investigators, and Institutional Review Boards, March 2020, updated 03 June 2020, and the EMA Guidance on the Management of Clinical Trials During the COVID 19 (Coronavirus) Pandemic, Version 3 (28 April 2020).

As the COVID-19 pandemic may peak in different regions at different times and restrictions implemented by local laws and recommendations may vary, any decision on procedural changes should be made on a case-by-case basis by the principal investigator in consultation with the study team and the medical team as needed, while maintaining patient safety and confidentiality as the priority.

Procedural changes due to COVID-19 may include the following:

- Visits at Weeks 4 and 8 in Part A and visits at Weeks 16, 32, and 40 in Part B may be converted to home healthcare visits or phone calls to extend flexibility to subjects during the COVID-19 public health emergency. Home healthcare visits/phone calls will be documented in the study records and eCRF. The data collected from home healthcare visits/phone calls may be handled differently in the final data analysis, with this documented in the statistical analysis plan. All other planned in-clinic visits must be done with the subject present at the site. Any conversion of visits must be approved by the

sponsor. If it is not possible to perform an on-site or a home-based visit due to local restrictions related to COVID-19, the site staff will contact the subject by phone to assess for AEs, concomitant medication use and any additional assessments that are possible to assess over the phone (e.g. fecal incontinence assessment).

- For home healthcare visits, collection of scheduled clinical laboratory samples (blood specimen collection or other diagnostic tests) will be performed by the investigator, qualified site staff, or a contracted qualified home health care professional who can visit the subject at home. All laboratory samples should still be sent to the designated laboratory as outlined in the laboratory manual.
- Sites may seek approval to extend a visit window in order to conduct an on-site visit. Assessments that cannot be completed during the protocol-specified window or within the visit window granted by the sponsor or designee will be considered missing data and such departures will be recorded in the study records.
- Missed clinic visits or subject withdrawals due to COVID-19 must be recorded on the eCRF (see Section 7.3). Subjects who discontinued from screening or run-in period due to COVID-19-related factors but were otherwise qualified to participate in the study may be rescreened. Any rescreening must be approved by the Takeda medical monitor.
- ECG procedures: For home healthcare visits, ECGs may be performed by a qualified health care professional who is authorized/certified to perform such tests routinely.
- Allow the use of a web-based back-up system on electronic devices. Allow Weeks 4, 8, 16, 32, and 40 electronic clinical outcomes assessments (eCOAs) typically scheduled for completion at the clinic to be completed at home if a site visit cannot occur.
- In specific circumstances and with sponsor approval, it may be allowed to transfer subjects to sites away from risk zones or closer to their homes (either to new sites or sites already participating in the study).
- Deviations from the protocol-specified procedures (eg, laboratory assessments) due to COVID-19 will be recorded as related to COVID-19.
- During the COVID-19 public health emergency, alternative IP delivery to study participants may be necessary to avoid unnecessary visits to sites while providing needed IP. If there is a high risk that an upcoming visit may be converted from an on-site to off-site visit, additional IP may be dispensed during a scheduled study visit. If needed, IP may also be shipped directly from investigational sites to participants' residences by a contracted logistics provider or distributor (direct-to-patient [DTP] shipment) in compliance with national laws or temporary national emergency measures and Takeda processes. IP should continue to be assigned by IRT in all cases. IRT may be accessed in advance of scheduled administration to allow time for DTP shipment or to allow for the dispensation of additional IP.

All subject discontinuations and alternative approaches to study procedures (ie, procedures not conducted per the Schedule of Activities) due to the COVID-19 pandemic must be documented in the study records. Data collected using alternative methods may be handled differently in the final data analyses. This will be documented in the Statistical Analysis Plan.

9. STATISTICAL CONSIDERATIONS

9.1 Statistical Analysis Process

The study will be analyzed by the sponsor or its agent.

The statistical analysis plan (SAP) will provide the statistical methods and definitions for the analysis of the efficacy and safety data, as well as describe the approaches to be taken for summarizing other study information such as subject disposition, demographics and baseline characteristics, IP exposure, and prior and concomitant medications. The SAP will also include a description of how missing, unused, and spurious data will be addressed.

The SAP will be finalized prior to the database lock, to preserve the integrity of the statistical analysis and study conclusions. Statistical issues related to the impact of the COVID-19 pandemic will be more fully developed in the SAP. Unless otherwise specified, the primary analysis will include all subjects with usable data in the respective analysis datasets. Subjects identified as being affected by COVID-19 will be classified prior to treatment unbinding.

All statistical analyses will be performed using SAS® Version 9.4 or higher (SAS Institute, Cary, NC 27513).

9.2 Planned Interim Analysis and DMC

9.2.1 DMC

An independent DMC will be installed to monitor accumulating safety data in this study including SI/SIBs to detect evidence of possible safety issues. (Additional information on the referral of SI/SIB to the DMC is provided in Section 7.6.4.) The DMC will also be involved in both planned interim analyses to assess if efficacy is sufficient to continue the study. The DMC is independent and will give recommendation to the sponsor with regards to the termination of study. Ideally, the DMC will include 3 clinicians (of which at least one is a pediatrician), a PK expert, and a statistician. The exact composition will be described in the DMC charter.

Unblinded data will be at the disposal of the DMC. The DMC review will consist of a closed session and an open session. During the closed session, the DMC will look at the data (blinded and if needed unblinded) and come to a recommendation for the sponsor. During the open session, the DMC recommendation is discussed with the sponsor (blinded). The sponsor remains blinded. The composition of the DMC, the responsibilities of all DMC members, and the relation between DMC and sponsor will be described in more detail in a separate DMC charter. The DMC chair or sponsor can request additional analyses or meetings. The DMC chair can invite additional internal or external experts to discuss specific issues.

For safety monitoring, the DMC will meet every 6 months. A DMC meeting can be cancelled when, compared to the previous DMC meeting, less than 15 additional subjects are randomized.

9.2.2 Interim Analyses

Two IA will be conducted during the study.

The primary objective of the first IA is to evaluate stopping the study for futility. The first IA will be conducted when 50% of target number of toilet-trained subjects who are at least 3 years of age (ie, 120 subjects) are randomized into the study and have either completed or had withdrawn from the Placebo-controlled part (Part A). Futility evaluation will be based on the conditional power (Lan and Wittes 1988) using a stopping threshold of 20% for each dose arm. Only if both comparisons, low dose and high dose versus placebo, based on the primary endpoint have a conditional power (probability) of less than 20% the study will be stopped for futility. In all other situations the study will continue as planned, with both high and low dosing arms and the placebo arms.

A second IA will be performed when all randomized and toilet-trained subjects have completed or have withdrawn from the Placebo-controlled Part (Part A). At this IA, the primary endpoint will be analyzed as planned. The DMC will then make a recommendation to the sponsor to stop or continue the remainder of the study (Part B). If one of the doses shows a statistically significant difference (using the Hochberg step-up procedure for adjustment of multiplicity) with placebo, the study will be continued.

To maintain data integrity, the results from the IAs based on unblinded data will be accessible only to the independent statistician and external DMC, such that they can make a recommendation to the sponsor. At all points, the study team directly involved in the conduct of the study will remain blinded to maintain the double-blind nature of the study (see Section 6.1.2). All outputs for the interim analysis will be prepared by an independent analysis team. More details will be explained in the charter for the DMC.

9.3 Sample Size and Power Considerations

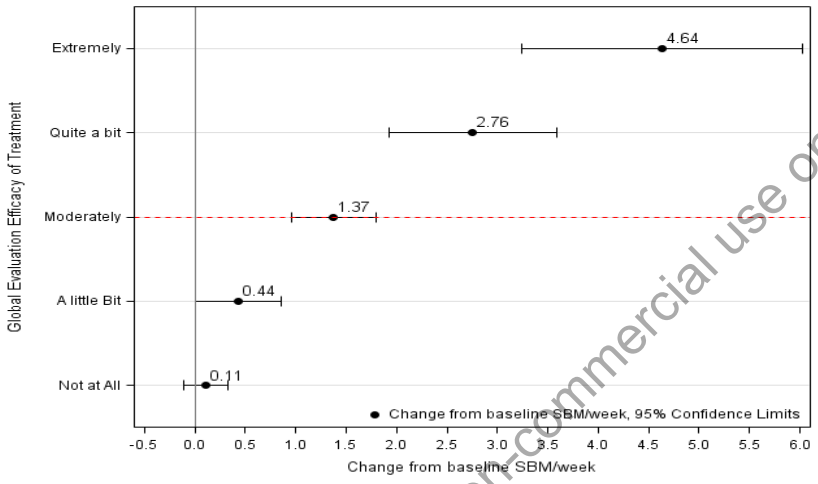
There is limited literature available with information of efficacy measures in children with functional constipation. For that reason, the data from a previous pediatric study with prucalopride (SPD555-C303) was used to estimate current primary endpoint - change from baseline in the average number of SBMs per week derived from the (diary) data over 12 weeks. In that 8-week study only the last 4 weeks (Weeks 5-8) of the treatment phase were used for evaluation of primary efficacy[1] and over this period the average change in SBMs/week was approximately 1.40 SBM/week for placebo and 1.52 SBM/week for prucalopride. The overall average change in SBMs/week was 1.5 with a pooled standard deviation of 2.30 (standard deviation in placebo arm was 2.02 and in prucalopride treatment arm 2.57). This change is based on a selection of subjects with <3 SBM/week at baseline, corresponding to the inclusion criteria of the current study.

[1] In study SPD555-C303, the first two weeks of the double-blind treatment phase were not used for the primary efficacy analysis because of a possible effect from the use of an enema or oral laxative agent during the screening phase to remove an impaction. Weeks 3 and 4 were used to evaluate the efficacy for a possible dose adjustment after 4 weeks of treatment. Therefore, the primary endpoint was evaluated over Weeks 5 to 8.

An attempt was made to get an estimate of the minimum clinically important difference (MCID) in average change from baseline of number of SBM/week, based on the data of the previous pediatric study with prucalopride (SPD555-C303).
For this, the change from baseline in number of SBM/week was estimated per outcome score of the global evaluation of efficacy of treatment scale (the anchor) after 8 weeks of treatment. This scale had 5 outcome scores: not at all -, a little bit -, moderately - quite a bit- and extremely effective).

Figure 2 presents the presents the average change from baseline over all subjects, per outcome of the global evaluation of efficacy of treatment.

Figure 2 Average Change from Baseline in Spontaneous Bowel Movements (Study SPD555-C303)



If we consider a moderate efficacy of treatment as a minimum clinically important difference, then it can be seen that those subjects have an average change from baseline of 1.37 SBM/week.

The 6 key efficacy studies with prucalopride in adults, showed a treatment difference of 1.66 SBM/week for the subgroup of patients with <3 SBM/week at baseline.

Based on all this information and since this is a pediatric study, a difference of 1.40 SBM/week between a prucalopride treatment arm and placebo was chosen as a (clinically meaningful) treatment effect for the sample size estimation.

As we expect to observe a higher treatment effect with one of the doses of prucalopride in current study, as compared to the previous pediatric study SPD555-C303, this might result in more variability and therefore a slightly higher value for the pooled standard deviation of the change from baseline will be assumed to be 2.50.

The sample size was estimated through statistical simulations based on the Hochberg step-up procedure to control the type I error rate for primary efficacy endpoint. These simulations showed that with 80 toilet-trained subjects who are at least 3 years of age per treatment arm, Part A of the study will have at least 90% power to detect a treatment difference of 1.40 in

primary efficacy endpoint between at least one active dose versus placebo assuming pooled SD of 2.5, using a two-sided two-sample t-test at a significance level of 5% based on the Hochberg step-up procedure to control the type I error rate for primary efficacy endpoint.

In addition, for exploratory purposes, it is targeted to enroll a maximum of 15 non-toilet-trained subjects.

9.4 Estimands

The primary estimand of interest will be composite estimand and corresponding analysis methods are described below. Additional details will be provided in the SAP.

- Population: modified intent-to-treat (mITT) (see Section 9.5 for population definitions).
- Primary efficacy endpoint: the average change from baseline in number of SBMs per week derived from the (e-Diary) data over 12 weeks.
- Any BM that occurs within 24 hours after intake of the rescue medication will not be considered as spontaneous.
- Intercurrent events (ICEs): Discontinuation of treatment due of lack of efficacy and/or AE is considered an ICE in this study. Usage of rescue medication is the other ICE and is captured through the variable definition.

The "composite strategy" will be utilized to address the ICEs, where discontinuation due to lack of efficacy or AE will be considered as undesired treatment outcome in the analysis. We will continue to collect data for subjects while on rescue medication.

- Population-level summary: 12-week average of the weekly average change from baseline in number of SBMs/week.
- Missing Data Handling: Missing data will be imputed using a hybrid imputation approach prior to analyses of primary and key secondary efficacy endpoints.

Subjects who discontinued due to lack of efficacy or AE during the course of the study will be considered as having undesired treatment outcome in the primary analysis. Subsequently, their missing data will be imputed using the worst observation carried forward (WOCF) under the missing not at random (MNAR) assumption. All other discontinuation/intermittent missingness will be imputed using multiple imputation under the missing at random (MAR) assumption. For multiple imputation, the weekly number of SBMs will be imputed by treatment group using a multivariate stepwise approach using a fully conditional specification regression method (Ratitch et al. 2013). Missing baseline weekly number of SBMs, if any, will be imputed using age and baseline global severity score. Missing post-baseline weekly number of SBMs will be imputed using all previous weeks in a stepwise fashion. Twenty multiply imputed datasets will be generated. Lastly, the imputed data under MNAR and MAR will be combined to obtain a complete dataset containing all subjects in the analysis population, and will be used to perform the pre-specified statistical modeling for primary and key secondary efficacy endpoints using mixed effects model for repeated measures (MMRM) for continuous endpoints and Cochran Mantel-Haenzel (CMH) test for binary endpoint.

- Primary Analysis: The primary estimand will be estimated from a MMRM based on restricted maximum likelihood using the imputed data from the hybrid imputation approach. The MMRM will include treatment group, age groups, study week, treatment-group-by-study-week interaction as fixed effects, baseline number of SBM/week as a covariate and subject as a random effect. An unstructured variance-covariance matrix will be used to model the within-subject errors for both treatment groups. The average change from baseline over 12 weeks will be estimated (LS means) by the above MMRM. The treatment difference in LS means between active treatment group versus placebo will be estimated. P-value, treatment difference in LS means and associated 95% confidence interval from the multiple imputed datasets will be combined using Rubin's rules, as implemented in the PROC MIANALYZE procedure.

Sensitivity analyses are discussed in Section 9.6.7.

Additional details regarding Tipping Point Analysis will be presented in the SAP.

9.5 Statistical Analysis Sets

Toilet-trained subjects who are at least 3 years of age

- Screened Set: The screened set will consist of all toilet-trained subjects who are at least 3 years of age and who have signed an informed consent form.
- Enrolled Set: The enrolled set will consist of all toilet-trained subjects who are at least 3 years of age and who have signed informed consent and also passed inclusion/exclusion criteria.
- Intent-to-treat (ITT) Analysis Set: The ITT analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age.
- mITT Analysis Set: The mITT analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age and who receive at least 1 dose of IP. The treatment arm will be the arm to which they were randomized, regardless of what treatment they received.
- Safety Analysis Set: The safety analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age and who receive at least 1 dose of IP. Subjects will be analyzed according to treatment received.
- Per-protocol (PP) Analysis Set: The PP analysis set will consist of all toilet-trained randomized subjects who are at least 3 years of age and who do not have major protocol deviations that may affect the primary efficacy endpoint. Subjects with the following deviations (including, but not limited to), will be excluded from the PP Analysis Set:
    - Violations of inclusion and/or exclusion criteria: A subject who violated any inclusion and/or exclusion criteria will be excluded from the PP Analyses Set.

- Compliance with study medication: A subject, who was less than 80% or more than 120% compliant (can occur when the subject/caregiver intentionally or unintentionally overdoses) with his/her assigned treatment, as assessed by tablet count or liquid volume from the date of first dispensing to the date of last dose, will be excluded.
- Incorrect timing of assessments: A primary/key secondary endpoint assessment performed outside a ±3 day window for the nominal visit time will be classified as a major protocol deviation.
- Prohibited concomitant medications that could have a potential effect on efficacy endpoints. Protocol violations based on prohibited concomitant medications will be defined at the at the final data review meeting before database lock.
- Taking wrong IP, ie, not the IP as randomized.
- Based on the deviations as determined at database closure subjects with other major protocol deviations might be excluded from the per-protocol set. All subjects excluded will be listed with the reason for exclusion.

– Completers Analysis Set: The Completers Analysis Set will consist of all toilet-trained subjects who are at least 3 years of age in the mITT Analysis Set who have completed the daily diary for at least 12 weeks during Part A of the study.

Non-toilet-trained Subjects

– Non-toilet-trained Enrolled Set: The enrolled set will consist of all non-toilet-trained subjects who have signed informed consent and also passed inclusion/exclusion criteria.

– Non-toilet-Trained Intent-to-treat (NTTITT) Analysis Set: The ITT analysis set will consist of all non-toilet-trained randomized subjects. The treatment arm will be the arm to which they were randomized, regardless of what treatment they received.

– Non-toilet-trained Modified Intent-to-treat (NTTmITT) Analysis Set: The mITT analysis set will consist of all non-toilet-trained randomized subjects who receive at least 1 dose of IP. The treatment arm will be the arm to which they were randomized, regardless of what treatment they received.

– Non-toilet-trained Safety Analysis Set (NTTSAF): The safety analysis set will consist of all non-toilet-trained randomized subjects who receive at least 1 dose of IP. Subjects will be analyzed according to treatment received.

PK analysis Set

– PK Analysis Set: The PK Analysis Set is defined as all subjects regardless of age in the Safety Analysis Sets and for whom at least 1 PK sample is evaluable.

9.6 Efficacy Analyses

9.6.1 Primary Efficacy Endpoint

The primary efficacy endpoint is defined as the average change from baseline in number of SBMs per week derived from the (diary) data over 12 weeks, collected during the placebo-controlled part (Part A).

A BM is defined as spontaneous (SBM) if not preceded within a period of 24 hours by the intake of rescue medication.

9.6.2 Primary Endpoint Hypothesis

The null and alternative hypotheses for the primary efficacy analysis are:

- Null hypothesis 1: There is no difference in mean change from baseline in number of SBMs/week over 12 weeks between the low dose and placebo.
- Alternative hypothesis 1: There is a difference in mean change from baseline in number of SBMs/week over 12 weeks between the low dose and placebo.
- Null hypothesis 2: There is no difference in mean change from baseline in number of SBMs/week over 12 weeks between the high dose and placebo.
- Alternative hypothesis 2: There is a difference in mean change from baseline in number of SBMs/week over 12 weeks between the high dose and placebo.

9.6.2.1 Primary Efficacy Analysis

The primary endpoint will be derived as described below:

- For each subject, the number of SBMs will be calculated (using the formula below) for each of the 12 weeks in the Placebo-controlled Part (Part A) of the study and for baseline.
- The (average) number of SBMs during the screening period (baseline) will be calculated as follows:

$$Number\ of\ SBM\ (baseline) = \frac{7 * (total\ frequency\ of\ SBMs\ during\ the\ screening\ period)}{(number\ of\ days\ with\ observation\ during\ the\ screening\ period)}$$

$$Note: excluding\ the\ days\ of\ the\ disimpaction\ procedure\ (3\ to\ 12\ days)$$

- For the calculation of weekly number of SBM, it is required that there are at least 4 days with completed diary data (ie, evaluable days). A day is considered evaluable (day with diary information) if at least some information on BM was recorded (ie, "no BM today" or the time of at least 1 BM was recorded). A minimum of 4 days each week are necessary to perform a weekly evaluation.

If there is no completed diary data available for at least 4 days, then weekly number of SBM will be set to missing and the missing weekly number of SBMs per week up to Week 12 will be imputed using hybrid imputation approach.

- The (average) number of SBMs per week post-baseline will be calculated as follows:

$$Number\ of\ SBMs\ (Week\ i) = \frac{7 * (total\ frequency\ of\ SBMs\ in\ Week\ i)}{(number\ of\ days\ with\ observation\ in\ Week\ i)}$$

- The change from baseline in number of SBM/week can be derived at each week during Placebo-controlled Part (Part A) of the study. The average change from baseline in number of SBM/week over the 12-week placebo-controlled Part A will be estimated through statistical modeling.

Note that BMs during the disimpaction period(s) which can take 3 to 12 days during the screening period and BMs within 24 hours after rescue medication intake during Part A, will not be counted.

Missing data will be imputed using a hybrid imputation approach prior to the analysis of the primary efficacy endpoint. The primary efficacy endpoint will be analyzed using a MMRM. The MMRM will include treatment group, age groups, study week, treatment-group-by-study-week interaction as fixed effects, baseline number of SBM/week as a covariate and subject as a random effect. An unstructured variance-covariance matrix will be used to model the within-subject errors for both treatment groups. The average change from baseline over 12 weeks will be estimated (LS means) by the above MMRM. The treatment difference in LS means between active treatment group versus placebo will be estimated. P-value, treatment difference in LS means and associated 95% confidence interval from the multiple imputed datasets will be combined using Rubin's rules, as implemented in the PROC MIANALYZE procedure. The restricted maximum likelihood method will be used, with an unstructured covariance structure (UN). If the UN covariance structure fails to converge, as an alternative, the Compound symmetry (CS) covariance matrix will be applied. For purpose of convergence, pooling of smaller centers might be needed, decisions on pooling will be made before unblinding.

The mITT will be used for efficacy analyses.

9.6.3 Key Secondary Efficacy Endpoints

- The average change from baseline in stool consistency (based on BSFS score), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
- The average change from baseline in straining (based on a 3-point Likert scale, assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
- The proportion of responders with a responder defined as a subject having an increase of ≥1 SBM/week compared to baseline and ≥3 SBMs/week for at least 9 out of the 12 weeks of placebo-controlled part (Part A), including 3 of the last 4 weeks.

9.6.3.1 Key Secondary Efficacy Analysis

Missing data will be imputed using a hybrid imputation approach prior to analyses of key secondary efficacy endpoints.

The continuous endpoints using the MMRM with the same covariates and factors as for the primary endpoint.

The binary endpoints using CMH test, controlling for the stratification variables age and baseline number of SBMs/week. An overall combined p-value, response rates and difference in response rates plus 95% confidence interval will be derived using Rubin's rules, as implemented in the PROC MIANALYZE procedure. The CMH test statistic will be normalized using the Wilson-Hilferty transformation before combining.

Additional details will be described in the Statistical Analysis Plan (SAP).

9.6.4 Other Secondary Efficacy Endpoints

- Other secondary efficacy endpoint in toilet-trained subjects who are at least 3 years of age:
  - Proportion of subjects with fecal incontinence per week during the 12-week treatment Period.

9.6.5 Exploratory Efficacy Endpoints

- Exploratory efficacy endpoints in toilet-trained subjects who are at least 3 years of age:
  - The average change in worst abdominal pain score over the past 24 hours (based on a Wong-Baker faces scale in subjects <8 years and the 11-point NRS in subjects ≥8 years), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.
  - Proportion of subjects with an average of ≥3 SBMs per week and increase of ≥1 SBM compared to baseline during the 12-week double-blind, placebo-controlled treatment phase.
  - Proportions of subjects with an average of ≤1 SBMs per week during the 12-week double-blind, placebo-controlled treatment phase.
  - Proportion of subjects with an average of ≥1 day(s) with rescue medication intakes per week.
  - Proportion of subjects assessed with retentive posturing at monthly visits during the 12-week treatment period.

    Retentive posturing is defined as the attempt to preserve continence by vigorous contraction of the gluteal muscles. Children with retentive posturing will be typically tight legged, tiptoed, and/or will have a back-arching posture.
  - Global evaluation and QoL Health economics and outcomes research endpoints: PGI-S, CGI-S, and PedsQL Gas and Bloating Domain GI Symptoms Scale (Placebo-controlled Part [Part A]):
    - Absolute values and change from baseline.

    - For PedsQL Gas and Bloating Domain GI Symptoms Scale-SRM: classified as no, small, moderate, and large effect.
  - Proportion of subjects with ≤2 signs/symptoms from the Rome IV Criteria following the 12-week double-blind, placebo-controlled treatment phase
- Exploratory efficacy endpoints in non-toilet-trained subjects who are at least 6 months of age:
  - Proportion of subjects with an average of ≥3 SBMs per week and increase of ≥1 SBM compared to baseline during the 12-week double-blind, placebo-controlled treatment phase.
  - Number of SBMs per week in categories ≤1 and >1 during the 12-week double-blind, placebo-controlled treatment phase.
  - The average change from baseline in stool consistency (based on BSFS score), assessed as the weekly average during the 12-week double-blind, placebo-controlled treatment phase.

9.6.5.1 Other Secondary and Exploratory Efficacy Analyses

For the other secondary and exploratory efficacy endpoints based on diary data, the weekly number of SBMs scores will be derived in a same way as for the primary endpoint.

The other and exploratory efficacy endpoints will be summarized descriptively by treatment group and evaluated over the mITT analysis set. Where possible data will be presented graphically (over time) to support the interpretation of the results.

For binary endpoints, treatment arms will be compared using the Cochran Mantel Haenszel test controlling for the stratification variables age and baseline number of SBMs/week. The difference in proportions will be presented with a 95% confidence interval.

For continuous endpoints, treatment arms will be compared using the same MMRM as used for primary endpoint analysis. No multiplicity adjustment will be performed for the analysis of other and exploratory efficacy endpoints.

Additional details will be described in the Statistical Analysis Plan (SAP).

9.6.5.2 Exploratory Efficacy Analysis for non-toilet-trained subjects

All the efficacy analyses will be descriptive with no formal hypothesis testing.

For exploratory and descriptive purposes, efficacy endpoint will be summarized by each treatment group. Frequency and percentage for each treatment group will be presented.

The NTTmITT Analysis Set will be used for efficacy analysis.

9.6.6 Multiplicity Adjustment for Primary and Secondary Endpoints

The global family-wise error rate will be controlled at an alpha of 0.05 for the 8 hypotheses resulting from comparing each prucalopride dose group (low dose and high dose) with placebo for the primary endpoint and the 3 key secondary endpoints using a Hochberg-step-up procedure [(Dmitrienko et al. 2016); FDA 2017, Guidance for Industry: Multiple Endpoints in Clinical Trials. US Department of Health and Human Services, FDA, CDER, CBER.]

This approach will use the Hochberg-step-up procedure for the primary endpoint dose-comparisons vs placebo and the truncated Hochberg step-up procedure for each of the three key secondary endpoint dose comparisons vs placebo, in a stepwise manner in the order below. Specifically, the truncation parameter will take the value 1 for the primary endpoint and the value 0.8 for the first and second key secondary endpoints. The first key secondary endpoint will be tested only if both comparisons for high dose vs placebo and low dose vs placebo for primary efficacy endpoint have demonstrated statistical significance.

Primary Endpoint:

The average change from baseline in number of SBMs[1] per week derived from the (e-Diary) data over 12 weeks, collected during the placebo-controlled part (Part A).

Key Secondary Endpoints:

1. The average change from baseline in stool consistency

2. The average change from baseline in straining

3. The proportion of responders with a responder defined as a subject having an increase of ≥1 SBM/week compared to baseline and ≥3 SBMs/week for at least 9 out of the 12 weeks of placebo-controlled part (Part A), including 3 of the last 4 weeks.

Details will be provided in the SAP.

Since the first IA is for futility only and the second IA will be based on complete data from Part A, there will be no alpha level adjustment for the final analysis in the presence of two IAs.

9.6.7 Missing Data and Sensitivity Analyses

The primary method for missing data handling will be based on a hybrid imputation approach under a composite estimand. Refer to Section 9.4 for details.

Missing Diary Days

For the calculation of weekly number of SBM, it is required that there are at least 4 days with completed diary data (ie, evaluable days), otherwise the weekly number of SBM will be set to

[1] A BM is defined as spontaneous (SBM) if not preceded within a period of 24 hours by the intake of rescue medication.

missing. In the case when there are at least 4 days with diary data, the (average) number of SBM per week will be calculated as follows:

$$\text{Number of SBMs (week i)} = 7 * \frac{total\ frequency\ of\ SBMs\ in\ week\ i}{number\ of\ days\ with\ observation\ in\ week\ i}$$

Sensitivity Analyses:

- Sensitivity Analyses: The following sensitivity analysis for missing data handling will be conducted to examine the robustness of the primary efficacy analysis results:
  - On treatment multiple imputation: For any subject with missing values, the estimates for the missing weekly frequencies will be based on the treatment group of that subject (ie, on-treatment multiple imputation). In other words, for a prucalopride subject, data from the placebo group will not be used to impute missing weekly frequencies. This is the difference between this method and the placebo multiple imputation method (see below). Twenty imputations will be performed.
  - Placebo multiple imputation: For any subject with missing values, the estimates for the missing weekly frequencies will be based on data of the placebo group. In other words, for a prucalopride subject, data from the placebo arm will be used to impute the missing weekly frequencies. Twenty imputations will be performed.
  - Completer analysis: This analysis will include only subjects who have an average number of SBMs available for all 12 weeks in the Placebo-controlled Part (Part A) of the study (the completers analysis set).
  - PP analysis: This analysis will include only subjects from the PP analysis set.
  - MMRM analysis with additional covariate and factors: The proposed MMRM model for primary efficacy analysis, including additional factors (center, tablet vs oral solution, global severity score) and covariate age. Note: When age is analyzed as a covariate, age group will not be included as a factor in the MMRM.
  - Tipping-point analysis: A tipping-point multiple imputation analysis will be conducted to assess the robustness of the missing data handling for primary efficacy endpoint. The tipping point is defined as the difference in the average number of SBM/week between a treatment arm and placebo arm at which the study conclusion (ie, the statistical significance) changes. To find this tipping point a systematic shift will be applied to the imputed values (for weeks with missing number of SBM/week) in each of the TAK-555 treatment arms respectively, assuming that the tipping point that reverses the study conclusion is between –3 and 0 with a step size of -0.2 (or adapt as appropriate).

The multiple imputation under MNAR assumption will be performed by searching for the tipping point that reverses the study conclusion.

9.7 Safety Analysis

The safety analysis will be performed using the Safety Analysis Set, according to the treatment the subject actually received.

All safety data will be summarized for the whole study (Part A and Part B combined), and also separately for the double-blind treatment part A.

Safety variables include AEs, clinical laboratory variables, vital signs, and ECG variables. For each safety variable, the last value collected before the first dose of investigational product will be used as baseline for all analyses of that safety variable. Last Value on Treatment will be defined as the last valid assessment obtained after Baseline and whilst on investigational product. Last Observed Value will be defined as the last valid assessment obtained after Baseline.

9.7.1 AEs

An AE (classified by preferred term) that occurs during this study will be considered a TEAE if it has a start date on or after the first dose of double-blind IP or if it has a start date before the date of the first dose of double-blind IP, but increases in severity on or after the date of the first dose of double-blind IP. An AE that occurs more than 5 days (5 x half-life of 24 hours) after the date of the last dose of double-blind IP will not be counted as a TEAE.

A summary of the number of subjects with TEAEs will be presented, including the number and percentage of subjects with any TEAEs, serious TEAEs, TEAEs related to IP and TEAEs leading to discontinuation of IP.

The number and percentage of subjects reporting TEAEs in each treatment group will be tabulated by system organ class (SOC) and preferred term; by SOC, preferred term, and maximum severity. Treatment-emergent adverse events considered related to investigational product will also be summarized by SOC and preferred term. If more than 1 AE occurs with the same preferred term for the same subject, then the subject will be counted only once for that preferred term using the most severe and most related occurrence for the summarization by severity and by relationship to investigational product. The number and percentage of subjects reporting TEAEs in each treatment group will also be tabulated by week (the TEAEs that start in that week).

The incidence of common TEAEs (≥2% of subjects in any treatment group) will be summarized by preferred term. Serious TEAEs, TEAEs leading to discontinuation of investigational product and serious TEAEs leading to death, will be summarized by SOC, preferred term and treatment group.

9.7.2 Vital Signs (Blood Pressure, Heart Rate) and Body Weight

Descriptive statistics for vital signs (blood pressure and pulse rate) and their changes from baseline at each post-baseline visit and at the end of study will be presented by treatment group.

The number and percentage of subjects with potentially clinically significant (PCS) post-baseline values will be tabulated by treatment group. The percentages will be calculated relative to the

number of subjects with baseline and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least 1 PCS post-baseline vital sign value. A supportive listing of subjects with postbaseline PCS values will be provided including the subject number, site, baseline, and postbaseline PCS values.

9.7.3 ECG

Descriptive statistics for ECG variables (eg, heart rate, PR interval, QRS interval, QT interval, and QTc interval) and their changes from baseline at each assessment time point will be presented by treatment group. QTc interval will be calculated using both Bazett (QTcB=QT/(RR)1/2) and Fridericia (QTcF=QT/(RR)1/3) corrections; and if RR is not available, it will be replaced with 60/h in the correction formula. ECG interpretation will be summarized by visit. A shift table from baseline to each visit for qualitative ECG results will be presented.

The number and percentage of subjects with post-baseline PCS values will be tabulated by treatment group. The percentages will be calculated relative to the number of subjects with available non-PCS baseline and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least 1 PCS post-baseline ECG value. A listing of all subjects with post-baseline PCS value will be provided including the subject number, site, baseline, and post-baseline PCS values. Frequencies of normal, clinically significant abnormal, and not clinically significant abnormal ECGs will be presented for each relevant visit.

A subject data listing containing all abnormal ECG observations will be provided.

9.7.4 Clinical Laboratory Tests

Laboratory data will be subjected to both a quantitative analysis (descriptive summary statistics) and qualitative analysis where frequencies of normal, above normal, and below normal values will be computed.

The following analyses will be performed:

- Standard descriptive summary statistics at each scheduled measuring time point and the last individual measuring time point.
- Standard descriptive summary statistics for the absolute change from baseline to each scheduled measuring time point after baseline and the last individual measuring time point.
- The number and percentage of subjects with post-baseline PCS values will be tabulated by treatment group. The percentages will be calculated relative to the number of subjects with available baseline values and at least 1 post-baseline assessment. The numerator is the total number of subjects with at least 1 post-baseline PCS value.
- Shift tables displaying shifts from baseline with respect to the PCS limits between baseline and each scheduled measuring time point after baseline and the last individual measuring time point will be provided.

A supportive listing of subjects with post-baseline PCS values will be provided including the subject number, site, baseline, and post-baseline values.

9.7.5 Safety Analyses (Non-Toilet-trained Subjects)

The safety analysis for non-toilet-trained subjects will be performed in the same way as for toilet-trained subjects as explained in Section 9.7.

The NTTSAF will be used for safety analysis

9.8 PK and PK/Pharmacodynamic Analyses

For subjects who consent to the sparse PK sampling, individual plasma concentrations with corresponding sampling times post-dose will be tabulated per visit. A graphical presentation will be used to visualize the plasma concentration-time relationship. Descriptive statistics will be calculated per visit and at specific post-dose time intervals. Plasma levels below the lower limit of quantification will be flagged.

Plasma concentrations measured in this study will be combined with the PK data from previous trials in pediatric subjects and a population PK analysis will be performed on the pooled data. The relationship between PK and efficacy/safety endpoints of interest may be explored. This analysis will be subject of a separate report.

9.8.1 Health-related Quality of Life Analyses (Toilet-trained Subjects)

During the Placebo-controlled Part [Part A] of the study, global evaluations and QoL Health economics and outcomes research endpoints, will be assessed. These include:

- PGI-S.
- CGI-S.
- PedsQL™ Gas and Bloating Module for subjects with functional gastrointestinal disorders.

The PGI-S and CGI-S will jointly be used in an anchor-based analysis to determine a clinically meaningful within-patient change threshold for change in the number of SBMs. Baseline will be defined as the PGI-S/CGI-S from screening (ie, prior to disimpaction). Subjects with assessments at both baseline and Week 12 will be classified, regardless of treatment assignment, by the magnitude of the change categories between baseline and Week 12; specifically, the categories will be (- indicating improvement and + indicating deterioration): -3 categorical shift, -2 categorical shift, -1 categorical shift, 0 categorical shift, +1 categorical shift, +2 categorical shift, +3 categorical shift. Shift tables from baseline to Week 12 will be presented to show the changes for PGI-S and CGI-S, respectively. In the event categories on the extreme (eg, -3 or +3) have zero or <10 subjects then those categories will be collapsed or pooled with the neighboring categories.

Once classified the following anchor-based empirical cumulative distribution function (eCDF) and probability density function (PDF; many times estimated using kernel density estimation)

curves, including the sample size and median score for each eCDF and PDF anchor curve for the primary endpoint; along with the sample size, and the 10th, the 25th, the 50th (median), the 75th, and the 90th percentile of the primary endpoint. In the event categories are collapsed then supplemental eCDF and PDF will be provided using the un collapsed categories.

Baseline for the PedsQL Gas and Bloating Module will be the screening assessment prior to disimpaction. For both baseline and postbaseline the total scale score is calculated as the sum of all items divided by the total number of nonmissing items. The subscale score is calculated as the sum of subscale items divided by the total number of non-missing subscale items. If more than 50% of the items in the (sub)scale are missing, the score for this (sub)scale will be set to "missing".

PedsQL Gas and Bloating module will be summarized as follows:

- Absolute values and change from baseline.
- SRM.

  The SRM is defined as the mean change from baseline divided by the SD of the change. Assessments performed
- The SRM will also be presented classified: <0.2, 0.2-<0.5, 0.5-<0.8, and ≥0.8, with classes regarded as no effect, small, moderate, and large, respectively.
- Absolute values.

  All global evaluations and QoL Health economics and outcomes research endpoints will be analyzed by each scheduled visit (ie, baseline, post disimpaction, Week 8, Week 24). In addition, the final on treatment assessment, defined as either the final scheduled time point for completers or the early discontinuation visit for early terminators, will be summarized. Descriptive statistics for the total scale, each subscale and each global evaluation scale, will be tabulated per treatment group over the mITT analysis set. For continuous endpoints, treatment arms will be compared using the analysis of covariance with the age and baseline number of SBMs/week as covariates. For categorical endpoint (only classification of the SRM), treatment arms will be compared using the CMH test adjusted for age and baseline number of SBMs/week.

9.8.2 Subgroup Analyses

For explorative and descriptive purposes, the primary and selected secondary endpoints (endpoints based on number of BMs and SBMs per week and endpoints based on consistency scoring) will also be summarized and compared between treatment groups within each subgroup as defined by the stratification variables: age group (<12 years, 12 to 17 years) and average number of SBM/week (≤1; >1) during the screening period.

10. REFERENCES

Bongers, M. E. J., van den Berg, M. M., Reitsma, J. B., Voskuijl, W. P. and Benninga, M. A. 2009. A Randomized Controlled Trial of Enemas in Combination With Oral Laxative Therapy for Children With Chronic Constipation. *Clinical Gastroenterology and Hepatology,* 7(10), 1069-74.

Briejer, M., Ghoos, E., Eelen, J. and Schuurkes, J. 1997a. Study on the involvement of serotonin 5-HT4 receptors in the effects of R093877 on colonic motility patterns in fasted conscious dogs. *Janssen Research Foundation Preclinical Research Report.*

Briejer, M., Van Bergen, P., Borgmans, C., De Winter, P. and Schuurkes, J. 1997b. R093877 is a selective and relatively potent 5-HT4 receptor agonist in guinea-pig and rat intestinal tissues. *Janssen Research Foundation Preclinical Research Report.*

Briejer, M. R., Akkermans, L. M. and Schuurkes, J. A. 1995. Gastrointestinal prokinetic benzamides: the pharmacology underlying stimulation of motility. *Pharmacological Reviews,* 47(4), 631-51.

Dmitrienko, A., Kordzakhia, G. and Brechenmacher, T. 2016. Mixture-based gatekeeping procedures for multiplicity problems with multiple sequences of hypotheses. *J Biopharm Stat,* 26(4), 758-80.

Drossman, D. A., Chang, L., Chey, W. D., Kellow, J., Tack, J. and Whitehead, W. J. 2016. *Rome IV. Functional gastrointestinal disorders - disorders of the gut-brain interaction.* Fourth ed. Raleigh, NC: The Rome Foundation.

Lan, K. K. and Wittes, J. 1988. The B-value: a tool for monitoring data. *Biometrics,* 44(2), 579-85.

Lewis, S. J. and Heaton, K. W. 1997. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol,* 32(9), 920-4.

Mugie, S. M., Benninga, M. A. and Di Lorenzo, C. 2011. Epidemiology of constipation in children and adults: A systematic review. *Best Practice & Research Clinical Gastroenterology,* 25(1), 3-18.

Mugie, S. M., Korczowski, B., Bodi, P., Green, A., Kerstens, R., Ausma, J., et al. 2014. Prucalopride is no more effective than placebo for children with functional constipation. *Gastroenterology,* 147(6), 1285-95.e1.

Nurko, S. and Saps, M. 2014. Treating constipation with prucalopride: one size does not fit all. *Gastroenterology,* 147(6), 1214-6.

Ratitch, B., Lipkovich, I. and O'Kelly, M. 2013. Combining Analysis Results from Multiply Imputed Categorical Data - PharmaSUG Paper SP03.

Schwartz, G. J. and Work, D. F. 2009. Measurement and estimation of GFR in children and adolescents. *Clin J Am Soc Nephrol,* 4(11), 1832-43.

Tabbers, M., DiLorenzo, C., Berger, M., Faure, C., Langendam, M. and Nurko, S. 2014. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPHGAN. *J Pediatr Gastroenterol Nutr,* 58, 258-74.

Wellens, A. and Schuurkes, J. 1996. Effect of R093877 on lidamine-delayed gastric emptying of a liquid test meal in conscious dogs. *Janssen Research Foundation Preclinical Research Report.*

Wong, S. W. and Lubowski, D. Z. 2007. Slow-Transit Constipation: Evaluation and Treatment. *ANZ Journal of Surgery,* 77(5), 320-8.

Appendix 1 Regulatory, Ethical, and Study Oversight Considerations

Appendix 1.1 Regulatory and Ethical Considerations

This study is conducted in accordance with current applicable regulations including ICH E6 and all updates, as well as local ethical and legal requirements.

Compliance with these regulations and guidelines also constitutes compliance with the ethical principles described in the Declaration of Helsinki.

The name and address of each third-party vendor (eg, CRO) used in this study will be maintained in the investigator's and sponsor's files, as appropriate.

Appendix 1.2 Sponsor's Responsibilities

Good Clinical Practice Compliance

The study sponsor and any third party to whom aspects of the study management or monitoring have been delegated will undertake their assigned roles for this study in compliance with all applicable industry regulations, current ICH GCP Guidelines, as well as all applicable national and local laws and regulations.

Visits to sites are conducted by representatives of the study sponsor and/or the company organizing/managing the research on behalf of the sponsor to inspect study data, subjects' medical records, and eCRFs in accordance with current GCP and the respective local and (inter)national government regulations and guidelines. Records and data may additionally be reviewed by auditors or by regulatory authorities.

The sponsor ensures that local regulatory authority requirements are met before the start of the study. The sponsor (or a nominated designee) is responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of IP for shipment to the site.

Indemnity/Liability and Insurance

The sponsor of this research adheres to the recommendations of the Association of British Pharmaceutical Industry Guidelines. If appropriate, a copy of the indemnity document is supplied to the investigator before study initiation, per local country guidelines.

The sponsor ensures that suitable clinical study insurance coverage is in place prior to the start of the study. An insurance certificate is supplied to the CRO and investigator as necessary.

Public Posting of Study Information

The sponsor is responsible for posting appropriate study information on applicable websites. Information included in clinical study registries may include participating investigators' names and contact information.

The timing for study registration and results summary posting must be in accordance with applicable local and national requirements.

Submission of Summary of Clinical Study Report to Competent Authorities of Member States Concerned and Ethics Committees

The sponsor will provide a summary of the clinical study report to the competent authority of the member state(s) concerned as required by regulatory requirement(s) and to comply with the Community guideline on GCP. This requirement will be fulfilled within 6 months of study completion date for pediatric studies and within 1 year for non-pediatric studies as per guidance.

Study Suspension, Termination, and Completion

The sponsor may suspend or terminate the study, or part of the study, at any time for any reason. If the study is suspended or terminated, the sponsor will ensure that applicable sites, regulatory agencies and Institutional Review Boards (IRBs)/Ethics Committees (ECs) are notified as appropriate. Additionally, the discontinuation of a registered clinical study which has been posted to a designated public website will be updated accordingly.

Appendix 1.3 Investigator's Responsibilities

Good Clinical Practice Compliance

The investigator must undertake to perform the study in accordance with ICH GCP E6 R2 (2016), and applicable regulatory requirements and guidelines.

It is the investigator's responsibility to ensure that adequate time and appropriately trained resources are available at the site prior to commitment to participate in this study. The investigator should also be able to estimate or demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant study-related tasks, and shall, upon request of the sponsor, provide documented evidence of any licenses and certifications necessary to demonstrate such qualification. Curriculum vitae for investigators and sub-investigators are provided to the study sponsor (or designee) before starting the study.

If a potential research subject has a primary care physician, the investigator should, with the subject's consent, inform them of the subject's participation in the study.

A coordinating principal investigator is appointed to review the final clinical study report for multicenter studies. Agreement with the final clinical study report is documented by the signed and dated signature of the principal investigator (single-site study) or coordinating principal investigator (multicenter study), in compliance with Directive 2001/83/EC as amended by Directive 2003/63/EC and ICH Guidance E3 (1995).

Protocol Adherence and Investigator Agreement

The investigator and any sub-investigators must adhere to the protocol as detailed in this document. The investigator is responsible for enrolling only those subjects who have met protocol eligibility criteria. Investigators are required to sign an investigator agreement to confirm acceptance and willingness to comply with the study protocol.

If the investigator suspends or terminates the study at their site, the investigator will promptly inform the sponsor and the IRB/EC and provide them with a detailed written explanation. The investigator will also return all IP, containers, and other study materials to the sponsor. Upon study completion, the investigator will provide the sponsor, IRB/EC, and regulatory agency with final reports and summaries as required by (inter)national regulations.

If the site closure is due to COVID-19, this should be captured on the CRF or eCRF.

Communication with local IRBs/ECs, to ensure accurate and timely information is provided at all phases during the study, may be done by the sponsor, applicable CRO, investigator, or for multicenter studies, the coordinating principal investigator according to national provisions and will be documented in the investigator agreement.

Documentation and Retention of Records

Case Report Forms

CRFs are supplied by the CRO and should be handled in accordance with instructions from the sponsor.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information is recorded onto eCRFs, which have been designed to record all observations and other data pertinent to the clinical investigation. Case report forms must be completed by the investigator or designee as stated in the site delegation log.

All data will have separate source documentation; no data will be recorded directly onto the eCRF.

The clinical research associate (CRA)/study monitor will verify the contents against the source data per the monitoring plan. If the data are unclear or contradictory, queries are sent for corrections or verification of data.

The eCRFs should be approved by the investigator per study specifications and the sponsor's data delivery requirements.

Recording, Access, and Retention of Source Data and Study Documents

Original source data to be reviewed during this study will include, but are not limited to subject's medical file, original clinical laboratory reports, and ECG conducted by site.

All key data must be recorded in the subject's source documents.

The investigator must permit authorized representatives of the sponsor; the respective national, local, or foreign regulatory authorities; the IRB/EC; and auditors to inspect facilities and to have direct access to original source records relevant to this study, regardless of media.

The CRA/study monitor (and auditors, IRB/EC or regulatory inspectors) may check the eCRF entries against the source documents. The consent form includes a statement by which the subject agrees to the monitor/auditor from the sponsor or its representatives, national or local regulatory authorities, or the IRB/EC, having access to source data (eg, subject's medical file, appointment books, original laboratory reports, X-rays etc).

These records must be made available within reasonable times for inspection and duplication, if required, by a properly authorized representative of any regulatory agency (eg, the US FDA, European Medicines Agency (EMA), United Kingdom [UK] Medicines and Healthcare products Regulatory Agency) or an auditor.

Essential documents must be maintained according to ICH GCP requirements and may not be destroyed without written permission from the sponsor.

Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of, for example, the US FDA (as well as other US national and local regulatory authorities), the EMA, the Medicines and Healthcare products Regulatory Agency, other regulatory authorities, the sponsor or its representatives, and the IRB/EC for each site.

Financial Disclosure

The investigator is required to disclose any financial arrangement during the study and for 1 year after, whereby the outcome of the study could be influenced by the value of the compensation for conducting the study, or other payments the investigator received from the sponsor. The following information is collected: any significant payments from the sponsor or subsidiaries such as a grant to fund ongoing research, compensation in the form of equipment, retainer for ongoing consultation or honoraria; any proprietary interest in IP; any significant equity interest in the sponsor or subsidiaries as defined in 21 CFR 54 2(b) (1998).

Appendix 1.4 Data Management Considerations

Data Collection

The study will be monitored according to GCP.

The investigators' authorized site personnel must enter the information required by the study eCRF Completion Guidelines or similar for all data requiring transcription of the source.

A study monitor will visit each site in accordance with the monitoring plan and review the eCRF data against the source data for completeness and accuracy. Discrepancies between source data

and data entered on the eCRF will be addressed by qualified site personnel. When a data discrepancy warrants correction, the correction will be made by authorized site personnel. Data collection procedures will be discussed with the site at the site initiation visit and/or at the investigator's meeting.

In the event an on-site monitoring visit cannot be accommodated, other monitoring methods such as remote monitoring may occur in accordance with the Monitoring Plan.

Data Management

Data are to be entered into a clinical database as specified in the CRO data management plan or similar. Quality control and data validation procedures are applied to ensure the validity and accuracy of the clinical database.

Data are to be reviewed and checked for omissions, errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification are to be communicated to the site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections are documented in an auditable manner.

Data Handling

Data that may potentially unblind the treatment assignment (ie, IP serum concentrations, treatment allocation, and IP preparation/accountability data) will be handled with special care during the data cleaning and review process. These data will be handled in such a way that, prior to unblinding, any data that may unblind study team personnel will be presented as blinded information or otherwise will not be made available. If applicable, unblinded data may be made available to quality assurance representatives for the purposes of conducting independent drug audits.

Appendix 1.5 Ethical Considerations

Informed Consent

It is the responsibility of the investigator to obtain written informed consent and assent from all study subjects prior to any study-related procedures including screening assessments. All consent and assent documentation must be in accordance with applicable regulations and GCP. Each subject or the subject's legally authorized representative, as applicable, is requested to sign and date the subject informed consent form or a certified translation if applicable, after the subject has received and read (or been read) the written subject information and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences, and the subject's rights and responsibilities. A copy of the informed consent and assent documentation (ie, a complete set of subject information sheets and fully executed signature pages) must be given to the subject or the subject's legally authorized representative, as applicable. This document may require translation into the local language. Signed consent forms must remain in each subject's study file and must be available for verification at any time.

Within the source documents, site personnel should document instruction of and understanding by the parent(s)/legally authorized representative(s)/caregiver(s) of the safe, responsible storage and administration of IP to the study subject.

The principal investigator provides the sponsor with a copy of the consent form (and assent form where applicable) that was reviewed by the IRB/EC and received their favorable opinion/approval. A copy of the IRB/EC's written favorable opinion/approval of these documents must be provided to the sponsor prior to the start of the study unless it is agreed to and documented (abiding by regulatory guidelines and national provisions) prior to study start that another party (ie, sponsor or coordinating principal investigator) is responsible for this action. Additionally, if the IRB/EC requires modification of the sample subject information and consent document provided by the sponsor, the documentation supporting this requirement must be provided to the sponsor.

Note that additional consent/assent is required for subjects agreeing to provide sparse PK samples.

Institutional Review Board or Ethics Committee

For sites outside the EU, it is the responsibility of the investigator to submit this protocol, the informed consent document (approved by the sponsor or their designee), relevant supporting information and all types of subject recruitment information to the IRB/EC for review, and all must be approved prior to site initiation.

The applicant for an EC opinion can be the sponsor or investigator for sites within the EU; for multicenter studies, the applicant can be the coordinating principal investigator or sponsor, according to national provisions.

Responsibility for coordinating with IRBs/ECs is defined in the investigator agreement. IP supplies will not be released until the sponsor/CRO has received written IRB/EC approval.

Prior to implementing changes in the study, the sponsor and the IRB/EC must approve any revisions of all informed consent documents and amendments to the protocol unless there is a subject safety issue. If required by local law, substantial amendments to the protocol must also be approved by the appropriate regulatory agency prior to implementation.

For sites outside the EU, the investigator is responsible for keeping the IRB/EC apprised of the progress of the study and of any changes made to the protocol at least annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. This can be the responsibility of the sponsor or investigator for sites within the EU; or for multicenter studies, the coordinating principal investigator, according to national provisions. The investigator must also keep the local IRB/EC informed of any serious and significant AEs as required by IRB/EC procedures.

Privacy and Confidentiality

All US-based sites and laboratories or entities providing support for this study, must, where applicable, comply with the Health Insurance Portability and Accountability Act (HIPAA) of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the CRO/sponsor.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the sponsor and/or its representatives reviews their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the sponsor; third parties with whom the sponsor may develop, register, or market prucalopride; national or local regulatory authorities; and the IRB(s)/EC(s) which gave approval for the study to proceed. The sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities. Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected, if permitted under local laws governing privacy.

The results of studies containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth, where allowed per local law, may be transferred to, and used in, other countries which may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include: to support regulatory submissions, to conduct new data analyses to publish or present the study results, or to answer questions asked by regulatory or health authorities.

Study Results/Publication Policy

The term "Publication" shall mean any paper, article, manuscript, report, poster, internet posting, presentation slides, abstract, outline, video, instructional material, presentation (in the form of a written summary), or other public disclosure of the study results, in printed, electronic, oral, or other form. The parties understand and agree that participation in the study may involve a commitment to publish the data from all sites participating in the study in a cooperative publication with other investigators prior to publication or oral presentations of the study results on an individual basis. The site agrees not to publish or present the site's study results until such time as either the aggregate multisite study results are published in a cooperative publication or for a period of one (1) year after termination or completion of the study at all participating sites, whichever shall first occur. After that time, the site may publish the site's study results in scientific journals or present the study results at symposia or other professional meetings in accordance with the following provisions:

If the study is part of a multicenter study, the first publication of the study results shall be made by the sponsor in conjunction with the sponsor's presentation of a joint, multicenter publication of the compiled and analyzed study results.

If such a multicenter publication is not submitted to a journal for publication by the sponsor within an 18-month period after conclusion, abandonment, or termination of the study at all sites, or after the sponsor confirms there shall be no multicenter study publication of the study results, an investigator may individually publish the study results from the specific site in accordance with this section. The investigator must, however, acknowledge in the publication the limitations of the single site data being presented.

At least sixty (60) days prior to submitting an abstract, manuscript, or other document for publication, a copy of the proposed publication will be provided to the sponsor by the site for review. Upon the sponsor's request, the site agrees to remove any and all confidential information (expressly excluding study results) identified in the publication and to delay such submission or presentation for an additional sixty (60) day period in order to allow the sponsor time to file any patent application(s). All publications of the study results shall appropriately reference the multi-site study publication, if any, or the fact that the study results are a subset of data resulting from a larger multi-site study.

The sponsor is committed to transparent dissemination of all scientific, technical and medical manuscripts generated from sponsor-supported research. Therefore, after January 1, 2018, the sponsor will require the submission of all sponsor-supported research manuscripts to journals that offer public availability via Open Access (including publisher platforms/repositories and self-archiving). Open Access refers to the free at point of entry, online availability of published research output with, where available, rights of re-use according to an End User License.

Unless otherwise required by the journal in which the publication appears, or the forum in which it is made, authorship will comply with the International Committee of Medical Journal Editors Recommendation for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals. Participation as an investigator does not confer any rights to authorship of publications.

Appendix 2 Clinical Laboratory Tests

The following clinical laboratory assessments will be performed:

Chemistry	
Albumin	Total protein
Creatinine	Creatine kinase
alkaline phosphatase	AST
ALT	Gamma glutamyltransferase
Total bilirubin	Direct bilirubin
Lactate dehydrogenase	Total cholesterol
Triglycerides	Glucose
Chloride	Calcium
Potassium	Prolactin
Sodium	Magnesium
Bicarbonate	Phosphorus
Urea	Uric acid
Hematology	
Hemoglobin	Hematocrit
Red blood cell (RBC) count	White blood cell (WBC) count
Differential WBC count	Platelet count
Urinalysis	
Protein	Glucose
Blood	pH
If abnormal, microscopic examination for WBC, RBC, and casts will be performed	
Serum pregnancy test	

Appendix 3 AEs: Definitions and Procedures for Recording, Evaluating, Follow-Up, and Reporting

Appendix 3.1 AE Definitions

An AE is any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this IP or medicinal product. An AE can therefore be any unfavorable and unintended sign (including a clinically significant laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not causality is suspected (ICH Guidance E2A 1995).

TEAE

A TEAE is defined as any event emerging or manifesting at or after the initiation of treatment with an IP or medicinal product or any existing event that worsens in either intensity or frequency following exposure to the IP or medicinal product.

SAE

An SAE is any untoward clinical manifestation of signs, symptoms or outcomes (whether considered related to IP or not) and at any dose:

- Results in death
- Is life-threatening. Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.
- Requires inpatient hospitalization or prolongation of hospitalization. Note: Hospitalizations that are the result of elective or previously scheduled investigations procedures or surgery for pre-existing conditions and have not worsened after initiation of treatment should not be classified as SAEs.

  For example, an admission for a previously scheduled ventral hernia repair would not be classified as an SAE; however, complication(s) resulting from a hospitalization for an elective or previously scheduled surgery that meet(s) serious criteria must be reported as SAE(s).
- Results in persistent or significant disability/incapacity
- Results in a congenital abnormality/birth defect
- Is an important medical event. Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include:

- Bronchospasm associated with anaphylaxis requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization; or the development of drug dependency or drug abuse.
- Reviewed and confirmed seroconversion for human immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis E virus, or parvovirus B19.

Unexpected Adverse Event

An unexpected AE is an AE whose nature, severity, specificity, or outcome is not consistent with the term, representation, or description used in the Reference Safety Information (RSI). "Unexpected" also refers to the AEs that are mentioned in the IB and/or prescribing information as occurring with a class of drugs or as anticipated from the pharmacological properties of the product, but are not specifically mentioned as occurring with the particular product under investigation.

The expectedness of AEs will be determined by the sponsor using the IB and/or prescribing information as the RSI. This determination will include considerations such as the number of AEs previously observed, but not on the basis of what might be anticipated form the pharmacological properties of a product.

Suspected Unexpected Serious Adverse Reaction

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is defined as any suspected adverse reaction to study treatment (ie, including active comparators) that is both serious and unexpected.

The event(s) must meet all of the following criteria:

- Suspected adverse reaction
- Serious
- Unexpected
- Assessed as related to study treatment

The sponsor will be responsible for reporting all SUSARs and any other applicable SAEs to regulatory authorities, investigators, and IRBs or IECs, as applicable, in accordance with national regulations in the countries where the study is conducted. Relative to the first awareness of the event by/or further provision to the sponsor or sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other serious events, unless otherwise required by national regulations. The sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in investigational medicinal product administration or in the overall conduct of the study. The investigational site will also forward a copy of all expedited reports to his or her IRB or IEC in accordance with national regulations.

AESI

An AESI (serious or non-serious) is one of scientific and medical concern specific to the compound or program, for which ongoing monitoring and rapid communication by the investigator to Takeda may be appropriate. Such events may require further investigation in order to characterize and understand them and would be described in protocols and instructions provided for investigators as to how and when they should be reported to Takeda.

The clinical database will be searched using the Standardized Medical Dictionary for Regulatory Activities Query Suicide/Self-injury (Narrow) to identify all suicide-related events.

Further, all identified AESIs will be recorded in the AE eCRF and additional details will be provided by the site using the "Safety Report Form". Any cases of suicide or suicide attempts are expected to be reported as an SAE and the site should complete both the "Safety Report Form" and the Suicide Questionnaire. All AESIs (serious and nonserious) will follow a similar reporting plan as is in place for SAEs. Non-serious and serious AESIs will be reported to Takeda on the "Safety Report Form" within 24 hours. The investigator will discontinue the IP for all identified cases of SI/SIB and the subjects will be referred to either a MHP or the ER per investigator discretion. The MHP completes an assessment and provides a recommendation to the site investigator which may include but is not limited to continuing psychiatric management per standard of care. Following this recommendation, the site investigator is expected to assess for causality, to send redacted clinical notes and to provide updated medical information on the AESI using the "Safety Report Form". All confirmed cases of SIB are to be discontinued from the study. The evaluations listed for Visit 23/ET will be performed as completely as possible. For confirmed cases of suicidal ideation, the site investigator should contact the sponsor before any planned rechallenge of IP. The subject should not be re-started on IP until confirmation with the study sponsor is obtained. All cases of SI/SIB are to be followed to closure (the subject's health has returned to his/her baseline status or all variables have returned to baseline), regardless of whether the subject is still participating in the study.

All AESIs will be reported to the DMC for further review within 7 business days of the AESI being reported to the sponsor on the "Safety Report Form". Important case details will be shared with the DMC within 30 days. The DMC, following its receipt of the cases, reserves the right to call for an ad hoc DMC meeting at any time and will provide recommendations to the sponsor which may include temporarily pausing the study or continuing the study as planned.

Additional details are provided in the DMC charter.

Symptoms of the Disease under Study

Symptoms of the disease under study should not be classed as AEs as long as they are within the normal day-to-day fluctuation or expected disease progression and are part of the efficacy or effectiveness data collected in the study. Significant worsening of symptoms should be recorded as an AE.

Pre-existing conditions prior to randomization or initiation of study medication are described in the medical history, and those that manifest with the same severity, frequency, or duration

after drug exposure, are not to be recorded as AEs. However, when there is an increase in the severity, duration or frequency of a pre-existing condition, the event must be described on the AE eCRF.

Clinical Laboratory and Other Safety Assessment

A change in the value of a clinical laboratory parameter, vital sign measure, or ECG assessment can represent an AE if the change is clinically relevant or if, during administration of IP, a shift of a parameter is observed from a value in the normative range to a value that is outside the normal range and considered clinically significant, or a further waning of an already clinically significant value. When evaluating such changes, the extent of deviation from the reference range, the duration until return to the reference range, either while continuing administration or after the end of administration with the IP, and the range of variation of the respective parameter within its reference range, should also be considered.

If, at the end of the treatment phase, there are abnormal clinical laboratory (such as hematology panel or clinical chemistry panel), vital sign, or ECG values which were not present at the pretreatment evaluation observed closest to the start of study treatment, further investigations should be performed until the values return to within the reference range or until a plausible explanation (eg, concomitant disease or expected disease evolution) is found for the abnormal values.

The investigator should assess, based on the above criteria and the clinical condition of the subject, whether a change in a clinical laboratory value, vital sign, or ECG parameter is clinically significant and represents an AE.

Appendix 3.2 Collection of Adverse Events

All AEs/SAEs are collected from the time the informed consent document is signed until the defined follow-up period stated in Section 8.1.4. This includes events occurring during the screening phase of the study, regardless of whether or not IP is administered.

All AEs must be monitored until the end of the study or until the event resolves, stabilizes, is otherwise explained, or the participant is lost to follow-up. The sponsor may request relevant additional information on reported adverse events that may include but are not limited to the following: redacted medical records, redacted admission/discharge notes, clinical notes, completed targeted questionnaire and/or clinical study adverse event form by the site investigators.

Appendix 3.3 Assessment of Adverse Events

Severity Categorization

The severity of AEs must be recorded during the course of the event including the start and stop dates for each change in severity. An event that changes in severity is captured as a new event.

Worsening medical conditions, signs or symptoms present prior to initiation of IP, must be recorded as new AEs.

For example, if a subject reports mild intermittent dyspepsia prior to initiation of dosing with the IP, and the dyspepsia becomes severe and more frequent after first dose a new AE of severe dyspepsia (with the appropriate date of onset) should be documented in the source.

The medical assessment of severity is determined by using the following definitions:

- Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- Moderate: A type of AE that is usually alleviated with specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject.
- Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Relationship Categorization

A physician/investigator must make the assessment of relationship to IP/rescue medication/disimpaction medication for each AE. The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the IP/rescue medication/disimpaction medication. If there is no valid reason for suggesting a relationship, then the AE should be classified as "not related". Otherwise, if there is any valid reason, even if undetermined or untested, for suspecting a possible cause-and-effect relationship between the IP/rescue medication/disimpaction medication and the occurrence of the AE, then the AE should be considered "related". The causality assessment must be documented in the source.

The following additional guidance may be helpful:

Table A1 Adverse Event Relationship Categorization

Related	The temporal relationship between the event and the administration of the IP/rescue medication/disimpaction medication is compelling enough and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.
Not related	The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no plausible temporal or biologic relationship exists between the IP/rescue medication/disimpaction medication and the event.

Outcome Categorization

The outcome of AEs must be documented in the source during the course of the study. Outcomes are as follows:

- Fatal
- Not Recovered/Not Resolved
- Recovered/Resolved
- Recovered/Resolved With Sequelae
- Recovering/Resolving
- Unknown

If applicable, action taken (ie, dose increased, dose not changed, drug interrupted by investigator, drug withdrawn, not applicable, or unknown) will also be recorded on the AE eCRF.

Appendix 3.4 Safety Reporting

Reference Safety Information

The RSI for this study is the IB which the sponsor has provided under separate cover to all investigators.

Reporting Procedures

All initial and follow-up SAE reports must be reported by the investigator to the Takeda Global Patient Safety Evaluation Department and the study medical monitor within 24 hours of becoming aware of the event. Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors (see Appendix 3.9) unless they result in an SAE.

The investigator must complete, sign, and date the "Safety Report Form", verify the accuracy of the information recorded on the form with the corresponding source documents (Note: Source documents are not to be sent unless requested), and fax or e-mail the form to the Takeda Global Patient Safety Evaluation Department using the details specified in the emergency contact information section of the protocol. A copy of the "Safety Report Form" (and any applicable follow-up reports) must also be sent to the study medical monitor using the details specified in the emergency contact information section of the protocol.

Appendix 3.5 SAE Collection Time Frame

All SAEs (regardless of relationship to IP) are collected from the time the subject signs the informed consent until the defined follow-up period stated in Section 8.1.4 and must be reported to the Takeda Global Patient Safety Evaluation Department and the study medical monitor within 24 hours of the first awareness of the event.

In addition, any SAE(s) considered "related" to the IP and discovered by the investigator at any interval after the study has completed must be reported to the Takeda Global Patient Safety Evaluation Department within 24 hours of the reported first becoming aware of the event.

Appendix 3.6 Serious Adverse Event Onset and Resolution Dates

The onset date of the SAE is defined as the date the event meets serious criteria. The resolution date is the date the event no longer meets serious criteria, the date the symptoms resolve, or the event is considered chronic. In the case of hospitalizations, the hospital admission and discharge dates are considered the onset and resolution dates, respectively.

In addition, any signs or symptoms reported by the subject after signing the informed consent form or leading up to the onset date of the SAE, or following the resolution date of the SAE, must be recorded as an AE, if appropriate.

Appendix 3.7 Fatal Outcome

Any SAE that results in the subject's death (eg, the SAE was noted as the primary cause of death) must have "fatal" checked as an outcome with the date of death recorded as the resolution date. For all other events ongoing at the time of death that did not contribute to the subject's death, the outcome should be considered not resolved, without a resolution date recorded.

For any SAE that results in the subject's death or any ongoing events at the time of death, unless another IP action was previously taken (eg, drug interrupted, reduced, withdrawn), the action taken with the IP should be recorded as "dose not changed" or "not applicable" (if the subject never received IP). The IP action of "withdrawn" should not be selected solely as a result of the subject's death.

Appendix 3.8 Pregnancy

All pregnancies are reported from the time informed consent is signed until the defined follow-up period stated in Section 8.1.4.

Any report of pregnancy for any female study participant must be reported within 24 hours to the sponsor using the "Pregnancy Report Form" using the details specified in the emergency contact information section of the protocol.

A copy of the sponsor's "Pregnancy Report Form" (and any applicable follow-up reports) must also be sent to the study medical monitor using the details specified in the emergency contact information section of the protocol. The pregnant female study participant must be withdrawn from the study.

Every effort should be made to gather information regarding the pregnancy outcome and condition of the infant. It is the responsibility of the investigator to obtain this information within 30 calendar days after the initial notification and approximately 30 calendar days and 1 year post-partum.

Pregnancy complications such as spontaneous abortion/miscarriage, elective abortion, or congenital abnormality are considered SAEs and must be reported using the "Safety Report Form".

In addition to the above, if the investigator determines that the pregnancy meets serious criteria, it must be reported as an SAE using the "Safety Report Form" as well as the "Pregnancy Report Form". The test date of the first positive serum/urine β-hCG test or ultrasound result will determine the pregnancy onset date.

Appendix 3.9 Abuse, Misuse, Overdose and Medication Error

Abuse, misuse, overdose, or medication error (as defined below) must be reported to the sponsor according to the SAE reporting procedure whether or not they result in an AE/SAE using the "Special Situation Report Form" as described in Appendix 3.4.

Any dosing error that results in an AE should be recorded on the eCRF as an AE.

Note: The 24-hour reporting requirement for SAEs does not apply to reports of abuse, misuse, overdose, or medication errors unless these result in an SAE.

The categories below are not mutually exclusive; the event can meet more than 1 category.

- Abuse – Persistent or sporadic intentional intake of IP when used for a non-medical purpose (eg, to alter one's state of consciousness or get high) in a manner that may be detrimental to the individual and/or society
- Misuse – Intentional use of IP other than as directed or indicated at any dose (Note: this includes a situation where the IP is not used as directed at the dose prescribed by the protocol.)
- Overdose – Intentional or unintentional intake of a dose of IP higher than the protocol-prescribed dose
- Medication Error – An error made in prescribing, dispensing, administration, and/or use of an IP. For studies, medication errors are reportable to the sponsor only as defined below.

Cases of subjects missing doses of the IP are not considered reportable as medication errors.

Medication errors should be collected/reported for all products under investigation.

The administration and/or use of the unassigned treatment is/are always reportable as a medication error.

The administration and/or use of an expired IP should be considered as a reportable medication error.

All IP provided to pediatric subjects should be supervised by the parent(s)/legally authorized representative(s)/caregiver(s).

Appendix 3.10 Urgent Safety Measures

An urgent safety measure is an immediate action taken, which is not defined by the protocol, in order to protect subjects participating in a clinical trial from immediate harm; these do not constitute de facto deviation from the protocol. Urgent safety measures may be taken by the sponsor or clinical investigator, and may include any of the following:

- Immediate change in study design or study procedures
- Temporary or permanent halt of a given clinical trial or trials
- Any other immediate action taken in order to protect clinical trial participants from immediate hazard to their health and safety

The investigator may implement urgent safety measures to protect study subjects from immediate hazard to their health or safety. The measures should implement immediately and does not require prior authorization from the sponsor. In the event(s) of an apparent direct hazard to the subject, the investigator will notify the sponsor immediately by phone and confirm notification to the sponsor in writing as soon as possible, and within 1 calendar day after the change is implemented. The sponsor will also ensure the responsible EC(s) and relevant competent authority(s) are notified of the urgent safety measures taken in such cases according to local regulations.

Appendix 3.11 Regulatory Agency, Institutional Review Board, Ethics Committee and Site Reporting

The sponsor and/or CRO is responsible for notifying the relevant regulatory authorities (US FDA, central IRBs/EU central ECs) of related, unexpected SAEs.

In addition, the sponsor is responsible for notifying active sites of all related, unexpected SAEs occurring during all interventional studies across the TAK-555 program.

The investigator is responsible for notifying the local IRB/EC of SAEs or significant safety findings that occur at his or her site as required by IRB/EC procedures (see Appendix 1.5).

Appendix 4 Contraceptive Guidance

Female participants:

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described below.

Highly Effective Contraceptive Methods That Are User Dependent[a] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] Oral Intravaginal Transdermal
Progestogen only hormonal contraception associated with inhibition of ovulation Oral Injectable
Highly Effective Methods That Are User Independent[a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] Intrauterine device (IUD) Intrauterine hormone-releasing system (IUS) Bilateral tubal occlusion
Vasectomized partner A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the woman of child-bearing potential (WOCBP) and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.
Sexual abstinence Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatment. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.
NOTES: a) Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies. b) Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraceptive method. In this case, two highly effective methods of contraception should be utilized during the treatment period and for at least [X, corresponding to time needed to eliminate study treatment plus 30 days for study treatments with genotoxic potential] after the last dose of study treatment

Male participants:

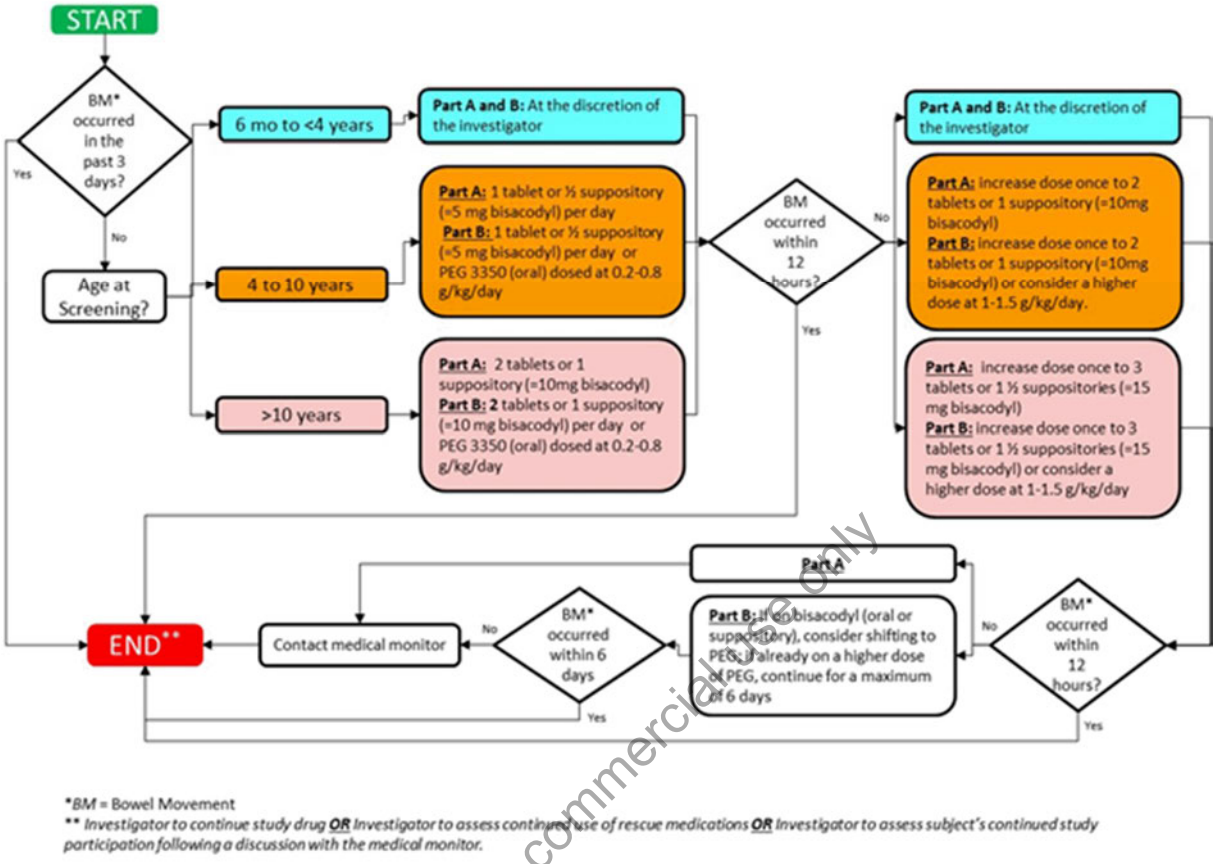
Male participants with female partners of childbearing potential are eligible to participate if they agree to ONE of the following throughout the study:

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Agree to use a male condom plus partner use of a contraceptive method with a failure rate of <1% per year when having penile-vaginal intercourse with a partner of childbearing potential who is not currently pregnant

Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration throughout the study.

Refrain from donating sperm for the duration of the study.

Appendix 5 Rescue Medication Algorithm



*BM = Bowel Movement
** *Investigator to continue study drug **OR** Investigator to assess continued use of rescue medications **OR** Investigator to assess subject's continued study participation following a discussion with the medical monitor.*

Note: It should be noted that the above guidelines on rescue medication remain subject to clinical discretion. If the subject has a BM after taking rescue medication but does not experience relief of symptoms, an additional dose(s)/escalated dose(s) of rescue medication may be recommended based on investigator discretion.

Appendix 6 Scales and Assessments

The following scales/assessments will be utilized in this study:

Study Part	Full Title of Scale/Assessment	Completed By
Screening	**e-Diary assessments:**	
	General e-diary questions: record bowel movements (all subjects) and abdominal pain (toilet-trained subjects only)	Self-completed for toilet-trained subjects ≥8 years of age, caregiver completed for toilet-trained subjects ≥3 but <8 years of age with subject input, where appropriate, and caregiver completed for non-toilet-trained subjects at least 6 months of age
A	**e-Diary assessments:**	
	General e-diary questions	Self-completed for toilet-trained subjects ≥8 years of age, caregiver completed for toilet-trained subjects ≥3 but <8 years of age with subject input, where appropriate, and caregiver completed for non-toilet-trained subjects at least 6 months in age
	Wong-Baker Faces Scale	Subjects aged ≥3 to <8 years or parent(s)/caregiver(s)/ legally authorized representative(s) (with subject input)
	Numerical Response Scale	Subjects aged ≥8 years
	BSFS	Self-completed for toilet-trained subjects ≥8 years of age, caregiver completed for toilet-trained subjects ≥3 but <8 years of age with subject input, where appropriate, and caregiver completed for non-toilet-trained subject at least 6 months in age
	In-clinic assessments:	
	PGI-S (toilet-trained subjects)	Subjects aged ≥8 years
	CGI-S (toilet-trained subjects)	Parent/caregiver/legally authorized representative for toilet-trained subjects 3 to <8 years
	PedsQL GI (Gas and Bloating Module Only) (toilet-trained subjects)	Parent report form for toddlers (2-4 years)[a] or young children (5-7 years) will be used for toilet-trained subjects ≥3 and <8 years, child-report for children (8-12 years) or the teen report form (13-18 years) will be used for toilet-trained subjects >8.
B	**e-Diary assessments:**	
	General e-diary questions: Use of study medication, use of other medication	Self-completed for toilet-trained subjects ≥8 years of age, caregiver completed for toilet-trained subjects ≥3 but <8 years of age with subject input, where appropriate, and caregiver completed for non-toilet-trained subjects at least 6 months of age

BSFS=Bristol Stool Form Scale; CGI-S=Caregiver Global Impression of Severity; PGI-S=Patient Global Impression of Severity; PedsQL GI=Pediatric Quality of Life Inventory (PedsQL™) Gastrointestinal Symptoms Scales™ Acute version 3.0
Note that all ages are ages at signing of informed consent.

[a] The parent report form for toddlers will only be applicable for toilet-trained children ≥3 years of age.

Appendix 7 Bedside Schwartz Equation

eGFR=40.7 x [height (m)/ SCr (mg/dL)]0.640 x [30/BUN (mg/dL)]0.202

or

eGFR=0.41 x height (cm)/SCr (mg/dL)

Where:

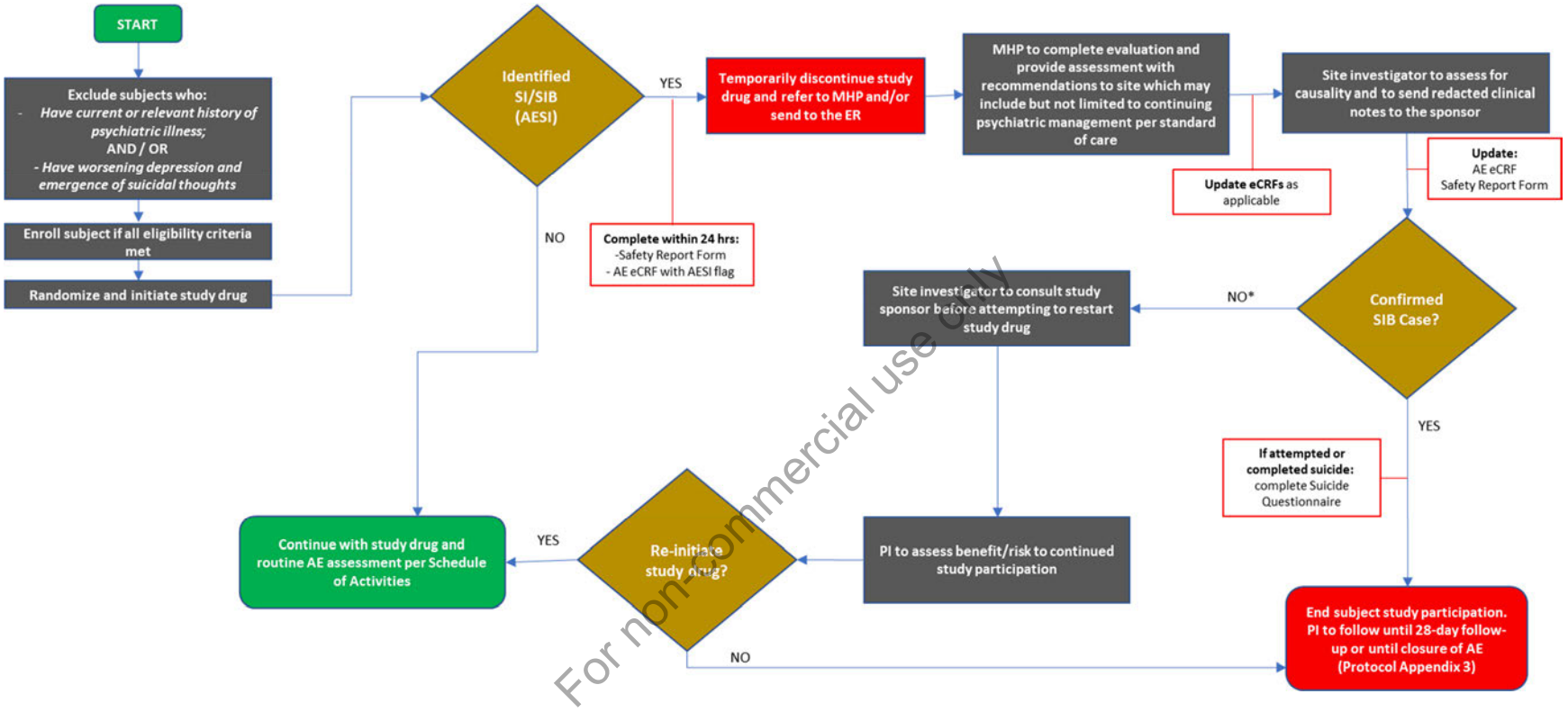
eGFR=mL/min/1.73 m²

SCr=mg/dL

Note The National Kidney Disease Education presently recommends reporting eGFR values for children greater than or equal to 75 mL/min/1.73 m² simply as "≥75 mL/min/1.73 m²," not as an exact number.

Source: (Schwartz and Work 2009)

Appendix 8 Site Workflow in Case of SI/SIB Assessment



Orange diamond: decision point; red box: subject disruption on study; green box: start/continue; grey box ; instructional; red-outlined box: forms

AESI = Adverse Event of Special Interest; DMC = Data Monitoring Committee; ER = Emergency Room; MHP = Mental Health Professional; SI/SIB = Suicidal Ideation/Suicidal Ideation Behavior

*SI cases continue with assessment

Appendix 9 e-Diary Text for Completion by Caregivers of Non-Toilet-Trained Subjects at Least 6 Months of Age

Design elements:

1) This diary will be completed once every evening.
2) Caregivers will answer all questions based on their own observations. For this reason, pain will not be assessed.
3) Training will be provided to the caregivers to cover navigation of the diary, as well as content to make sure the caregiver understands each question.
4) The full e-Diary will be used in Part A. Specific elements will be used for the e-Diary during screening (record bowel movement [date and time, consistency, and straining]) and Part B e-Diary (use of study medication and use of other medication).

Questions within the e-Diary:

- *"When did the child take the Study Medication today?"*

  [Capture time]

- Provide a checkbox under the time field:

  "The child did not take the Study Medication today."

[If no bowel movements have been recorded within the past 24 hours]

- *"No bowel movements have been recorded in the past 24 hours. Has the child had any bowel movements in the last 24 hours (since this time last evening)?"*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to rescue medication question]

[If at least one bowel movement has been recorded within the past 24 hours, show the caregiver the dates and times of this/these bowel movements.]

- *"You have recorded the following bowel movements. Has the child had any bowel movements in the last 24 hours that are not listed here?"*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to rescue medication question]

[Loop as many times as necessary to capture information for all bowel movements. Note that the date and time of the bowel movement will be populated on the BSFS and straining question screens during the loop.]

- *"When did the child have a bowel movement (in the last 24 hours)?*

  [Capture date and time]

- *"Which of these choices most closely resembles the child's bowel movement?"*

  Response options: BSFS

- *"How much did the child strain (push hard) during the bowel movement?"*

  Response options: Not at all, A little, A lot

Reminder text:

Unless it has been recommended by the child's study doctor, medication to help the child have a bowel movement (other than the study medication) should only be given if he or she has not had a bowel movement within the past 72 hours (3 days) or longer.

Only medications discussed with the study doctor should be given.

Please call the study doctor for further instructions if you are not sure what medications can be given to help the child have a bowel movement.

- *Other than the Study Medication, did the child receive any medication to help produce a bowel movement in the last 24 hours (since this time last evening)?*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to completion screen]

  - *When did the child receive a medication (other than the Study Medication) to help produce a bowel movement?*

    [Capture date and time]

Appendix 10 e-Diary Text for Completion by Caregivers of Toilet-Trained Subjects 3 to <8 Years of Age

Design elements:

1) The design will allow for real-time data collection but also allow for all data to be recorded once per evening to capture the most accurate data with built-in flexibility.
2) A home screen will offer four options. Selection of the first three will allow for real-time entry of information related to medication intake and bowel movements. Selection of the fourth option will be required once every evening to ensure that all data pertaining to that diary day are captured.
    - Record Use of Study Medication
    - Record a Bowel Movement
    - Record Use of Other Medication
    - Complete Evening Diary
3) Caregivers will do all data entry based on their own observations and information provided by the subject. Importantly, only subjects should provide the response for the abdominal pain item.
4) Training will be provided to the caregivers to cover navigation of the diary, as well as content to make sure the caregiver (and subject who will assist) understand each question.
5) The full e-Diary will be used in Part A. Specific elements will be used for the e-Diary during screening (record bowel movement [date and time, consistency, straining], abdominal pain) and Part B e-Diary (use of study medication and use of other medication).

Questions within each section of the e-Diary:

<u>Record Use of Study Medication</u>

- *"When did the child take the Study Medication today?"*

  [Capture time]

  Default to current time. Training will be provided on confirming or changing the time as needed.

<u>Record a Bowel Movement</u>

- *"When did the child have a bowel movement?"*

  [Capture date and time]

  Default to current date and time. Training will be provided on confirming or changing the date and time as needed.
- *"Which of these choices most closely resembles the child's bowel movement?"*

Response scale: BSFS

- *"How much did the child strain (push hard) during the bowel movement?"*

  Response options: Not at all, A little, A lot

Record Use of Other Medication

Reminder text:

Unless it has been recommended by the child's study doctor, other medication to help the child have a bowel movement (other than the study medication) should only be given if he or she has not had a bowel movement within the past 72 hours (3 days) or longer.

Only medications discussed with the study doctor should be given.

Please call the study doctor for further instructions if you are not sure what medications can be given to help the child have a bowel movement.

- *When did the child receive a medication (other than the Study Medication) to help produce a bowel movement?*

  [Capture date and time]

  Default to current date and time. Training will be provided on confirming or changing the date and time as needed, as well as the types of medications that should be recorded here.

Complete Evening Diary

[If date and time the subject took the Study Medication has not been provided since last completion of the evening diary.]

- *"When did the child take the Study Medication today?"*

  [Capture time]

  - Provide a checkbox under the date and time fields:

    "The child did not take the Study Medication today."

[If no bowel movements have been recorded within the past 24 hours.]

- *"You have not recorded any bowel movements for your child in the past 24 hours. Has the child had any bowel movements in the last 24 hours (since this time last night)?"*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to abdominal pain assessment]

[Loop the next three questions as many times as necessary to capture information for all bowel movements. Note that the date and time of the bowel movement will be populated on the BSFS and straining question screens during the loop.]

- *"When did the child have a bowel movement ?*

  [Capture date and time]

- *"Which of these choices most closely resembles the child's bowel movement?"*

  Response scale: BSFS

- *"How much did the child strain (push hard) during the bowel movement?"*

  Response options: Not at all, A little, A lot

[If at least one bowel movement has been recorded within the past 24 hours, show the caregiver the dates and times of this/these bowel movements.]

- *"You have recorded the following bowel movements. Did the child have any bowel movements in the last 24 hours that are not listed here?"*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to abdominal pain assessment]

- *"When did the child have a bowel movement?"*

  [Capture date and time]

- *"Which of these choices most closely resembles the child's bowel movement?"*

  Response scale: BSFS

- *"How much did the child strain (push hard) during the bowel movement?"*

  Response options: Not at all, A little, A lot

[Loop back to screen with bowel movement list until the caregiver answers "no".]

- *Please ask you child the following question: How has your tummy felt today?*

  Response scale: Wong-Baker Faces Scale

[If no rescue medication use was recorded within the past 24 hours.]

Reminder text:

Unless it has been recommended by the child's study doctor, medication to help the child have a bowel movement (other than the study medication) should only be given if he or she has not had a bowel movement within the past 72 hours (3 days) or longer.

Only medications discussed with the study doctor should be given.

Please call the study doctor for further instructions if you are not sure what medications can be given to help the child have a bowel movement.

- *Other than the Study Medication, did the child receive any medication to help produce a bowel movement in the last 24 hours (since this time last night)?*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to completion screen]

- *When did the child receive a medication (other than the Study Medication) to help produce a bowel movement?*

  [Capture date and time]

Appendix 11 e-Diary Text for Self-Completion by Toilet-Trained Subjects at Least 8 Years of Age

Design elements:

1) The design will allow for real-time data collection but also allow for all data to be recorded once per evening to capture the most accurate data with built-in flexibility.
2) A home screen will offer four options. Selection of the first three will allow for real-time entry of information related to medication intake and bowel movements. Selection of the fourth option will be required once every evening to ensure that all data pertaining to that diary day are captured.
    - Record Use of Study Medication
    - Record a Bowel Movement (Poop)
    - Record Use of Other Medication
    - Complete Evening Diary
3) Training will cover navigation of the diary, as well as content to make sure the subject (and caregiver who may assist) understand each question.
4) It is anticipated that caregivers may help with data entry, particularly medication-related information for younger children. Caregivers will be trained that the subject should answer all symptom-related questions (without influencing subjective responses).
5) The full e-Diary will be used in Part A. Specific elements will be used for the e-Diary during screening (record bowel movement [date and time, consistency, straining], abdominal pain) and Part B e-Diary (use of study medication and use of other medication).

Questions within each section of the e-Diary:

<u>Record Use of Study Medication</u>

- *"When did you take your Study Medication today?"*

  [Capture time]

  Default to current time. Training will be provided on confirming or changing the time as needed.

<u>Record a Bowel Movement</u>

- *"When did you poop?"*

  [Capture date and time]

  Default to current date and time. Training will be provided on confirming or changing the date and time as needed.

- *"Which of these choices looks most like your poop?"*

  Response scale: BSFS

- *"How much did you strain (push hard) when you pooped?"*

  Response options: Not at all, A little, A lot

Record Use of Other Medication

Reminder text:

Unless it has been recommended by the study doctor, you should only take a medication to help you poop (other than the Study Medication) if you have not pooped in the last 72 hours (3 days) or longer.

You should only take medications that have been talked about with your study doctor.

Please call (or ask someone else, such as a parent, to call) your study doctor for further instructions if you are not sure what medications you can take to help you poop.

- *When did you take a medication (other than the Study Medication) to help you poop?*

  [Capture date and time]

  Default to current date and time. Training will be provided on confirming or changing the date and time as needed, as well as the types of medications that should be recorded here.

Complete Evening Diary

[If date and time the subject took the Study Medication has not been provided since last completion of the evening diary.]

- *"When did you take your Study Medication today?"*

  [Capture time]

  - Provide a checkbox under the time field:

    "I did not take my Study Medication today."

[If no bowel movements have been recorded within the past 24 hours.]

- *"Have you pooped in the last 24 hours (since this time last night)?"*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to abdominal pain assessment]

[Loop through the next 3 questions as many times as necessary to capture information for all bowel movements. Note that the date and time of the bowel movement will be populated on the BSFS and straining question screens during the loop.]

- *"When did you poop ?*

  [Capture date and time]

- *"Which of these choices looks most like your poop?"*

  Response scale: BSFS

- *"How much did you strain (push hard) when you pooped?"*

  Response options: Not at all, A little, A lot

[If at least 1 bowel movement has been recorded within the past 24 hours, show the subject the dates and times of this/these bowel movements.]

- *"You have recorded the following poops. Did you have any poops in the last 24 hours (since this time last evening) that are not listed here?"*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to abdominal pain assessment]

- *"When did you poop?"*

  [Capture date and time]

- *"Which of these choices looks most like your poop?"*

  Response scale: BSFS

- *"How much did you strain (push hard) when you pooped?"*

  Response options: Not at all, A little, A lot

[Loop back to screen with bowel movement list until the subject answers "no".]

- *How would you rate the worst belly pain you felt in the last 24 hours?*

  Response scale: 0-to-10 numeric rating scale with 0 = no belly pain and 10 = worst possible belly pain

[If no rescue medication use was recorded within the past 24 hours.]

Reminder text:

Unless it has been recommended by the study doctor, you should only take a medication to help you poop (other than the Study Medication) if you have not pooped in the last 72 hours (3 days) or longer.

You should only take medications that have been talked about with your study doctor.

Please call (or ask someone else, such as a parent, to call) your study doctor for further instructions if you are not sure what medications you can take to help you poop.

- *Other than the Study Medication, have you taken any medication to help you poop in the last 24 hours (since this time last evening)?*

  Response options: Yes, No

  [If response = "yes", continue with below; if response = "no", skip to completion screen]

- *When did you last take a medication (other than the Study Medication) to help you poop?*

  [Capture date and time]

Appendix 12 PedsQL Gastrointestinal Symptoms: Overview of Questionnaires

Modified PedsQL™ Gastrointestinal Symptoms Modules: Includes Gas and Bloating Module

Child's Age at Baseline / Day 0	Questionnaires Required to be completed During Screening / Part A (Study Visit 1/Screening, Study Visit 2/Baseline, Study Visit 6/Week 4, Study Visit 10 /Week 8, and Study Visit 14/Week 12)	Who completes?
3-4 years old		
	Parent Report for Toddlers (2-4): Gas and Bloating Module	Parent
5-7 years old		
	Parent Report for Young Children (5-7): Gas and Bloating Module	Parent
8-12 years old		
	Child Report (8-12): Gas and Bloating Module	Subject
13-17 years old		
	Teen Report (13-18): Gas and Bloating Module	Subject

Each assessment will utilize a 7-day recall period. The assessments will not be completed for non-toilet-trained subjects.

Appendix 13 PedsQL Gastrointestinal Symptoms Modules

PedsQL™

Gastrointestinal Symptoms Scales

Acute Version 3.0

TEEN REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you.

Please tell us **how much of a problem** each one has been for you during the **past 7 days** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.

If you do not understand a question, please ask for help.

*In the past **7 days**, how much of a **problem** has this been for you …*

GAS AND BLOATING (PROBLEMS WITH…)	NEVER	ALMOST NEVER	SOME-TIMES	OFTEN	ALMOST ALWAYS
1. My stomach feels full of gas	0	1	2	3	4
2. My stomach feels very full	0	1	2	3	4
3. My stomach gets big and hard	0	1	2	3	4
4. I have a lot of gas	0	1	2	3	4
5. I pass a lot of gas	0	1	2	3	4
6. My stomach feels gassy	0	1	2	3	4
7. My stomach makes noises	0	1	2	3	4

PedsQL™

Gastrointestinal Symptoms Scales

Acute Version 3.0

CHILD REPORT (ages **8-12**)

DIRECTIONS

On the following page is a list of things that might be a problem for you.

Please tell us **how much of a problem** each one has been for you

during the **past 7 days** by circling:

0 if it is **never** a problem

1 if it is **almost never** a problem

2 if it is **sometimes** a problem

3 if it is **often** a problem

4 if it is **almost always** a problem

There are no right or wrong answers.

If you do not understand a question, please ask for help.

*In the past **7 days**, how much of a **problem** has this been for you …*

GAS AND BLOATING (PROBLEMS WITH…)	NEVER	ALMOST NEVER	SOME-TIMES	OFTEN	ALMOST ALWAYS
My stomach feels full of gas	0	1	2	3	4
My stomach feels very full	0	1	2	3	4
My stomach gets big and hard	0	1	2	3	4
I have a lot of gas	0	1	2	3	4
I pass a lot of gas	0	1	2	3	4
My stomach feels gassy	0	1	2	3	4
My stomach makes noises	0	1	2	3	4

PedsQL™

Gastrointestinal Symptoms Scales

Acute Version 3.0

PARENT REPORT for YOUNG CHILDREN (ages 5-7)

DIRECTIONS

On the following page is a list of things that might be a problem for your child.

Please tell us **how much of a problem** each one has been for your child during the **past 7 days** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.

If you do not understand a question, please ask for help.

*In the past **7 days**, how much of a **problem** has this been for your child …*

GAS AND BLOATING *(PROBLEMS WITH…)*	NEVER	ALMOST NEVER	SOME-TIMES	OFTEN	ALMOST ALWAYS
1. Stomach feels full of gas	0	1	2	3	4
2. Stomach feels very full	0	1	2	3	4
3. Stomach gets big and hard	0	1	2	3	4
4. Has a lot of gas	0	1	2	3	4
5. Passes a lot of gas	0	1	2	3	4
6. Stomach feels gassy	0	1	2	3	4
7. Stomach makes noises	0	1	2	3	4

PedsQL™

Gastrointestinal Symptoms Scales

Acute Version 3.0

PARENT REPORT for TODDLERS (ages 2-4)

DIRECTIONS

On the following page is a list of things that might be a problem for your child.
Please tell us **how much of a problem** each one has been for your child
during the **past 7 days** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

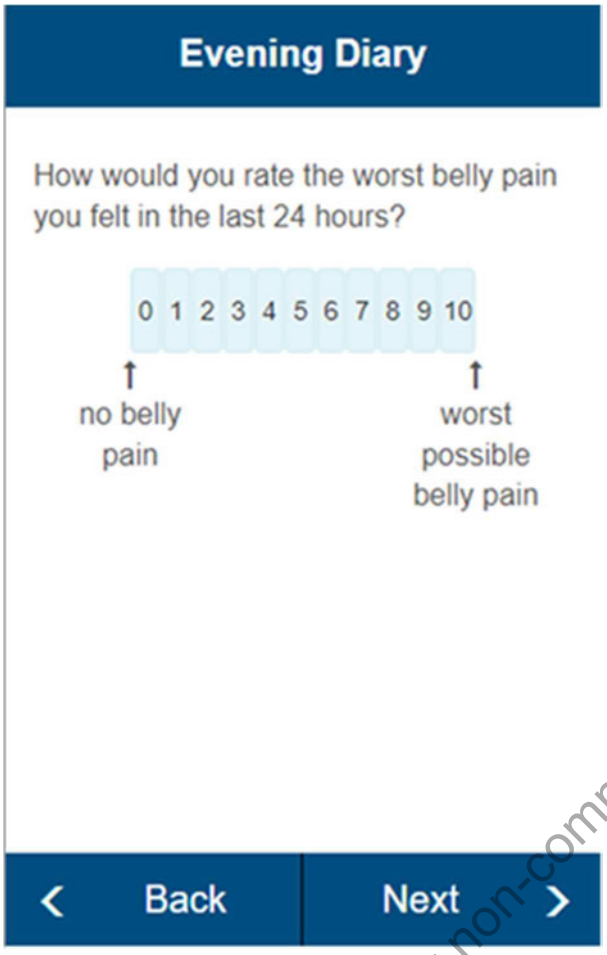
There are no right or wrong answers.
If you do not understand a question, please ask for help.

*In the past **7 days**, how much of a **problem** has this been for your child …*

GAS AND BLOATING *(PROBLEMS WITH…)*	NEVER	ALMOST NEVER	SOME-TIMES	OFTEN	ALMOST ALWAYS
Stomach feels full of gas	0	1	2	3	4
Stomach feels very full	0	1	2	3	4
Stomach gets big and hard	0	1	2	3	4
Has a lot of gas	0	1	2	3	4
Passes a lot of gas	0	1	2	3	4
Stomach feels gassy	0	1	2	3	4
Stomach makes noises	0	1	2	3	4

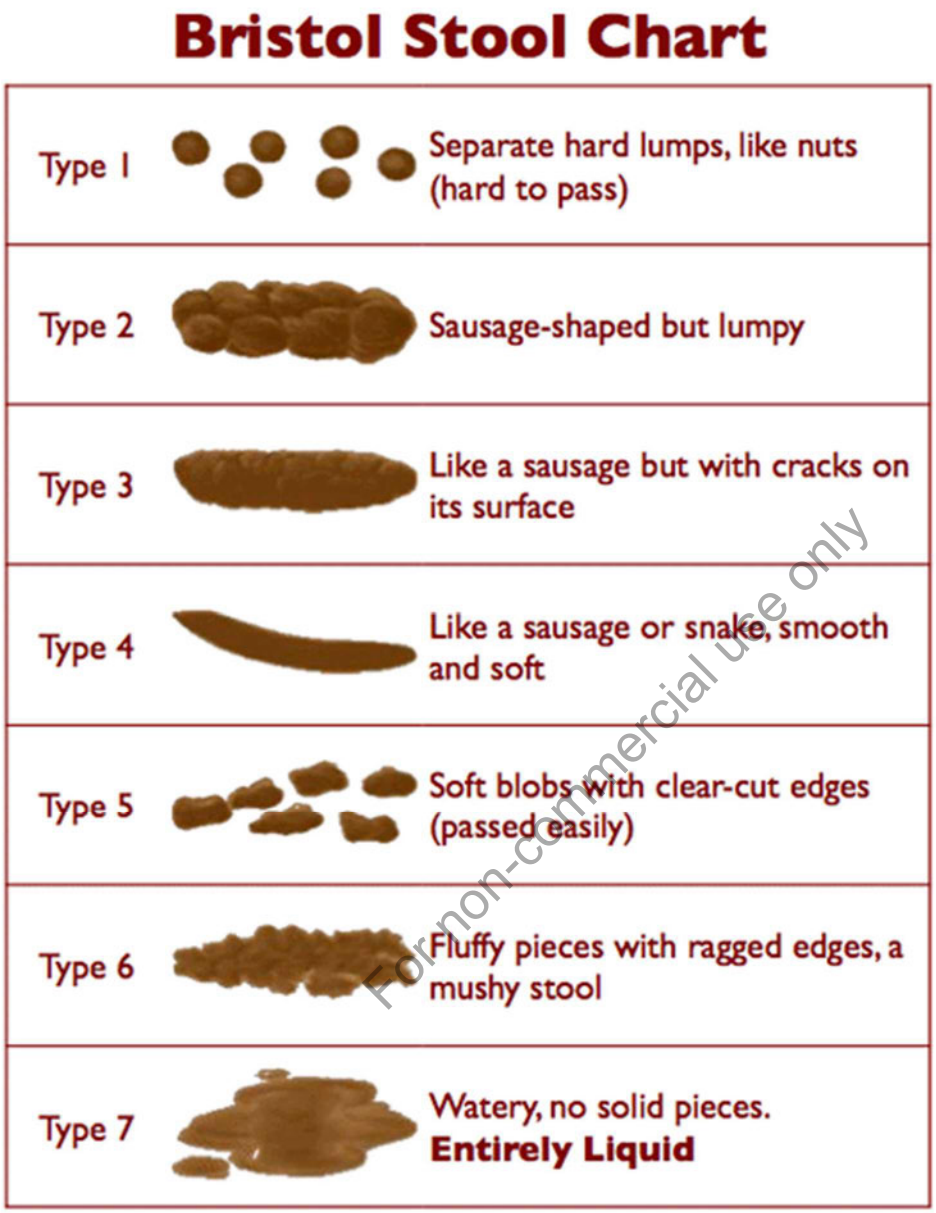
Appendix 14 Wong-Baker Faces Scale

Appendix 15 NRS



Note: Subjects will only be able to select whole numbers on the NRS.

Appendix 16 BSFS



Source: (Lewis and Heaton 1997)

Appendix 17 Global Impression of Severity

Caregiver Global Impression of Severity (CGI-S)

Based on what you have observed and heard from your child, overall, how would you rate the severity of his or her constipation over the past 7 days?

None

Mild

Moderate

Severe

Patient Global Impression of Severity (PGI-S)

Overall, how would you rate the severity of your constipation over the past 7 days?

None

Mild

Moderate

Severe

Appendix 18 Abbreviations

Abbreviation	Definition
5-HT_4	5-hydroxytryptamine type 4
ADHD	attention-deficit/hyperactivity disorder
ADR	adverse drug reaction
AE	adverse event
AESI	adverse event of special interest
ALT	alanine aminotransferase (synonymous with SGPT)
AST	aspartate aminotransferase (synonymous with SGOT)
AUC	area under the curve
β-hCG	beta-human chorionic gonadotropin
BM	bowel movement
BMI	body mass index
BSFS	Bristol Stool Form Scale
BUN	blood urea nitrogen
BW	body weight
CGI-S	Caregiver Global Impression of Severity
C_{max}	maximum observed plasma concentration
CMH	Cochrane Mantel Haenszel
COVID-19	Coronavirus Disease 2019
CRA	clinical research associate
CrCl	creatinine clearance
CRF	case report form
CRO	contract research organization
CS	compound symmetry
DMC	data monitoring committee
DTP	direct-to-patient
EC	ethics committee
eCDF	empirical cumulative distribution function
ECG	electrocardiogram
eCOA	electronic clinical outcome assessment
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EMA	European Medicines Agency
ESPGHAN	European Society for Pediatric Gastroenterology, Hepatology, and Nutrition
EU	European Union
ER	emergency room
ET	early termination
FDA	Food and Drug Administration
FGID	Functional Gastrointestinal Disorders
GCP	Good Clinical Practice
GFR	glomerular filtration rate
HIPAA	Health Insurance Portability and Accountability Act
IA	interim analysis/analyses
IB	investigator's brochure
IBS	irritable bowel syndrome
IBS-C	irritable bowel syndrome with constipation
ICE	intercurrent event
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IDMC	independent data monitoring committee

Abbreviation	Definition
IP	investigational product
IRB	institutional review board
IRT	interactive response technology
ITT	intent-to-treat
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
LLN	lower limit of normal
MAR	missing at random
MCID	minimal clinically important difference
MHP	mental health professional
mITT	modified intent-to-treat
MMRM	mixed-effects model for repeated measures
MNAR	missing not at random
NASPGHAN	North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition
NDA	new drug application
NRS	numerical rating scale
NTTITT	non-toilet-trained intent-to-treat
NTTmITT	non-toilet-trained modified intent-to-treat
NTTSAF	non-toilet-trained safety
PCS	potentially clinically significant
PDF	probability density function
PedsQL™	Pediatric Quality of Life Inventory™
PedsQL GI	Gastrointestinal Symptoms Scales
PEG	polyethylene glycol
PGI-S	Patient Global Impression of Severity
PI	prediction interval
PK	pharmacokinetic(s)
PMR	post-marketing requirement
PP	per-protocol
PREA	Pediatric Research Equity Act
QD	once a day
QoL	quality of life
QTcB	QT interval corrected for heart rate using Bazett's formula
QTcF	QT interval corrected for heart rate using Fridericia's formula
RBC	red blood cell
RSI	reference safety information
SAE	serious adverse event
SAP	statistical analysis plan
SBM	spontaneous bowel movement
SD	standard deviation
SI	suicidal ideation
SIB	suicidal behavior
SOC	system organ class
SRM	standardized response mean
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse events
t_{max}	time to maximum observed plasma concentration
UADE	unanticipated adverse device effect
UK	United Kingdom
ULN	upper limit of normal

Abbreviation	Definition
UN	unstructured
US	United States
vs	versus
WBC	white blood cell
WOCBP	woman of child-bearing potential
WOCF	worst observation carried forward

Appendix 19 Protocol History

Document	Date	Global/Country/Site Specific
Amendment 3	27 October 2022	Global
Amendment 2	20 October 2021	Global
Amendment 1	18 December 2020	Global
Original protocol	21 October 2020	Global

Protocol Amendment 2			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
1	Section 1.1 Synopsis Section 5.2 Exclusion Criteria	It was clarified that exclusion criterion #11 discusses any investigational product.	Clarification.
2	Section 1.3 Schedule of Activities Section 8.1.1.1 Screening Visit (Visit 1)	The screening visit window was aligned with wording in the protocol.	Correction.
3	Section 1.3 Schedule of Activities	Time points when visits should be confirmed on the electronic clinical outcomes assessments (eCOA) device.	Clarification.
4	Section 1.3 Schedule of Activities Section 6.7.3.1 Rescue Medication	The frequency for providing rescue medications was revised to align with operational processes. Further, rescue medications will not be collected. Accountability will be limited to diary data where subjects are instructed to record rescue medications use.	Alignment with vendor process.
5	Section 1.3 Schedule of Activities	PGI-S and CGI-S assessments were added at screening.	Addition to have predose baseline data.
6	Section 6.2.3 Dosing	It was added that if a subject weighing ≥50 kg cannot tolerate the tablets, they can be switched to oral solution for the duration of study participation. Further, switching from oral solution to tablets is not allowed.	Clarification of study process.
7	Section 6.2.3 Dosing	Additional instructions were added to make it clear that the oral dose cannot be mixed with orange juice or any other beverage and should be administered through an oral syringe as instructed.	Clarification of study process.
8	Section 6.5 Drug Accountability Section 6.7.3.1 Rescue Medication	Updates were made to reflect the drug accountability process followed.	Alignment with vendor process.

Protocol Amendment 2			
Summary of Changes Since the Last Version of the Approved Protocol			
Change Number	**Sections Affected by Change**	**Description of Each Change and Rationale**	
	Location	***Description***	***Rationale***
9	Section 7.3 Reasons for Discontinuation	It was specified that If a subject chooses to withdraw from study participation due to personal concerns or unavoidable circumstances related to the COVID-19 pandemic (other than a COVID-19–related AE), this should be specified as the reason for subject withdrawal in the eCRF.	Clarification.
10	Section 8.2.2.4 Retentive Posturing Section 8.2.2.5 Fecal Incontinence	It was clarified that retentive posturing and fecal incontinence assessments only apply to toilet-trained subjects at least 3 years of age and that responses will be logged in the source notes and electronic case report form.	Clarification.
11	Section 9.8.1 Health-related Quality of Life Analyses (Toilet-trained Subjects)	Additional details on the anchor-based analysis were added.	Clarification and update of the statistical analysis. Missing details for the analysis for the anchor-based analysis of the PGI-S/CGI-S were added.
12	Throughout protocol	Coronavirus disease-2019 (COVID-19)-related flexibility in study conduct has been added.	The sponsor allows flexibility in the conduct of the study to reduce burden on the subject and the site that is impacted by COVID-19.
13	Appendix 12	An excerpt of the PedsQL GI including the only the Gas and Bloating Module for each age group has been included.	

Protocol Amendment 1		
Summary of Changes Since the Last Version of the Approved Protocol		
Amendment Number	**Amendment Date**	**Global**
1	**18 Dec 2020**	
Protocol Amendment Summary and Rationale: The primary reasons for this amendment are to update the protocol implementing the comments and suggestions made by the Food and Drug Administration (FDA) in their administrative letter dated 03 December 2020. In this amendment, minor grammatical, editorial, formatting, and administrative changes not affecting the conduct of the study are included for clarification and administrative purposes only.		
Description of Each Change and Rationale		**Sections Affected by Change**
Description	***Rationale***	***Location***
Clarification that the primary endpoint is based on data collected during the	Minor clarification based on an FDA	Section 1.1 Synopsis Section 3.2.1 Primary Endpoint

Protocol Amendment 1		
Summary of Changes Since the Last Version of the Approved Protocol		
Amendment Number **1**	**Amendment Date** **18 Dec 2020**	**Global**
placebo-controlled part (Part A)	comment.	Section 9.6.1 Primary Efficacy Endpoint Section 9.6.6 Multiplicity Adjustment for Primary and Secondary Endpoints
Addition of a sub header to clearly distinguish between other and exploratory efficacy endpoints.	Minor clarification based on an FDA comment.	Section 1.1 Synopsis Section 9.6.5 Exploratory Efficacy Endpoints
Clarification that no paper administration will be implemented in this study.	Minor clarification based on an FDA comment.	Section 8.2.2.1 E-Diary
Update of the NRS figure provided in appendix and to the NRS description to clarify that only whole numbers can be selected on this scale.	Minor clarification based on an FDA comment.	Section 8.2.2.1 E-Diary Appendix 14
Correction of text describing Part B of the study as placebo controlled.	Correction based on an FDA comment.	Section 6.7.3.1 Rescue Medication
Correction of the population used for the primary estimand of interest from ITT to mITT.	Correction based on an FDA comment.	Section 9.4 Estimands
The description of the PK endpoint was slightly changed to make it consistent with the language used in the SAP.	Minor update for consistency with the SAP.	Section 1.1 Synopsis Section 3.2.6 PK Endpoint
Correction of the timing of the call and number of BMs from >3 to ≥3 and in the description of the first telephone contact of the screening period.	Minor correction for consistency.	Section 8.1.1.1 Screening Visit (Visit 1)
Clarification that the ages used will be those as the signing of informed consent.	Minor clarification.	Section 8.2.2.1 E-Diary Appendix 6
Correction to the description of the NRS where a score of 10 rather than 11 presents the worst pain imaginable.	Minor correction.	Section 8.2.2.1 E-Diary
Description of Each Change and Rationale		**Sections Affected by Change**
Description	***Rationale***	***Location***
Update the description of the PedsQL GI to make it clear that these questionnaire modules will be assessed over the previous 7 days.	Minor clarification.	Section 8.2.2.3 Pediatric Quality of Life Inventory Gastrointestinal Symptom Scales (PedsQL GI; Gas and Bloating Module) Appendix 11
Correction to the younger toilet-trained subject lower limit in the PedsQL GI from >3 to ≥3.	Minor correction.	Section 8.2.2.3 Pediatric Quality of Life Inventory Gastrointestinal Symptom Scales (PedsQL GI; Gas and Bloating Module) Appendix 6
Correction of the blood volumes based on information from the clinical	Update with most	Section 8.2.5 Volume of Blood to Be Drawn

Protocol Amendment 1		
Summary of Changes Since the Last Version of the Approved Protocol		
Amendment Number **1**	**Amendment Date** **18 Dec 2020**	**Global**
laboratory.	recent information.	from Each Subject
Update of the interval in between DMC meetings from 3 to 6 months.	Update with most recent information.	Section 9.2.1 Data Monitoring Committee
Correction of the incorrect timing definition for exclusion from the PP Analysis Set.	Minor correction.	Section 9.5 Statistical Analysis Sets
Updates (flow changes and improved streamlining) to the e-Diary questions to reflect the most recent study materials.	Update with most recent information.	Appendix 8 Appendix 9 Appendix10