CLINICAL TRIAL PROTOCOL

A Multi-center, Single-arm Trial Exploring the Safety and Clinical Effectiveness of RBX2660 Administered by Colonoscopy to Adults with Recurrent *Clostridioides difficile* Infection

Trial 000416

CDI-SCOPE

IND Number:	029,020
UTN Number:	U1111-1277-5271
Investigational Medicinal Product:	RBX2660, microbiota suspension
Indication:	Prevention of recurrence of *Clostridioides difficile* infection (CDI) in individuals 18 years of age and older, following antibiotic treatment for recurrent CDI (rCDI)
Phase:	3b
Name and Address of Sponsor:	Ferring Pharmaceuticals A/S Amager Strandvej 405 2770 Kastrup Denmark Telephone number: +45 88 33 88 34
GCP Statement:	This trial will be performed in compliance with GCP.

SYNOPSIS

TITLE OF TRIAL

A Multi-center, Single-arm Trial Exploring the Safety and Clinical Effectiveness of RBX2660 Administered by Colonoscopy to Adults with Recurrent *Clostridioides difficile* Infection

SIGNATORY INVESTIGATOR

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TRIAL SITES

This trial will be conducted at approximately 10-20 sites in the United States (US).

PLANNED TRIAL PERIOD	CLINICAL PHASE
First subject first visit (FSFV): April 2023 Last subject last visit (LSLV): May 2024	3b

BACKGROUND AND SCIENTIFIC JUSTIFICATION FOR CONDUCTING THE TRIAL

Clostridioides difficile (previously *Clostridium difficile* and hereafter denoted *C. difficile*) infection (CDI) is a common cause of healthcare-associated infections and is also commonly acquired in community settings such as nursing homes. CDI contributes to considerable morbidity, mortality, and healthcare expenditure. In recent years, CDI has demonstrated an escalation in overall prevalence and severity of disease. A study of data from 2017 reported that *C. difficile* accounted for nearly half a million infections in the United States (US). A major complication from CDI is recurrent infection, despite absence of additional risk factors.

While mature colonic microbiota in healthy adults are generally resistant to *C. difficile* colonization, the widely accepted model for CDI pathogenesis is that the use of broad-spectrum antimicrobials suppresses and disrupts the microbial communities that normally prevent expansion of *C. difficile*. Because *C. difficile* spores are largely resistant to antibiotics, they can germinate into vegetative forms after antibiotic treatment has been discontinued. If residual normal intestinal microbiota cannot restrain the infection, *C. difficile* bacteria proliferate and produce toxins that cause destruction of colonic epithelial cells, inflammation, and disease symptoms.

Conventional treatments for CDI utilize antibiotics, which further disrupt the gut bacteria flora and limit the recovery of the gut microbiota following CDI. This decreased diversity of the gut microbiota following antibiotic treatment can predispose patients to re-colonization with

C. difficile and lead to recurrent infections. Recurrent CDI (rCDI) is defined as recurrence of symptoms within 8 weeks after response to, and cessation of, specific antibiotic treatment, with a positive diagnostic test for *C. difficile* and exclusion of other enteropathogens. Recurrent CDI (rCDI) presents most commonly as repeated bouts of diarrhea that respond to antibiotic treatment but recur within days to 8 weeks of stopping the antibiotic treatment. Repeated bouts of infection can continue for years, leading to persistent use of antibiotics, markedly reduced quality of life (QoL), repeated hospitalizations, and even death.

It is estimated that the risk of a first recurrence after an initial response to antibiotic therapy for a primary CDI ranges from 20 to 30%. In patients with prior rCDI, the risk of an additional recurrence increases to 40 to 60%. Analyses of recurrence history over time also suggest that the rate of recurrence for any number of recurrences after the first recurrence is similar. Effective alternative treatment options to repeated or continuous antibiotics or bezlotoxumab, a human monoclonal antibody indicated for prevention of recurrence of CDI in adults at high risk for recurrence of CDI, are lacking.

Administration of human stool from healthy donors, i.e., microbiome restoration therapy (MRT), is believed to restore the diversity of the gut microbiome and thereby suppress *C. difficile* outgrowth, and is emerging as an important treatment option for rCDI. Multiple studies have used colonoscopy to deliver MRT in the form of fecal microbiota transplantation (FMT) to rCDI patients with promising success rates. This is also reflected in the American College of Gastroenterology (ACG) guidelines on the prevention, diagnosis, and treatment of CDI, which recommend that patients with rCDI should be treated with MRT delivered by colonoscopy to prevent further recurrence of the infection.

Microbiome changes in patients with rCDI include reduced diversity, reduced abundance of *Bacteroidetes* and *Firmicutes* phylae, increased abundance of the *Proteobacteria* phylum (especially in the *Enterobacteriaceae* family), and differences in species involved in bile acid metabolism. Numerous studies have shown that successful MRT in patients with rCDI is associated with a shift towards an increase in abundance of *Bacteroidetes* and *Firmicutes* phylae and a corresponding reduction in the *Proteobacteria* phylum. Complete replacement by donor microbiota does not seem to be necessary for clinical success. In addition, bile acid restoration has been hypothesized as one possible contributing factor to how microbiome restoration could prevent rCDI.

RBX2660 is a microbiota suspension prepared from human stool which is collected from pre-screened and qualified donors. In order to ensure consistency and quality, the suspension is tested and processed to a stable, cryopreserved drug product under Good Manufacturing Practice (GMP) conditions. RBX2660 is supplied as a pre-packaged single-dose 150 mL microbiota suspension of 1×10^8 to 5×10^{10} colony-forming units (CFU)/mL of diverse spore-forming and non-spore-forming bacteria, including Bacteroides.

The safety and efficacy of RBX2660 to prevent recurrence of CDI in subjects with rCDI has been investigated when delivered as an enema in 5 prospective clinical trials (2 completed

placebo-controlled trials [phase 2b Trial 2014-01 and pivotal phase 3 Trial 2017-01], 2 completed open-label trials [phase 2 Trial 2013-001 and 2015-01], and one open-label trial [phase 3 Trial 2019-01; database lock 25 August 2023, reporting ongoing]) and one retrospective study (Study 2019-02), all of which contribute to the overall totality of evidence supporting the safety and efficacy of RBX2660. On 30 November 2022, RBX2660 was approved as REBYOTA by the US Food and Drug Administration (FDA) for the indication "Prevention of recurrence of *Clostridioides difficile* infection (CDI) in individuals 18 years of age and older, following antibiotic treatment for recurrent CDI (rCDI)" when delivered by rectal administration as an enema.

The purpose of this trial is to explore the safety and clinical effectiveness of RBX2660 when delivered by colonoscopy to adults with rCDI. The experience of physicians will be documented through a physician-experience questionnaire to explore the usability of RBX2660 in clinical practice for colonoscopic administration. Furthermore, to explore the patient-experience of RBX2660 treatment, each trial subject will be offered to undergo a structured interview.

OBJECTIVES

Primary Objective

- To explore the safety of RBX2660 when delivered by colonoscopy in subjects with recurrent *Clostridioides difficile* infection (rCDI)[a].

Secondary Objectives

- To explore the effectiveness of RBX2660 when delivered by colonoscopy in preventing recurrence[b] of *Clostridioides difficile* infection (CDI) in subjects with rCDI.
- To explore the usability[c] of RBX2660 in clinical practice when delivered by colonoscopy in subjects with rCDI.
- To explore the patient-experience of RBX2660 when delivered by colonoscopy in subjects with rCDI.

[a] Recurrent CDI (rCDI) for trial entry is defined as a documented diagnosis of 1) CDI diarrhea: the passage of ≥3 unformed/loose stools in 24 consecutive hours for ≥2 consecutive days, that began within 8 weeks after completion of previous CDI treatment and 2) ≥1 stool test positive for toxigenic *C. difficile* or *C. difficile* toxin, documented at the time of the CDI diarrhea for the qualifying CDI episode.

[b] Recurrence of CDI during the trial, i.e., treatment failure, is defined as the presence of CDI diarrhea within 8 weeks after RBX2660 treatment. CDI diarrhea during the trial is defined as passage of ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days and a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin, as determined by a toxin protein detecting assay at the time of the diarrhea.

[c] The extent to which a physician conducting therapeutic colonoscopy can use commercially available RBX2660 safely, effectively, and efficiently.

ENDPOINTS

Primary Endpoint

- RBX2660-related treatment-emergent adverse events (TEAEs) after RBX2660 treatment delivered by colonoscopy through 8 weeks, or treatment failure.

Secondary Endpoints

- Recurrence of *Clostridioides difficile* infection (CDI) within 8 weeks after RBX2660 treatment delivered by colonoscopy.
- Time to CDI recurrence from baseline through 8 weeks after RBX2660 treatment delivered by colonoscopy.
- Physician-experience, as determined by questionnaire, documenting subjective experience of investigators on usability of RBX2660 in clinical practice when delivered by colonoscopy.
- Physician perception of patient benefit, as determined by Clinician Global Impression of Improvement (CGI-I) at 8 weeks, or at treatment failure, after RBX2660 treatment delivered by colonoscopy.
- Patient-experience interview at 8 weeks, or at treatment failure, after RBX2660 treatment delivered by colonoscopy.
- Safety up to 6 months after RBX2660 treatment delivered by colonoscopy:
    - TEAEs, including type, intensity, and causality.
    - Serious adverse events (SAEs).
    - Adverse events of special interest (AESIs): septic shock, toxic megacolon, colonic perforation, and emergency colectomy, and new onsets of obesity, glucose intolerance, metabolic syndrome, or autoimmune conditions.
    - Adverse events leading to death or intensive care unit (ICU) admission.

METHODOLOGY

This is a multi-center, single-arm trial to explore the safety and clinical effectiveness of RBX2660 (microbiota suspension) when administered by colonoscopy to adults with rCDI.

Subjects aged 18 years and above, with a diagnosis of rCDI and a stool test positive for the presence of toxigenic *C. difficile* or *C. difficile* toxin to confirm the medical record documentation of rCDI, will be eligible for screening in this trial. After signing the informed consent form (ICF), subjects will undergo a screening evaluation prior to treatment at baseline (day 1). To be screened in this trial, subjects must be taking or have just received a prescription for a course of antibiotics to control rCDI symptoms, type and dosage at the discretion of the prescribing physician. This means that subjects who have already completed their prescribed course of antibiotics to treat rCDI are not eligible for screening unless they have another recurrence that requires treatment with antibiotics. Antibiotic therapy for the qualifying rCDI episode must include a minimum of 10 consecutive days and must not exceed a total of 60 days (including intermittent dosing/pulse)

of antibiotic use before a mandatory antibiotic washout period can be initiated. The antibiotic washout period consists of a minimum of 1 day (≥24 hours) to a maximum of 3 days (≤72 hours) immediately before treatment at baseline. Before the antibiotic washout period can start, CDI symptoms must be under control, i.e., no longer meeting the symptomatic criteria for CDI diarrhea for the 2 consecutive days immediately before the antibiotic washout period. The CDI symptoms must furthermore be under control throughout the antibiotic washout period and up to start of a mandatory bowel preparation (by a method at the investigator's discretion), in order for subjects to receive treatment with RBX2660. Consented subjects who do not meet the eligibility criteria will be considered as screening failures.

At the screening visit, subjects will be instructed on how to document stool patterns in an electronic stool diary. Subjects are required to start completing the stool diary immediately after the screening visit, on the same day, and continue until start of bowel preparation. The stool diary will be reviewed on an ongoing basis by trial site staff and at the baseline visit before administration of RBX2660. In order for subjects to receive treatment with RBX2660, the stool diary completed from the screening visit up to start of bowel preparation must demonstrate that CDI symptoms were under control, i.e., no longer meeting the symptomatic criteria for CDI diarrhea, for the 2 consecutive days immediately before initiating the antibiotic washout period, and throughout the antibiotic washout period up to start of bowel preparation. At baseline, immediately after the antibiotic washout period and bowel preparation, subjects will receive treatment with RBX2660, administered by colonoscopy. RBX2660 should be administered to the right side of the colon (i.e., between the ileocecal valve and the hepatic flexure of the colon). At the baseline visit, subjects will be instructed on how to document solicited events in an electronic solicited events diary. Subjects are required to start completing the solicited events diary immediately after receiving treatment with RBX2660 at the baseline visit, on the same day, and continue until the first follow-up visit at week 1 when it will be reviewed by the trial site staff.

Follow-up visits will occur at 1, 2, 4, and 8 weeks, and 3 and 6 months after treatment. The follow-up visits at 1, 2 and 4 weeks, and 3 months after treatment can be either virtual or on-site, as judged by the investigator. An on-site CDI recurrence visit is required if CDI recurrence (treatment failure) is suspected at any time within 8 weeks after treatment with RBX2660. CDI recurrence will be determined by use of an algorithm relating to diarrhea and *C. difficile* toxin test results from a toxin protein detecting assay. External adjudication will not be used.

Subjects who have completed the follow-up 4 visit at 8 weeks after RBX2660 treatment, or who have been documented as treatment failures (recurrence of CDI) at a CDI recurrence visit, will be contacted by Clinical Outcomes Solution (COS) which is a third-party contract research organization (CRO) for a patient-experience interview by telephone within 14 days of attending the respective visit. Participation in the patient-experience interview is optional and signed informed consent must have been obtained through the ICF for the trial, as appropriate, before participation. The patient-experience interview is designed to examine the impact of living with CDI on a subject's health-related quality of life, and to assess what a meaningful positive change

after RBX2660 treatment would be from the subject's perspective. The focus of the interview will be on the general subject impact of rCDI.

To capture the investigators' subjective experience on the usability of RBX2660 in clinical practice, all investigators in this trial will be required to complete a physician-experience questionnaire after each attempted colonoscopy. The questionnaire will capture each physician's opinion on the practical aspects of the usability of RBX2660 when delivered by colonoscopy. In addition, investigators will be asked to rate each subject's total improvement according to the Clinician Global Impression of Improvement (CGI-I) scale at the follow-up 4 visit, or at a CDI recurrence visit for subjects who have been documented as treatment failures (recurrence of CDI).

Subjects who are documented treatment failures, defined as presence of CDI diarrhea, including a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin as determined by a toxin protein detecting assay, within 8 weeks after RBX2660 treatment should be treated according to standard of care (SoC) at the investigator's discretion. These subjects will continue the follow-up assessments for safety through 6 months after RBX2660 treatment according to the trial flow chart. Subjects who experience CDI after the follow-up 4 visit at week 8 should be diagnosed and treated according to SoC at the investigator's discretion. These subjects will continue the follow-up assessments for safety through 6 months after RBX2660 treatment according to the trial flow chart.

As part of the trial, subjects will be required to provide stool samples for exploratory research of potential biomarkers pertaining to the mechanism of action of microbiome restoration in patients with rCDI. Subjects are required to collect and ship stool samples in association with each of the scheduled follow-up visits, and any CDI recurrence visit, after RBX2660 treatment. Subjects will collect the stool samples in pre-labelled containers provided by the trial site. The stool samples will be used for future analyses of potential biomarkers and will be analysed and reported separately from the current trial.

Adverse events, safety laboratory variables (clinical chemistry and hematology), vital signs, and concomitant medications will be assessed throughout the trial.

NUMBER OF SUBJECTS

Approximately 40 subjects are planned to receive treatment with RBX2660.

CRITERIA FOR INCLUSION / EXCLUSION

Inclusion Criteria

Subjects must meet all of the criteria listed below to be eligible for participation in this trial.

1. Signed informed consent obtained before any trial-related procedures at screening.

2. Subject aged ≥18 years old at screening.
3. Medical record documentation of recurrent *Clostridioides difficile* infection (rCDI) per the trial definition that includes ≥1 recurrence after a primary *C. difficile* infection (CDI) episode, at screening.
4. Stool test positive for the presence of toxigenic *C. difficile* or *C. difficile* toxin to confirm the medical record documentation of rCDI.
5. Eligible for fecal microbiota transplantation (FMT) as judged by the investigator or current treatment guidelines for rCDI in the United States (US).
6. Candidate for colonoscopy as judged by the investigator.
7. Currently taking or have just received a prescription for a course of antibiotics to ensure control of CDI-related diarrhea, at screening. The antibiotic course should be for a minimum of 10 consecutive days, and a maximum of 60 days in total (including intermittent dosing/pulse) before the antibiotic washout period.
8. Adherence to at least one of the following conditions throughout the trial period:

   The subject, <u>if female</u>, must:

   a) be post-menopausal (women ≥45 years with no menstrual period for ≥12 months without an alternative medical cause), or
   b) have been surgically sterilized, or
   c) have had a hysterectomy prior to screening, or
   d) use an adequate method of contraception (i.e., implants, injectables, hormonal intrauterine devices, combined hormonal contraceptives, having a vasectomized sexual partner, or total abstinence from heterosexual relations with no plans of becoming pregnant through insemination or in vitro fertilization).

   The subject, <u>if male</u>, must:

   a) use a non-hormonal single-barrier contraception (i.e., condom), and
   b) use an adequate method of contraception if his female partner is of childbearing potential (i.e., not post-menopausal) as defined above.

   This is however not required if the male subject is documented surgically sterile, remains sexually abstinent, when this is in line with his preferred and usual lifestyle, or if he has a female partner who is surgically sterilized, had a hysterectomy, or is post-menopausal.
9. Willing to abstain from consuming probiotics (including over-the-counter and prescription) from screening through 8 weeks after RBX2660 treatment.
10. Willing to comply with trial procedures, including attending scheduled visits, completion of the electronic stool diary and solicited events diary, providing stool samples for future exploratory research, and adherence to treatment plan.

Exclusion Criteria

Subjects meeting any of the criteria listed below will not be eligible for participation in this trial.

1. Use or planned use of systemic antibiotics for an indication other than the qualifying rCDI episode.
2. Current disease symptoms (e.g., diarrhea) caused by a confirmed intestinal pathogen other than *C. difficile*.
3. Current uncontrolled chronic diarrhea not related to CDI.
4. Current refractory CDI, i.e., CDI diarrhea (defined as the passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for ≥2 consecutive days) not improving with antibiotics used to treat CDI.
5. Fecal microbiota transplantation, treatment with other microbiota-based therapies, or treatment with RBX2660 other than the IMP:
   a) within 6 months before screening, or
   b) between screening and baseline.
6. Receipt of CDI vaccine or treatment with CDI monoclonal antibodies:
   a) within 12 months before screening, or
   b) between screening and baseline.
7. Compromised immune system including, but not limited to, inherited/primary and acquired immune disorders as judged by the investigator.
8. Solid-organ transplant recipient.
9. Initiation or escalation of systemic immunosuppressive agents, at the discretion of the investigator, for any condition:
   a) during the 8 weeks before screening, or
   b) between screening and baseline.

   Subjects on a stable dose of systemic immunosuppressants (e.g., ≤20 mg/day prednisone equivalent daily dose) may be considered eligible at the discretion of the investigator.
10. Current or planned therapy that may cause diarrhea.
11. Current or planned systemic cancer treatment (e.g., chemotherapy, radiotherapy, or other).
12. Diagnosis of short bowel syndrome.
13. Current uncontrolled gastrointestinal motility disorders.
14. Evidence of active, severe, or fulminant colitis.
15. Diagnosis of toxic megacolon.

16. Have a current colostomy or ileostomy.
17. Major gastrointestinal tract surgery (this does not include appendectomy or cholecystectomy):
    a) within 60 days before screening, or
    b) between screening and baseline.
18. Planned surgery requiring perioperative antibiotics.
19. Life expectancy of <6 months, as judged by the investigator at screening.
20. Current abuse of alcohol or drugs, as judged by the investigator at screening.
21. Pregnant (as confirmed by a positive pregnancy test), breastfeeding, or intending to become pregnant, during trial participation.
22. Participation in any interventional clinical trial during the past 12 weeks before screening.
23. Known or suspected hypersensitivity to polyethylene glycol 3350.
24. Severe food allergy, characterized as previous episodes of severe allergic reactions in the form of anaphylaxis after intake of food allergens.
25. Inability to participate in the trial for other reasons, as judged by the investigator at screening.

MEDICINAL PRODUCTS

The investigational medicinal product (IMP) is RBX2660. RBX2660 is a 150 mL liquid microbiota suspension in an ethylene vinyl acetate (EVA) bag fitted with a spike port. Each RBX2660 consists of a suspension of 1×10^8 to 5×10^{10} CFU/mL in an excipient solution of polyethylene glycol 3350 and 0.9% Sodium Chloride Irrigation, US Pharmacopoeia (USP). One RBX2660 treatment course consists of the content of one RBX2660 microbiota EVA bag, delivered by colonoscopy.

IMP	Presentation
RBX2660	Microbiota suspension in an EVA bag fitted with a spike port.

EVA: ethylene vinyl acetate; IMP: investigational medicinal product

DURATION OF TREATMENT

One RBX2660 treatment course consists of the content of one RBX2660 microbiota EVA bag, delivered by colonoscopy. Subjects will be followed for 8 weeks after treatment for safety and CDI recurrence, and up to 6 months after treatment for safety.

STATISTICAL METHODS

There are no pre-specified hypotheses, therefore there are no pre-planned significance tests or sample size calculations.

The outcomes of this trial will be reported by descriptive statistics as indicated below.

Primary Endpoint

The number of subjects with RBX2660-related TEAEs after RBX2660 treatment through 8 weeks, or treatment failure, will be reported, based on the safety analysis set.

The safety analysis set comprises all subjects treated with RBX2660.

Secondary Endpoints

- For the secondary endpoints on recurrence of CDI within 8 weeks after RBX2660 treatment, i.e., treatment failure, the observed frequency of treatment success (absence of CDI diarrhea i.e., no recurrence) within 8 weeks after treatment, will be reported.
- For the secondary endpoint on time to CDI recurrence from baseline through 8 weeks after RBX2660 treatment, a Kaplan-Meier plot will be prepared to illustrate treatment success.
- For the secondary endpoints on investigator-experience and perception of patient benefit, responses to the questions in the questionnaires will be reported. Responses will be reported quantitatively, as frequencies of different identified scores, where applicable.
- For the secondary endpoint on patient-experience, scores of responses to questions asked in the patient-experience interview and, where relevant, descriptions of the responses will be reported. Responses will be reported both quantitatively, as frequencies of different identified themes, but also qualitatively, as relevant subject quotes arising from the interviews.

The full analysis set will be used for reporting of these endpoints. The full analysis set comprises all subjects treated with RBX2660 and is identical to the safety analysis set.

Secondary Safety Endpoints

The secondary safety endpoints will be summarized descriptively using the safety analysis set.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

List of Abbreviations

ACG	American College of Gastroenterology
AESI	adverse event of special interest
ATC	Anatomical Therapeutic Chemical
CDI	*Clostridioides difficile* infection
C. difficile	*Clostridioides difficile*
CGI-I	Clinician Global Impression of Improvement
COS	Clinical Outcomes Solution
COVID-19	coronavirus disease 2019
CRO	contract research organization
CRP	C-reactive protein
DMC	Data Monitoring Committee
EDC	electronic data capture system
EVA	ethylene vinyl acetate
FAS	full analysis set
FDA	Food and Drug Administration, USA
FMT	fecal microbiota transplantation
FSFV	first subject first visit
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GVD	Global Value Dossier
HTA	health technology agency
ICF	informed consent form
ICH	International Council for Harmonisation
ICH-GCP	International Council for Harmonisation-Good Clinical Practice
ICMJE	International Committee of Medical Journal Editors
ICU	intensive care unit
IQR	interquartile range
IMP	investigational medicinal product
IND	investigational new drug (application)
IRB	institutional review board
ITT	intention-to-treat
LSLV	last subject last visit

MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intention-to-treat
MRT	microbiome restoration therapy
NLM	National Library of Medicine
NIH	National Institutes of Health
PP	per protocol
PT	preferred term
QoL	quality of life
rCDI	recurrent *Clostridioides difficile* infection
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SMT	Safety Management Team
SoC	standard of care
SOC	system organ class
TEAE	treatment-emergent adverse event
US	United States
USP	US Pharmacopoeia
UTN	Universal Trial Number

Definition of Terms

CDI diarrhea during trial (CDI recurrence)	Passage of ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days and a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin, as determined by a toxin protein detecting assay at the time of the diarrhea.
Company name	Ferring is used as an abbreviation for Ferring Pharmaceuticals.
Control of CDI symptoms	No longer meeting the symptomatic criteria for CDI diarrhea, i.e., passage of ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days.
Medication error	A medication error is an unintended failure in the drug treatment process that leads to, or has the potential to lead to, harm to the patient. A failure in the drug treatment process refers to human or process mediated failures (i.e., failures due to mechanical or technical issues such as temperature excursions due to refrigerator failures are not considered medication errors).
Recurrent CDI for trial entry	Documented diagnosis of 1) CDI diarrhea: the passage of ≥3 unformed/loose stools in 24 consecutive hours for ≥2 consecutive days, that began within 8 weeks after completion of previous CDI treatment and 2) ≥1 stool test positive for toxigenic *C. difficile* or *C. difficile* toxin to confirm the medical record documentation of rCDI.
Treatment failure (CDI recurrence)	Presence of CDI diarrhea (passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for ≥2 consecutive days and a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin, as determined by a toxin protein detecting assay at the time of the diarrhea) within 8 weeks after RBX2660 treatment.
Treatment success	Absence of CDI diarrhea (passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for ≥2 consecutive days and a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin, as determined by a toxin protein detecting assay at the time of the diarrhea) for 8 weeks after RBX2660 treatment.
Usability	The extent to which a physician conducting therapeutic colonoscopy can use commercially available RBX2660 safely, effectively, and efficiently.

1 INTRODUCTION

1.1 Background

Clostridioides difficile (previously *Clostridium difficile* and hereafter denoted *C. difficile*) infection (CDI) is a common cause of healthcare-associated infections and is also commonly acquired in community settings such as nursing homes.[1] CDI contributes to considerable morbidity, mortality, and healthcare expenditure. In recent years, CDI has demonstrated an escalation in overall prevalence and severity of disease.[2,3,4,5] A study of data from 2017 reported that *C. difficile* accounted for nearly half a million infections in the United States (US).[6] A major complication from CDI is recurrent infection, despite absence of additional risk factors.

While mature colonic microbiota in healthy adults are generally resistant to *C. difficile* colonization, the widely accepted model for CDI pathogenesis is that the use of broad-spectrum antimicrobials suppresses and disrupts the microbial communities that normally prevent expansion of *C. difficile*.[7] Because *C. difficile* spores are largely resistant to antibiotics, they can germinate into vegetative forms after antibiotic treatment has been discontinued. If residual normal intestinal microbiota cannot restrain the infection, *C. difficile* bacteria proliferate and produce toxins that cause destruction of colonic epithelial cells, inflammation, and disease symptoms.[4]

Conventional treatments for CDI utilize antibiotics, which further disrupt the gut bacteria flora and limit the recovery of the gut microbiota following CDI. This decreased diversity of the gut microbiota following antibiotic treatment can predispose patients to re-colonization with *C. difficile* and lead to recurrent infections.[2,3,8] Recurrent CDI (rCDI) is defined as recurrence of symptoms within 8 weeks after response to, and cessation of, specific antibiotic treatment, with a positive diagnostic test for *C. difficile* and exclusion of other enteropathogens.[3,9,10] Recurrent CDI (rCDI) presents most commonly as repeated bouts of diarrhea that respond to antibiotic treatment but recur within days to 8 weeks of stopping the antibiotic treatment. Repeated bouts of infection can continue for years, leading to persistent use of antibiotics, markedly reduced quality of life (QoL), repeated hospitalizations, and even death.

It is estimated that the risk of a first recurrence after an initial response to antibiotic therapy for a primary CDI ranges from 20 to 30%. In patients with prior rCDI, the risk of an additional recurrence increases to 40 to 60%.[11] Analyses of recurrence history over time also suggest that the rate of recurrence for any number of recurrences after the first recurrence is similar.[12,13] Effective alternative treatment options to repeated or continuous antibiotics or bezlotoxumab, a human monoclonal antibody indicated for prevention of recurrence of CDI in adults at high risk for recurrence of CDI, are lacking.[5,14,15,16]

Administration of human stool from healthy donors, i.e., microbiome restoration therapy (MRT), is believed to restore the diversity of the gut microbiome and thereby suppress *C. difficile* outgrowth, and is emerging as an important treatment option for rCDI. Multiple studies have used colonoscopy to deliver MRT in the form of fecal microbiota transplantation (FMT) to rCDI patients with promising success rates.[17,18,19,20,21,22] This is also reflected in the American College of Gastroenterology (ACG) guidelines on the prevention, diagnosis, and treatment of CDI, which

recommend that patients with rCDI should be treated with MRT delivered by colonoscopy to prevent further recurrence of the infection.[23]

Microbiome changes in patients with rCDI include reduced diversity, reduced abundance of *Bacteroidetes* and *Firmicutes* phylae, increased abundance of the *Proteobacteria* phylum (especially in the *Enterobacteriaceae* family), and differences in species involved in bile acid metabolism.[24,25,26,27,28] Numerous studies have shown that successful MRT in patients with rCDI is associated with a shift towards an increase in abundance of *Bacteroidetes* and *Firmicutes* phylae and a corresponding reduction in the *Proteobacteria* phylum.[8,29,30,31] Complete replacement by donor microbiota does not seem to be necessary for clinical success. In addition, bile acid restoration has been hypothesized as one possible contributing factor to how microbiome restoration could prevent rCDI.[32,33,34]

RBX2660 is a microbiota suspension prepared from human stool which is collected from pre-screened and qualified donors. In order to ensure consistency and quality, the suspension is tested and processed to a stable, cryopreserved drug product under Good Manufacturing Practice (GMP) conditions. RBX2660 is supplied as a pre-packaged single-dose 150 mL microbiota suspension of 1×10^8 to 5×10^{10} colony-forming units (CFU)/mL of diverse spore-forming and non-spore-forming bacteria, including Bacteroides.

The safety and efficacy of RBX2660 to prevent recurrence of CDI in subjects with rCDI has been demonstrated when delivered as an enema in 5 prospective clinical trials (2 completed randomized placebo-controlled trials [phase 2b Trial 2014-01 and pivotal phase 3 Trial 2017-01], 2 completed open-label trials [phase 2 Trial 2013-001 and 2015-01], and one open-label trial [phase 3 Trial 2019-01]; database lock 25 August 2023, reporting ongoing]) and one retrospective study (Study 2019-02).[35,36,37,38,39,40] In the 4 completed trials, 495 unique subjects received treatment with RBX2660, with some having received up to 4 treatments. In the open-label phase 3 Trial 2019-01 (database lock 25 August 2023, reporting ongoing), an additional 697 subjects received treatment with RBX2660.

The primary efficacy analysis was completed for the 2 randomized placebo-controlled trials; phase 2b Trial 2014-01 and pivotal phase 3 Trial 2017-01. Trial 2014-01 was a regimen-finding trial comparing 2 doses of RBX2660, 1 dose of RBX2660 and placebo, and 2 doses of placebo. All doses were given 1 week apart. The treatment success rates in both the 2 dose RBX2660 group (N=25/45; 55.6%) and in the 1 dose RBX2660 group (N=25/44; 56.8%) were higher than observed in the 2 dose placebo group (19/44; 43.2%). Therefore, a single dose of RBX2660 was chosen for the pivotal 2017-01 trial.

In Trial 2017-01, the primary statistical analysis for treatment success used a Bayesian hierarchical model that formally integrated data from Trial 2014-01. In the modified ITT (mITT) population, the rate of treatment success in the RBX2660 arm (70.4%) was superior to the rate of treatment success in the placebo arm (58.1%) through 8 weeks after completing blinded treatment. The posterior probability of superiority (0.986354) exceeded the threshold of 0.9750338, but did not exceed the

higher threshold of 0.9993275. This analysis demonstrated a 98.6% probability of superiority for RBX2660 compared with placebo, a value that is significant at the 2-sided α=0.05 level.

Efficacy data (treatment success) from the non-randomized, open-label trials were leveraged as supportive evidence of efficacy and ranged from 50.0%-82.3%.

Safety data from the 5 prospective trials demonstrate that RBX2660 was well-tolerated, and that most treatment-emergent adverse events (TEAEs) were mild or moderate in intensity. No potentially life-threatening TEAEs were considered as only related to RBX2660. Instead, most TEAEs were related to pre-existing conditions or CDI during the first 6 months after administration of the first enema.

Taken as a whole, a favorable safety profile for RBX2660 was demonstrated by the analysis of the 5 prospective trials and indicated a high level of efficacy through 8 weeks after completed treatment. On 30 November 2022, RBX2660 was approved as REBYOTA by the US Food and Drug Administration (FDA) for the indication "Prevention of recurrence of *Clostridioides difficile* infection (CDI) in individuals 18 years of age and older, following antibiotic treatment for recurrent CDI (rCDI)" when delivered by rectal administration as an enema.

For further information regarding RBX2660, please refer to the current edition of the Investigator's Brochure.[41]

1.2 Scientific Justification for Conducting the Trial

Microbiome restoration therapy (MRT), in the form of FMT is provided at numerous centers around the world but remains a highly heterogenous treatment without being approved as a medicinal product. There is a wide variability in all stages (donor screening, donor type, stool processing, volume of stool instilled, formulation, dose, and route of administration) of the FMT procedures that are practiced. The aim of the RBX2660 development program has been to formally develop a safe and efficacious stabilized product with a microbial diversity as close to the composition of raw stool as possible, that is produced by a standardized, quality-controlled process, in order to restore a healthy gut microbiome and prevent further recurrences in adults following antibiotic treatment for rCDI.

Multiple studies have used colonoscopy to deliver MRT in the form of FMT to rCDI patients with promising success rates.[17,18,19,20,21,22] This is also reflected in the ACG guidelines on the prevention, diagnosis, and treatment of CDI, which recommend that patients with rCDI should be treated with MRT delivered by colonoscopy to prevent further recurrence of the infection.[23] RBX2660 has been conceived and designed to be administered to the distal colon by enema, with the benefit for patients and physicians that it can be more easily used in a wider clinical setting. This trial will be initiated to explore whether RBX2660 could be suitable for administration by the practice of colonoscopy. More specifically, the purpose of this trial is to explore the safety and clinical effectiveness of RBX2660 when delivered by colonoscopy to adults with rCDI. The experience of physicians will be documented through a physician-experience questionnaire to explore the usability of RBX2660 in clinical practice for colonoscopic administration. Furthermore, to explore

the patient-experience of RBX2660 treatment, each trial subject will be offered to undergo a structured interview.

1.3 Benefit / Risk Aspects

The intended clinical benefit for this trial population treated with RBX2660 delivered by colonoscopy is to prevent CDI recurrence within 8 weeks after treatment, with a treatment that is developed from systematically screened donor stool and that is associated with mild to moderate and transient adverse events.

Safety data from the 5 prospective clinical trials demonstrate that RBX2660 is well-tolerated, when delivered as an enema, and that most TEAEs were mild or moderate in intensity. The majority of the TEAEs were related to pre-existing conditions or CDI during the first 6 months after administration of the first enema. The results support a favorable safety profile for RBX2660 when delivered as an enema and this trial aims to explore whether the same is true when RBX2660 is delivered by colonoscopy.

Based on the safety and efficacy demonstrated in the RBX2660 development program, and the extensive screening and testing system of donors, as well as the known effectiveness of FMT for rCDI, it is considered that the anticipated risks to subjects in the current trial are acceptable in relation to the potential benefits of RBX2660.

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Objectives

Primary Objective

- To explore the safety of RBX2660 when delivered by colonoscopy in subjects with recurrent *Clostridioides difficile* infection (rCDI).

Secondary Objectives

- To explore the effectiveness of RBX2660 when delivered by colonoscopy in preventing recurrence of *Clostridioides difficile* infection (CDI) in subjects with rCDI.
- To explore the usability of RBX2660 in clinical practice when delivered by colonoscopy in subjects with rCDI.
- To explore the patient-experience of RBX2660 when delivered by colonoscopy in subjects with rCDI.

2.2 Endpoints

Primary Endpoint

- RBX2660-related treatment-emergent adverse events (TEAEs) after RBX2660 treatment delivered by colonoscopy through 8 weeks, or treatment failure.

Secondary Endpoints

- Recurrence of *Clostridioides difficile* infection (CDI) within 8 weeks after RBX2660 treatment delivered by colonoscopy.
- Time to CDI recurrence from baseline through 8 weeks after RBX2660 treatment delivered by colonoscopy.
- Physician-experience, as determined by questionnaire, documenting subjective experience of investigators on usability of RBX2660 in clinical practice when delivered by colonoscopy.
- Physician perception of patient benefit, as determined by Clinician Global Impression of Improvement (CGI-I) at 8 weeks, or at treatment failure, after RBX2660 treatment delivered by colonoscopy.
- Patient-experience interview at 8 weeks, or at treatment failure, after RBX2660 treatment delivered by colonoscopy.
- Safety up to 6 months after RBX2660 treatment delivered by colonoscopy:
    - TEAEs, including type, intensity, and causality.
    - Serious adverse events (SAEs).
    - Adverse events of special interest (AESIs): septic shock, toxic megacolon, colonic perforation, and emergency colectomy, and new onsets of obesity, glucose intolerance, metabolic syndrome, or autoimmune conditions.

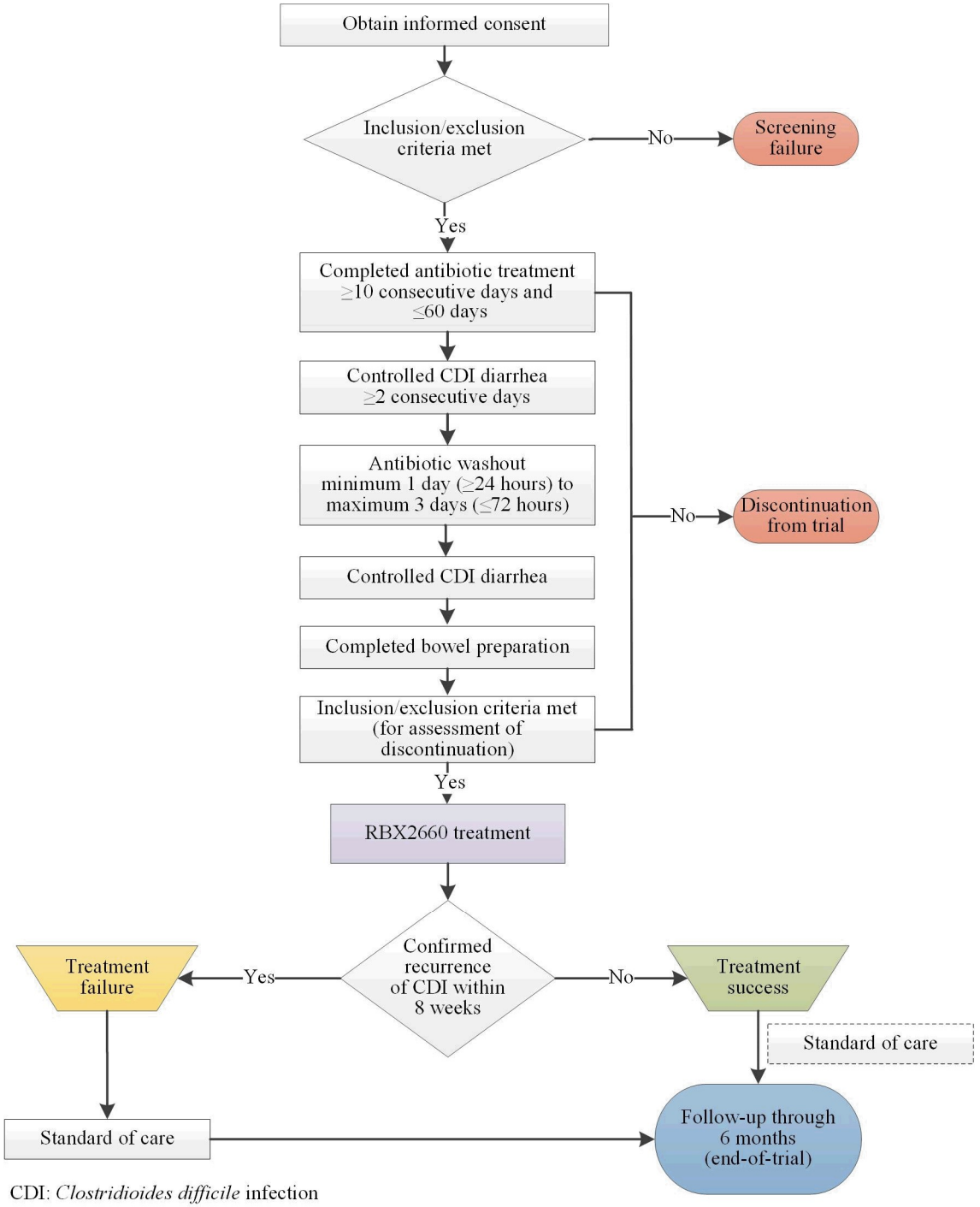
- Adverse events leading to death or intensive care unit (ICU) admission.

3 INVESTIGATIONAL PLAN

3.1 Overall Trial Design

3.1.1 Trial Design Diagram

A trial design diagram is presented in Figure 3-1.



CDI: *Clostridioides difficile* infection

Figure 3-1 Trial Design Diagram

3.1.2 Overall Design and Control Methods

This is a multi-center, single-arm trial to explore the safety and clinical effectiveness of RBX2660 (microbiota suspension) when administered by colonoscopy to adults with rCDI.

Subjects aged 18 years and above, with a diagnosis of rCDI and a stool test positive for the presence of toxigenic *C. difficile* or *C. difficile* toxin to confirm the medical record documentation of rCDI, will be eligible for screening in this trial. After signing the informed consent form (ICF), subjects will undergo a screening evaluation prior to treatment at baseline (day 1). To be screened in this trial, subjects must be taking or have just received a prescription for a course of antibiotics to control rCDI symptoms, type and dosage at the discretion of the prescribing physician. This means that subjects who have already completed their prescribed course of antibiotics to treat rCDI are not eligible for screening unless they have another recurrence that requires treatment with antibiotics. Antibiotic therapy for the qualifying rCDI episode must include a minimum of 10 consecutive days and must not exceed a total of 60 days (including intermittent dosing/pulse) of antibiotic use before a mandatory antibiotic washout period can be initiated. The antibiotic washout period consists of a minimum of 1 day (≥24 hours) to a maximum of 3 days (≤72 hours) immediately before treatment at baseline. Before the antibiotic washout period can start, CDI symptoms must be under control, i.e., no longer meeting the symptomatic criteria for CDI diarrhea for the 2 consecutive days immediately before the antibiotic washout period. The CDI symptoms must furthermore be under control throughout the antibiotic washout period and up to start of a mandatory bowel preparation (by a method at the investigator's discretion), in order for subjects to receive treatment with RBX2660. Consented subjects who do not meet the eligibility criteria will be considered as screening failures.

At the screening visit, subjects will be instructed on how to document stool patterns in an electronic stool diary. Subjects are required to start completing the stool diary immediately after the screening visit, on the same day, and continue until start of bowel preparation. The stool diary will be reviewed on an ongoing basis by trial site staff and at the baseline visit before administration of RBX2660. In order for subjects to receive treatment with RBX2660, the stool diary completed from the screening visit up to start of bowel preparation must demonstrate that CDI symptoms were under control, i.e., no longer meeting the symptomatic criteria for CDI diarrhea, for the 2 consecutive days immediately before initiating the antibiotic washout period, and throughout the antibiotic washout period up to start of bowel preparation. At baseline, immediately after the antibiotic washout period and bowel preparation, subjects will receive treatment with RBX2660, administered by colonoscopy. RBX2660 should be administered to the right side of the colon (i.e., between the ileocecal valve and the hepatic flexure of the colon). At the baseline visit, subjects will be instructed on how to document solicited events in an electronic solicited events diary. Subjects are required to start completing the solicited events diary immediately after receiving treatment with RBX2660 at the baseline visit, on the same day, and continue until the first follow-up visit at week 1 when it will be reviewed by the trial site staff.

Follow-up visits will occur at 1, 2, 4, and 8 weeks, and 3 and 6 months after treatment. The follow-up visits at 1, 2 and 4 weeks, and 3 months after treatment can be either virtual or on-site, as

judged by the investigator. An on-site CDI recurrence visit is required if CDI recurrence (treatment failure) is suspected at any time within 8 weeks after treatment with RBX2660. CDI recurrence will be determined by use of an algorithm relating to diarrhea and *C. difficile* toxin test results from a toxin protein detecting assay. External adjudication will not be used.

Subjects who have completed the follow-up 4 visit at 8 weeks after RBX2660 treatment, or who have been documented as treatment failures (recurrence of CDI) at a CDI recurrence visit, will be contacted by Clinical Outcomes Solution (COS) which is a third-party contract research organization (CRO) for a patient-experience interview by telephone within 14 days of attending the respective visit. Participation in the patient-experience interview is optional and signed informed consent must have been obtained through the ICF for the trial, as appropriate, before participation. The patient-experience interview is designed to examine the impact of living with CDI on a subject's health-related quality of life, and to assess what a meaningful positive change after RBX2660 treatment would be from the subject's perspective. The focus of the interview will be on the general subject impact of rCDI.

To capture the investigators' subjective experience on the usability of RBX2660 in clinical practice, all investigators in this trial will be required to complete a physician-experience questionnaire after each attempted colonoscopy. The questionnaire will capture each physician's opinion on the practical aspects of the usability of RBX2660 when delivered by colonoscopy. In addition, investigators will be asked to rate each subject's total improvement according to the Clinician Global Impression of Improvement (CGI-I) scale at the follow-up 4 visit, or at a CDI recurrence visit for subjects who have been documented as treatment failures (recurrence of CDI).

Subjects who are documented treatment failures, defined as presence of CDI diarrhea, including a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin as determined by a toxin protein detecting assay, within 8 weeks after RBX2660 treatment should be treated according to standard of care (SoC) at the investigator's discretion. These subjects will continue the follow-up assessments for safety through 6 months after RBX2660 treatment according to the trial flow chart (Table 6-1). Subjects who experience CDI after the follow-up 4 visit at week 8 should be diagnosed and treated according to SoC at the investigator's discretion. These subjects will continue the follow-up assessments for safety through 6 months after RBX2660 treatment according to the trial flow chart (Table 6-1).

As part of the trial, subjects will be required to provide stool samples for exploratory research of potential biomarkers pertaining to the mechanism of action of microbiome restoration in patients with rCDI. Subjects are required to collect and ship stool samples in association with each of the scheduled follow-up visits, and any CDI recurrence visit, after RBX2660 treatment. Subjects will collect the stool samples in pre-labelled containers provided by the trial site. The stool samples will be used for future analyses of potential biomarkers and will be analysed and reported separately from the current trial.

Adverse events, safety laboratory variables (clinical chemistry and hematology), vital signs, and concomitant medications will be assessed throughout the trial.

3.1.3 Trial Schedule

First subject first visit (FSFV): April 2023

Last subject last visit (LSLV): May 2024

3.2 Planned Number of Trial Sites and Subjects

It is planned to treat around 40 subjects with RBX2660 at approximately 10-20 sites in the US.

3.3 Interim Analysis

There is no interim analysis planned.

3.4 Database Lock

There will be a database lock when the last subject has completed the 8-week follow-up visit (follow-up 4 visit) for analysis and reporting of data collected up to 8-weeks. This database lock will be referred to as the 8-week database lock. In addition, there will be a database lock at end-of-trial, referred to as final database lock, for final analysis and reporting (Section 13.1).

3.5 Data Monitoring Committee (DMC)

No Data Monitoring Committee (DMC) is planned for this trial. During the trial, the Ferring Safety Management Team (SMT) for RBX2660 will monitor and evaluate safety data on a regular basis.

The Ferring SMT for RBX2660 will review data, including adverse events and laboratory values for subjects participating in this trial at regular intervals in order to identify any safety signal or alert. This includes the ability to recommend stopping the trial based on pre-defined stopping criteria (Section 4.5). In addition, serious adverse events (SAEs) from the trial are captured on an ongoing basis in the Ferring global safety database and are evaluated for causality and expectedness compared to the known safety profile of RBX2660. The Ferring SMT consists of at least two medically qualified members and other relevant competencies, and is responsible for reviewing clinical trial data on a regular basis, i.e., as a minimum every third month when at least one subject has been treated with IMP, in addition to ad hoc meetings in case of safety concerns. The Ferring SMT may recommend changes to the protocol at any time up to LSLV. The Ferring SMT may also recommend to terminate the trial.

Details of the SMT responsibilities will be included in an SMT charter.

3.6 Discussion of Overall Trial Design and Choice of Control Groups

3.6.1 Trial Design

This is a multi-center, single-arm, exploratory trial, which will be conducted in accordance with the protocol, International Council for Harmonisation-Good Clinical Practice (ICH-GCP), and applicable regulatory requirements.

Being exploratory in nature, the sample size is selected to be large enough to provide indicative descriptive data relating to the chosen endpoints. There is no comparator in this trial. The safety and

efficacy of RBX2660 delivered by enema has been demonstrated in previous controlled and open-label trials.

The antibiotic washout period of a minimum of 1 day (≥24 hours) to a maximum of 3 days (≤72 hours) prior to RBX2660 administration after successfully resolved CDI diarrhea has been chosen to minimize any potential deleterious effect of the antibiotics on RBX2660, and to ensure that the qualifying rCDI episode has been cured such that any new CDI episode within 8 weeks after RBX2660 treatment would qualify as a recurrence.

To ensure that RBX2660 reaches the entire colon, RBX2660 will be administered to the right side of the colon, i.e., between the ileocecal valve and the hepatic flexure of the colon. In order to facilitate the administration, bowel preparation prior to the colonoscopy, by a method at the investigator's discretion, will be mandated. Bowel preparation before the colonoscopy will also ensure proper antibiotic washout and furthermore allow the investigator to visualize the colon.

The trial also aims to collect data relating to the usability of RBX2660, from the perspective of the healthcare professional who administers the product by colonoscopy. This will be achieved by use of a structured questionnaire after each administration. The perception of the investigators about the benefits to their patients will also be explored by means of a questionnaire.

In addition, the experience of trial subjects will be elicited by means of an optional interview by telephone approximately 8 weeks following RBX2660 treatment, or at recurrence of CDI.

3.6.2 Selection of Endpoints

Collectively, the endpoints chosen for this trial will provide a description of the safety, clinical effectiveness, usability, perceptions of benefit and patient experience in relation to treatment with RBX2660.

The primary endpoint of this trial relates to the safety of RBX2660 when delivered by colonoscopy. Safety for the purpose of primary endpoint reporting will be assessed during the time window from administration up until 8 weeks, or until treatment failure if occurring within 8 weeks (after which other interventions may be indicated for the subject, which will obscure the safety picture attributable to RBX2660 alone).

Clinical effectiveness will also be assessed by use of secondary endpoints during the first 8 weeks after administration. The time frames and definitions used in this trial are aligned with those used in the phase 2 and phase 3 RBX2660 clinical development program, and have been accepted as adequate and clinically relevant by the FDA. Both the time frame of recurrence within 8 weeks and the definition of rCDI are in line with the US guidelines of the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America.[3,9] In addition, there are data in the literature to support that the majority of CDI recurrences occur within 30 days after completion of treatment.[42,43] This is also the basis for the acceptance from the Scientific Societies of the 8-week timeframe as clinically relevant.

An important aspect of this trial relates to the usability of RBX2660 for administration via a colonoscope. The physician-experience questionnaire will deliver secondary endpoint data on the experiences of the healthcare professionals administering RBX2660 in relation to a variety of aspects of usability. In addition, the perception of treating physicians on the benefits to their patients will be collected by means of CGI-I after 8 weeks from RBX2660 treatment.

Subject experience will be determined by an optional patient-experience interview by telephone at approximately 8 weeks, or at recurrence, after RBX2660 treatment as a secondary endpoint.

3.6.3 Selection of Doses in the Trial

RBX2660 is available as a single-dose preparation (150 mL microbiota suspension) that has been shown to be safe and efficacious when administered by enema. A course of treatment is equal to one dose. This is the dose that will be used in this trial, albeit delivered to other locations in the colon than would be reached by rectal administration as an enema. This trial will provide empirical data to determine if this dose is safe and clinically effective when delivered by colonoscopy.

3.6.4 Selection and Timing of Dose for Each Subject

Subjects who are eligible for treatment must have completed a course of antibiotics sufficient to control their CDI symptoms and an antibiotic washout period prior to administration of RBX2660. The course of antibiotics is to treat the qualifying episode of rCDI and the washout period is to ensure that the antibiotics do not impair the effectiveness of RBX2660 in preventing further recurrences of CDI.

3.6.5 Selection of the Trial Population

The key inclusion criteria for this trial are selected to ensure that the trial population mimics the patient population who would otherwise be expected to undergo treatment with FMT delivered by colonoscopy.

Thus, to be eligible for this trial, adult patients must:

- have documented evidence of rCDI (≥1 recurrence after a primary CDI episode),
- be undergoing antibiotic treatment for the qualifying rCDI episode,
- be eligible for FMT as judged by the investigator or current treatment guidelines for rCDI in the US, and
- be a candidate for colonoscopy as judged by the investigator.

3.6.6 Follow-up Procedures

No routine follow-up procedures are planned after end-of-trial. Standard safety follow-up measures after end-of-trial may occur in some individual subjects with adverse events as applicable. For details, please refer to Section 8.6.

No expanded access- or enforcement discretion programs with RBX2660 are planned during the trial or after the follow-up 6 visit (end-of-trial) in subjects who have completed the trial.

4 SELECTION OF TRIAL POPULATION

4.1 Trial Population

4.1.1 Inclusion Criteria

Subjects must meet all of the criteria listed below to be eligible for participation in this trial.

1. Signed informed consent obtained before any trial-related procedures at screening.
2. Subject aged ≥18 years old at screening.
3. Medical record documentation of recurrent *Clostridioides difficile* infection (rCDI) per the trial definition that includes ≥1 recurrence after a primary *C. difficile* infection (CDI) episode, at screening.
4. Stool test positive for the presence of toxigenic *C. difficile* or *C. difficile* toxin to confirm the medical record documentation of rCDI.
5. Eligible for fecal microbiota transplantation (FMT) as judged by the investigator or current treatment guidelines for rCDI in the United States (US).
6. Candidate for colonoscopy as judged by the investigator.
7. Currently taking or have just received a prescription for a course of antibiotics to ensure control of CDI-related diarrhea, at screening. The antibiotic course should be for a minimum of 10 consecutive days, and a maximum of 60 days in total (including intermittent dosing/pulse) before the antibiotic washout period.
8. Adherence to at least one of the following conditions throughout the trial period:

   The subject, <u>if female</u>, must:

   a) be post-menopausal (women ≥45 years with no menstrual period for ≥12 months without an alternative medical cause), or

   b) have been surgically sterilized, or

   c) have had a hysterectomy prior to screening, or

   d) use an adequate method of contraception (i.e., implants, injectables, hormonal intrauterine devices, combined hormonal contraceptives, having a vasectomized sexual partner, or total abstinence from heterosexual relations with no plans of becoming pregnant through insemination or in vitro fertilization).

   The subject, <u>if male</u>, must:

   a) use a non-hormonal single-barrier contraception (i.e., condom), and

   b) use an adequate method of contraception if his female partner is of childbearing potential (i.e., not post-menopausal) as defined above.

   This is however not required if the male subject is documented surgically sterile, remains sexually abstinent, when this is in line with his preferred and usual lifestyle, or if he has a female partner who is surgically sterilized, had a hysterectomy, or is post-menopausal.

9. Willing to abstain from consuming probiotics (including over-the-counter and prescription) from screening through 8 weeks after RBX2660 treatment.
10. Willing to comply with trial procedures, including attending scheduled visits, completion of the electronic stool diary and solicited events diary, providing stool samples for future exploratory research, and adherence to treatment plan.

4.1.2 Exclusion Criteria

Subjects meeting any of the criteria listed below will not be eligible for participation in this trial.

1. Use or planned use of systemic antibiotics for an indication other than the qualifying rCDI episode.
2. Current disease symptoms (e.g., diarrhea) caused by a confirmed intestinal pathogen other than *C. difficile*.
3. Current uncontrolled chronic diarrhea not related to CDI.
4. Current refractory CDI, i.e., CDI diarrhea (defined as the passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for ≥2 consecutive days) not improving with antibiotics used to treat CDI.
5. Fecal microbiota transplantation, treatment with other microbiota-based therapies, or treatment with RBX2660 other than the IMP:
   a) within 6 months before screening, or
   b) between screening and baseline.
6. Receipt of CDI vaccine or treatment with CDI monoclonal antibodies:
   a) within 12 months before screening, or
   b) between screening and baseline.
7. Compromised immune system including, but not limited to, inherited/primary and acquired immune disorders as judged by the investigator.
8. Solid-organ transplant recipient.
9. Initiation or escalation of systemic immunosuppressive agents, at the discretion of the investigator, for any condition:
   a) during the 8 weeks before screening, or
   b) between screening and baseline.

   Subjects on a stable dose of systemic immunosuppressants (e.g., ≤20 mg/day prednisone equivalent daily dose) may be considered eligible at the discretion of the investigator.
10. Current or planned therapy that may cause diarrhea.
11. Current or planned systemic cancer treatment (e.g., chemotherapy, radiotherapy, or other).
12. Diagnosis of short bowel syndrome.

13. Current uncontrolled gastrointestinal motility disorders.
14. Evidence of active, severe, or fulminant colitis.
15. Diagnosis of toxic megacolon.
16. Have a current colostomy or ileostomy.
17. Major gastrointestinal tract surgery (this does not include appendectomy or cholecystectomy):
    a) within 60 days before screening, or
    b) between screening and baseline.
18. Planned surgery requiring perioperative antibiotics.
19. Life expectancy of <6 months, as judged by the investigator at screening.
20. Current abuse of alcohol or drugs, as judged by the investigator at screening.
21. Pregnant (as confirmed by a positive pregnancy test), breastfeeding, or intending to become pregnant, during trial participation.
22. Participation in any interventional clinical trial during the past 12 weeks before screening.
23. Known or suspected hypersensitivity to polyethylene glycol 3350.
24. Severe food allergy, characterized as previous episodes of severe allergic reactions in the form of anaphylaxis after intake of food allergens.
25. Inability to participate in the trial for other reasons, as judged by the investigator at screening.

4.2 Method of Assigning Subjects to Treatment Groups

4.2.1 Recruitment

The participating subjects will be recruited among the patients attending the trial sites when they are experiencing at least their first recurrence after a primary CDI episode with at least 1 completed round of antibiotic therapy. Advertisements targeting patients not necessarily being treated at a trial site may be used if approved by the institutional review board (IRB), as applicable to local regulations.

Potential subjects are to be fully informed as to this trial's purpose, requirements, and anticipated risks, and are to be given the chance to review the ICF and receive satisfactory answers to all questions. Subjects who have given written informed consent will receive a unique screening number and perform the screening visit.

All screening assessments must be completed and evaluated to confirm that applicable eligibility criteria are met by the subject. A site-specific screening log, for recording details of all subjects screened and for confirmation of eligibility or recording of reasons for screening failure, as applicable, must be maintained at the trial site. The screening number, which will be allocated sequentially in the order in which the subjects are screened, must be entered into the subject's medical record.

Screened subjects who are considered as screening failures or who discontinue from the trial before receiving RBX2660 treatment may be re-screened once. To be eligible for re-screening, the CDI symptoms for the qualifying rCDI episode from which the subject screen failed or discontinued must be under control, i.e., no longer meeting the symptomatic criteria for CDI diarrhea for 2 consecutive days, and a new qualifying rCDI episode must have begun within 8 weeks. Subjects who are re-screened will receive a new screening number.

4.3 Restrictions

4.3.1 Prior and Concomitant Therapies

Prior medications are defined as any medication (prescription or non-prescription), nutritional supplement, probiotics, or herbal preparation taken or used within 30 days before screening.

Concomitant medications are defined as any medication (prescription or non-prescription), nutritional supplement, or herbal preparation taken or used from screening through the follow-up 6 (end-of-trial) visit.

Medication information will be collected at the screening visit and changes in medications, including changes in dose, will be recorded in the electronic data capture system (EDC), along with the main reason for their prescription, throughout the trial.

4.3.2 Prohibited Therapy

Prohibited medications are medications that are not allowed to be used during the trial. Any use of prohibited medications must be medically indicated and supported with documentation e.g., SoC after CDI recurrence (treatment failure). Any use of prohibited medications during the trial will be considered as protocol deviations.

Prohibited medications up to 8 weeks after RBX2660 treatment include the following:

- Systemic antibiotics other than for control of the qualifying rCDI episode.
- Fecal microbiota transplant preparations.
- Other microbiota based therapies.
- CDI vaccine.
- CDI monoclonal antibodies.
- Probiotics, including over-the-counter and prescription.
- Changing the dose of antimetabolites (e.g., azathioprine, 6-mercaptopurine, or low-dose methotrexate for autoimmune disease), calcineurin inhibitors (e.g., tacrolimus or cyclosporine), or mycophenolate mofetil, except for drug therapeutic monitoring adjustments for calcineurin inhibitors.
- Systemic steroids >20 mg/day (prednisone-equivalent daily dose).
- Cancer treatment (e.g., chemotherapy, radiotherapy, or other).

- Therapies that may cause diarrhea, with the exception of bowel preparation immediately before colonoscopy.

In addition, the following is considered as prohibited therapy throughout the trial:

- Abuse of alcohol or drugs that would prevent compliance with the trial protocol.

4.4 Discontinuation Criteria

4.4.1 Discontinuation from Treatment

At the baseline visit, a subject will not be entitled to receive RBX2660 treatment in case of:

- failure to complete antibiotic treatment for a minimum of 10 consecutive days, and a maximum of 60 days (including intermittent dosing/pulse) before the antibiotic washout period, or
- failure to comply to the antibiotic washout period of a minimum of 1 day (≥24 hours) to a maximum of 3 days (≤72 hours) immediately before the baseline visit, or
- failure to show controlled CDI diarrhea with documentation in the stool diary, defined as <3 unformed/loose stools/day (i.e., Bristol Stool Scale type 6-7) for the 2 consecutive days immediately before start of the antibiotic washout period and throughout the antibiotic washout period up to start of bowel preparation, or
- failure to complete bowel preparation during the antibiotic washout period.

At the baseline visit, the inclusion and exclusion criteria will, if not met, also qualify as discontinuation criteria. Subjects not meeting the relevant inclusion and exclusion criteria at the baseline visit will not receive RBX2660 treatment.

At the investigator's discretion, the investigator has the right to discontinue subjects from receiving RBX2660 treatment in the event of incidental additional diagnoses or due to safety concerns, indicating that administering treatment endangers the safety of the subject.

Subjects who do not receive RBX2660 treatment will be discontinued from the trial without further follow-up (Section 4.4.2).

4.4.2 Discontinuation from Trial

The subjects have the right to withdraw from the trial at any time for any reason, without the need to justify their decision. However, the investigator should record the reason for the subject's withdrawal, if possible. The investigator also has the right to discontinue subjects from the trial due to safety concerns prior to administration of RBX2660, or due to significant non-compliance with trial procedures.

Every effort should be made to invite subjects withdrawn/discontinued from the trial to an end-of-trial visit as soon as possible after a decision of withdrawal/discontinuation has been taken. For any discontinuation, the investigator will obtain all the required details and document the date of the premature termination and the main reason in the EDC.

In case the subject has withdrawn consent, no new data can be recorded. Correction of previous data and/or recording data related to visits/procedures done before but made available after withdrawal of consent (e.g., laboratory results) will be allowed unless the subject disapproves it. The subject can request destruction of samples which would otherwise have been kept in storage.

If the reason for withdrawal is an adverse event, or an adverse event based on the result of a clinically significant laboratory abnormality, this must be recorded. The investigator should make efforts to document the outcome.

For a subject who is suspected of being lost to follow-up, a minimum of 3 attempts must be made to contact the subject using 2 different contact methods (e.g., telephone, email, text, or letter). The contact attempts and methods must be recorded. The date to use for the subject as lost to follow-up is the date of last actual encounter with the subject, such as the last telephone call contact or visit.

Subjects who do not receive RBX2660 treatment will be discontinued from the trial without further follow-up.

4.5 Trial Stopping Criteria

Occurrence of the following may warrant consideration of pausing screening, stopping the trial, or other action taken:

- There is probable cause that the IMP contributed to a pathogenic intestinal infection in the stool of any (≥1) subject (e.g., due to transfer from a donor).
- One or more (≥1) SAEs assessed as unexpected and possibly, probably, or definitely related to the IMP.
- One or more (≥1) occurrences of gram negative or anaerobic blood stream infection within 14 days after IMP treatment and without any alternate etiology.
- Two or more (≥2) colonic perforations.

The SMT (Section 3.5) will review each occurrence and provide a recommendation as whether to pause screening, stop the trial, or take other action. The responsibilities and composition of the Ferring SMT are provided in a separate SMT charter, which will be available before FSFV.

Any review of the trial stopping criteria will be initiated within 72 hours after receiving information about any event(s) potentially fulfilling the stopping criteria.

5 TREATMENTS

5.1 Treatments Administered

5.1.1 Investigational Medicinal Product

All eligible subjects will receive one RBX2660 treatment course. One RBX2660 treatment course consists of the content of one RBX2660 microbiota ethylene vinyl acetate (EVA) bag, delivered by colonoscopy.

5.2 Characteristics and Source of Supply

5.2.1 Investigational Medicinal Product

RBX2660 is provided by Ferring and will be handled according to the principles of GMP.

RBX2660 is a 150 mL liquid microbiota suspension in an EVA bag fitted with a spike port. Each RBX2660 consists of a suspension of 1×10^8 to 5×10^{10} CFU/mL in an excipient solution of polyethylene glycol 3350 and 0.9% Sodium Chloride Irrigation, US Pharmacopoeia (USP).

An overview of the presentation of the IMP is provided in Table 5-1.

Table 5-1 Characteristics of Investigational Medicinal Product

IMP	Presentation
RBX2660	Microbiota suspension in an EVA bag fitted with a spike port.

EVA: ethylene vinyl acetate; IMP: investigational medicinal product

5.3 Packaging and Labelling

Packaging and labelling of the IMP will be performed under the responsibility of the clinical trial supply department at Ferring in accordance with GMP and national regulatory requirements.

All medicinal products will be labelled with trial-specific labels containing a lot number to ensure traceability.

5.4 Conditions for Storage and Use

The investigator will ensure that the IMP is stored under appropriate conditions as indicated on the product label in a secure location with controlled access. The storage compartment shall be monitored regularly and the temperature shall be documented.

The storage conditions for the IMP will be as described on the trial-specific or commercial box labels. Deviations in temperature must be reported to Ferring as instructed in the trial supply manual.

The IMP should be ordered at least 4 days before treatment. The IMP will be shipped to the trial site overnight. Prior to IMP administration, the IMP should be thawed for approximately 24 hours in the refrigerator, until completely thawed. The IMP must be used within the expiration date stated on the product label.

5.5 Blinding / Unblinding

Not applicable. This is a single-arm trial.

5.6 Treatment Compliance

5.6.1 Dispensing and Accountability

The IMP will only be dispensed to investigators for administration to subjects who meet the eligibility criteria and are allocated to treatment in the trial. The investigator (or their designated personnel, e.g., a trial nurse) will maintain a dispensing log detailing the dates and quantities of IMPs dispensed and used for each subject, as well as the batch numbers or other identifiers used in the trial. The monitor will verify the drug accountability during the trial.

5.6.2 Assessment of Compliance

The IMP will be administered by colonoscopy at the trial site. Compliance will be recorded by the investigator.

5.6.3 IMP Medication errors

IMP medication errors with and without clinical consequences will be tracked in the EDC and reviewed on an ongoing basis by Ferring Global Safety.

5.7 Auxiliary Supplies

Ferring will not provide any auxiliary supplies for this trial.

5.8 Return and Destruction of Medicinal Products

All used and unused (non-dispensed) IMP will be disposed as instructed by the Ferring clinical trial supply department, after drug accountability has been finalized.

6 TRIAL PROCEDURES

6.1 Trial Flow Chart

The overall trial design is shown in Section 3.1. The assessments scheduled for each visit are presented in the flow chart in Table 6-1, listed in Sections 6.1.1 to 6.1.10, and further described in Section 7.

Table 6-1 Trial Flow Chart

Visit	Screening	Baseline RBX2660	Follow-up 1 Virtual[a]	Follow-up 2 Virtual[a]	Follow-up 3 Virtual[a]	Follow-up 4	Follow-up 5 Virtual[a]	Follow-up 6 End-of-trial	CDI Recurrence Visit[b]
Day/Week/Month	**Up to Day −33**	**Day 1**	**Week 1[c]**	**Week 2[c]**	**Week 4[c]**	**Week 8[c]**	**Month 3[c]**	**Month 6[c]**	**≤8 weeks of treatment**
Assessment Window	**N/A**	**N/A**	**±3 days**	**±3 days**	**±3 days**	**±3 days**	**±14 days**	**±14 days**	**N/A**
Written informed consent	X								
Inclusion/exclusion criteria	X	X[d]							
Discontinuation criteria		X[d]							
Demographics	X								
Medical history, including CDI history	X								
Vital signs	X	X				X		X	X
Physical examination[e]	X					X		X	X
Body weight and height	X	X[f]				X[f]		X[f]	X[f]
Stool sample for *C. difficile* toxin testing						X[g]			X[h]
Stool sample kit for potential biomarkers hand out and training	X								
Safety laboratory assessment (clinical chemistry, including CRP and hematology)	X	X				X		X	X
Urine or serum pregnancy test[i]	X	X[j]				X		X	
Patient-experience interview[k]						X			X[l]
Physician-experience questionnaire		X							
CGI-I questionnaire						X			X[l]
Stool diary training/review	X[m]	X							
Stool diary completion by subject	X[n]								
Solicited events diary training/review		X[o]	X						
Solicited events diary completed by the subject		X[p]							

Antibiotic treatment ongoing or prescribed	X[q]								
Antibiotic washout confirmed		X[r]							
Colonoscopy preparation instructions, including bowel preparation	X[s]								
Bowel preparation confirmed		X							
IMP order	X[t]								
IMP administration		X[u]							
CDI symptoms assessed		X	X	X	X	X			X
Prior/concomitant medications[v]	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X
IMP accountability		X							

CDI: *Clostridioides difficile* infection; CGI-I: Clinician Global Impression of Improvement; CRP: C-reactive protein; IMP: investigational medicinal product; N/A: not applicable

a. Virtual or on-site visit, as judged by the investigator.
b. An on-site CDI recurrence visit is required if CDI recurrence (treatment failure) is suspected at any time within 8 weeks after treatment due to ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days.
c. The referenced day/week is calculated from the date of the baseline visit, e.g. week 1 is 7 days after the baseline visit. All visits should be planned to maintain the visit schedule relative to the baseline visit.
d. At the baseline visit, the inclusion and exclusion criteria will, if not met, also qualify as discontinuation criteria. Subjects not meeting the relevant inclusion and exclusion criteria at the baseline visit will be discontinued from the trial.
e. Modified physical examination (no genitourinary examination unless medically indicated).
f. Weight only.
g. In case CDI recurrence is suspected at the follow-up 4 visit, a stool sample for laboratory analysis of *C. difficile* toxin will be collected. A toxin protein detecting assay must be used to detect *C. difficile* toxin in order to confirm CDI recurrence.
h. A toxin protein detecting assay must be used to detect *C. difficile* toxin in order to confirm CDI recurrence. The stool sample must be provided before starting any antibiotic treatment, and can be collected before the visit.
i. Female subjects of child-bearing potential only.
j. Negative result must be obtained before RBX2660 administration.
k. After RBX2660 treatment, the interview will be conducted by telephone within 14 days of attending the respective visit, for subjects who have provided specific consent.
l. Performed in case of CDI recurrence, i.e., within 8 weeks after RBX2660 treatment.
m. Instructions for access to and completion of the stool diary. The subject diary data will be collected electronically, and reviewed before treatment at baseline.
n. Subjects are required to start completing the stool diary immediately after the screening visit, on the same day, and until start of bowel preparation immediately before the baseline visit. This may require completion of the stool diary for up to 33 days.
o. Instructions for access to and completion of the solicited events diary. The subject diary data will be collected electronically, and reviewed at the follow-up 1 visit at week 1.
p. Subjects are required to start completing the solicited events diary immediately after receiving RBX2660 treatment at the baseline visit, on the same day, and until the follow-up 1 visit at week 1.
q. Confirmation that antibiotics for control of CDI symptoms are either ongoing or just prescribed.
r. An antibiotic washout period of a minimum of 1 day (≥24 hours) to a maximum of 3 days (≤72 hours) is required before treatment with RBX2660. Antibiotics must be administered for a minimum of 10 consecutive days and a maximum of 60 days in total (including intermittent dosing/pulse), and CDI symptoms must be under control (i.e., no longer meeting the symptomatic criteria for CDI diarrhea, which is the passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for 2 consecutive days) before the antibiotic washout period can start.

s. Provide subjects with colonoscopy preparation instructions, including instructions for bowel preparation, at the investigator's discretion.
t. The IMP should be ordered at least 4 days before treatment at the baseline visit.
u. RBX2660 should be administered by colonoscopy to the right side of the colon (i.e., between the ileocecal valve and the hepatic flexure of the colon).
v. Medications used within 30 days before screening until the follow-up 6 visit/end-of-trial.

6.1.1 Screening (Up to Day −33)

Potential subjects will be scheduled to come to the site for the screening assessments. Screening must occur within 33 days before RBX2660 treatment at baseline (day 1). The investigator is obliged to keep logs of all screened subjects. Each subject will receive a unique screening number. Before or at the screening visit, all subjects must receive a detailed explanation (verbal and written information) of the trial and must sign the ICF after having had sufficient time to consider the participation in the trial. No trial-related procedures may be performed before the subject signs the ICF.

The following must take place/be collected during the screening visit:

- Signed and dated written ICF. Subjects will receive a copy of the signed and dated ICF before any trial-related procedures.
- Allocation of a screening number.
- Check of inclusion and exclusion criteria, including a stool test positive for the presence of toxigenic *C. difficile* or *C. difficile* toxin to confirm the medical record documentation of rCDI and confirmation that antibiotic treatment for control of CDI symptoms is either ongoing or just prescribed.
- Collection of demographics (gender, age, ethnicity, race).
- Collection of relevant medical history, including CDI history.
- Assessment of vital signs (temperature, blood pressure, pulse rate, respiratory rate).
- Modified physical examination (no genitourinary examination unless medically indicated).
- Measurement of body weight and height.
- Blood collection for safety laboratory assessment: clinical chemistry, including C-reactive protein (CRP), and hematology variables.
- Urine or serum pregnancy test for female subjects of child-bearing potential.
- Instructions for access to and completion of the stool diary.
- Recording of use of any prior (within 30 days prior to screening) and concomitant medications.
- Provide subjects with the stool sampling kit and instructions for collection and shipping of stool samples for exploratory research of potential biomarkers.
- Provide subjects with colonoscopy preparation instructions, including instructions for bowel preparation, at the investigator's discretion.

Subjects considered eligible for the trial based on the inclusion and exclusion criteria assessed at this time point may proceed to the baseline visit, scheduled within 33 days from the screening visit. For eligible subjects, the following should take place at least 4 days before the baseline visit:

- IMP ordering. The IMP should be ordered at least 4 days before treatment at the baseline visit.

6.1.2 Baseline

The baseline visit is performed on the day of administration of RBX2660 treatment (day 1). The baseline visit must occur within 33 days of the screening visit.

The following must take place/be collected during the baseline visit prior to treatment:

- Check of inclusion and exclusion criteria. Subjects who do not meet the inclusion and exclusion criteria at the baseline visit will be discontinued from the trial as described in Section 4.4.
- Check of discontinuation criteria (Section 4.4.2):
  - Completed antibiotic treatment for a minimum of 10 consecutive days, and a maximum of 60 days (including intermittent dosing/pulse) before the antibiotic washout period.
  - Compliance to the antibiotic washout period (discontinuation of the antibiotics for a minimum of 1 day [≥24 hours] to a maximum of 3 days [≤72 hours]) immediately before the baseline visit.
  - Controlled CDI diarrhea, defined as <3 unformed/loose stools/day (i.e., Bristol Stool Scale type 6-7) for the 2 consecutive days immediately before start of the antibiotic washout period, throughout the antibiotic washout period, and up to start of bowel preparation, as documented in the stool diary.
  - Completed bowel preparation.
- Assessment of vital signs (temperature, blood pressure, pulse rate, respiratory rate).
- Measurement of body weight.
- Confirmation that a stool sample for exploratory research of potential biomarkers has been collected and shipped during the antibiotic washout period before the baseline visit.
- Blood collection for safety laboratory assessment: clinical chemistry, including CRP, and hematology variables.
- Urine or serum pregnancy test for female subjects of child-bearing potential. Negative result must be obtained before RBX2660 administration.
- Assessment of CDI symptoms.
- Instructions for access to and completion of the solicited events diary.
- Recording of changes to concomitant medications.
- Recording of adverse events.

Once the above items have been performed, the following must take place/be recorded:

- RBX2660 administration by colonoscopy. RBX2660 should be administered to the right side of the colon (i.e., between the ileocecal valve and the hepatic flexure of the colon).
- IMP accountability.
- Completion of the physician-experience questionnaire (by the investigator).

The subject is requested to call the trial site if symptoms recur any time during the trial. In case CDI recurrence is suspected due to ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days, subjects are required to attend a CDI recurrence visit (Section 6.1.9).

6.1.3 Follow-up 1

A follow-up visit (follow-up 1) is performed 7 days ±3 days from RBX2660 treatment, either virtually or on-site as judged by the investigator.

The following must take place/be recorded during the follow-up 1 visit:

- Assessment of CDI symptoms.
- Review of the solicited events diary.
- Recording of changes to concomitant medications.
- Recording of adverse events

6.1.4 Follow-up 2

A follow-up visit (follow-up 2) is performed 2 weeks ±3 days from RBX2660 treatment, either virtually or on-site as judged by the investigator.

The following must take place/be recorded during the follow-up 2 visit:

- Assessment of CDI symptoms.
- Recording of changes to concomitant medications.
- Recording of adverse events.

6.1.5 Follow-up 3

A follow-up visit (follow-up 3) is performed 4 weeks ±3 days from RBX2660 treatment, either virtually or on-site as judged by the investigator.

The following must take place/be recorded during the follow-up 3 visit:

- Assessment of CDI symptoms.
- Recording of changes to concomitant medications.
- Recording of adverse events.

6.1.6 Follow-up 4

A follow-up visit (follow-up 4) is performed on-site 8 weeks ±3 days from RBX2660 treatment. The follow-up 4 visit is the timepoint for the primary endpoint. As such, every attempt should be made to ensure that subjects understand the importance of this follow-up visit and are scheduled within the appropriate visit window.

The following must take place/be recorded during the follow-up 4 visit:

- Assessment of vital signs (temperature, blood pressure, pulse rate, respiratory rate).
- Modified physical examination (no genitourinary examination unless medically indicated).
- Measurement of body weight.
- Blood collection for safety laboratory assessment: clinical chemistry, including CRP, and hematology variables.
- Urine or serum pregnancy test for female subjects of child-bearing potential.
- Assessment of CDI symptoms.
- Recording of changes to concomitant medications.
- Recording of adverse events.
- Completion of the CGI-I questionnaire (by the investigator).

In addition to the above, subjects who have provided specific consent will be contacted by a CRO for a patient-experience interview by telephone within 14 days of attending the follow-up 4 visit after RBX2660 treatment (Section 7.1.4).

In case CDI recurrence is suspected at the follow-up 4 visit, a stool sample for laboratory analysis of *C. difficile* toxin will be collected as described in Section 7.1.1.2. Subjects will be instructed in appropriate handling and storage of the stool sample. The results should be recorded under the follow-up 4 visit in the EDC.

6.1.7 Follow-up 5

A follow-up visit (follow-up 5) is performed 3 months ±14 days from RBX2660 treatment, either virtually or on-site as judged by the investigator.

The following must take place/be recorded during the follow-up 5 visit:

- Recording of changes to concomitant medications.
- Recording of adverse events.

6.1.8 Follow-up 6 / End-of-trial

A follow-up visit (follow-up 6) is performed on-site 6 months ±14 days from RBX2660 treatment. The follow-up 6 visit is the end-of-trial for subjects who complete the trial. For subjects who discontinue early from the trial (Section 4.4.2), the end-of-trial assessments should be performed at the earliest convenience following discontinuation, if possible.

The following must take place/be recorded during the follow-up 6 visit:

- Assessment of vital signs (temperature, blood pressure, pulse rate, respiratory rate).
- Modified physical examination (no genitourinary examination unless medically indicated).
- Measurement of body weight.

- Blood collection for safety laboratory assessment: clinical chemistry, including CRP, and hematology variables.
- Urine or serum pregnancy test for female subjects of child-bearing potential.
- Recording of changes to concomitant medications.
- Recording of adverse events.

At the end-of-trial for each subject, exit date and reason for exit will be collected.

6.1.9 CDI Recurrence Visit

An on-site CDI recurrence visit is required if CDI recurrence (treatment failure) is suspected at any time within 8 weeks after treatment with RBX2660 due to ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days. If the suspected CDI recurrence was detected at a scheduled on-site follow-up visit, the site should enter data under the CDI recurrence visit in the EDC.

The following must take place/be recorded during the CDI recurrence visit:

- Assessment of CDI symptoms and recording of start date and time of CDI symptoms including number of unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days.
- Stool sample collection for laboratory analysis of *C. difficile* toxin as described in Section 7.1.1.2. The stool sample must be provided before starting any antibiotic treatment, and can be collected before the visit. Subjects will be instructed in appropriate handling and storage of the stool samples.
- Assessment of vital signs (temperature, blood pressure, pulse rate, respiratory rate).
- Modified physical examination (no genitourinary examination unless medically indicated).
- Measurement of body weight.
- Blood collection for safety laboratory assessment: clinical chemistry, including CRP, and hematology variables.
- Recording of changes to concomitant medications.
- Recording of adverse events.
- Completion of the CGI-I questionnaire (by the investigator) for subjects who have been documented as treatment failures.

In addition to the above, subjects who have been determined as treatment failures (recurrence of CDI) and who have provided specific consent will be contacted by a third-party CRO for a patient-experience interview by telephone within 14 days of attending the CDI recurrence visit (Section 7.1.4).

6.1.10 Unscheduled Visits

Subjects may be called in for additional unscheduled visits due to safety reasons at the discretion of the investigator or the sponsor, unless a subject has withdrawn their consent. Subjects may also contact the trial site due to safety reasons for an unscheduled visit. An unscheduled visit may include additional collection of blood samples for safety reasons. An unscheduled visit may also include additional assessments deemed necessary by the investigator such as laboratory samples or other procedures which were missed at a previous visit. All unscheduled visits should be described (including the reason for the visit) and documented in the medical/source record.

7 TRIAL ASSESSMENTS

7.1 Assessments Related to Endpoints

7.1.1 Recurrence of CDI

7.1.1.1 CDI Symptoms

CDI symptoms, such as stool frequency, changes in bowel movements, and other symptoms indicative of CDI recurrence, will be assessed from receiving RBX2660 treatment at baseline until the follow-up 4 visit at week 8.

7.1.1.2 Stool Sample for *C.difficile* Toxin Testing

If CDI recurrence is suspected within 8 weeks after treatment with RBX2660, due to ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days, the subject must provide a fresh stool sample for analysis of *C. difficile* toxin. The stool sample must be provided before starting any antibiotic treatment and can be collected before attending the CDI recurrence visit. Subjects will be instructed in appropriate handling and storage of the stool samples.

In case CDI recurrence is suspected at the follow-up 4 visit at week 8, a stool sample for laboratory analysis of *C. difficile* toxin will be collected.

For the determination of CDI recurrence, the presence of *C. difficile* toxin must be determined by using a toxin protein detecting assay. The test will be conducted at the local laboratory at the trial site or another laboratory available to the trial site.

7.1.1.3 Determination of Treatment Outcome

Treatment outcome will be determined according to the definitions below and Table 7-1. Management of possible CDI recurrence (treatment failure) is described in Section 7.1.1.4.

- Treatment failure (CDI recurrence): Presence of CDI diarrhea (passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for ≥2 consecutive days and a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin, as determined by a toxin protein detecting assay at the time of the diarrhea) within 8 weeks after RBX2660 treatment.
- Treatment success: Absence of CDI diarrhea (passage of ≥3 unformed/loose stools [i.e., Bristol Stool Scale type 6-7] in 24 consecutive hours for ≥2 consecutive days and a stool test collected before start of any antibiotic treatment, which is positive for *C. difficile* toxin, as determined by a toxin protein detecting assay at the time of the diarrhea) for 8 weeks after RBX2660 treatment.
- Indeterminate treatment outcome: Neither the protocol-specified definition for treatment success nor treatment failure are met. Examples are provided below:
    - Not available – test not conducted or test result not available.

- Not valid – e.g., antibiotics started before collection of stool sample for testing, test method failure (without re-testing), or a negative test result considered unreliable due to e.g., an excessive delay from collection of stool sample to testing.

    - In case a suspected CDI recurrence is treated with antibiotics, and the stool sample collected before commencement of the antibiotic treatment is negative for *C. difficile* toxin as determined by a toxin protein detecting assay at the time of the diarrhea, the subject will be considered as having an indeterminate treatment outcome.

Table 7-1 Determination of Treatment Outcome

Diarrhea Before 8 Weeks[a]	Toxin Test Result Before 8 Weeks[b]	Clinical Treatment Outcome	Statistical Treatment Outcome at Week 8
Not present	Not tested (no need for test)	Treatment success	Treatment success
Present	Negative	Treatment success[c, d]	Treatment success[c]
Present	Positive	Treatment failure[e]	Treatment failure
Present	Not available[f]	Indeterminate	Indeterminate
Present	Not valid[g]	Indeterminate	Indeterminate
Unknown	Unknown	Discontinued/ Lost to follow-up	Indeterminate

a. Passage of ≥3 unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 consecutive hours for ≥2 consecutive days.
b. The stool sample for *C. difficile* toxin testing must be provided before starting any antibiotic treatment. The presence of *C. difficile* toxin must be determined by using a toxin protein detecting assay.
c. Unless a subsequent suspected CDI recurrence has an outcome of treatment failure or indeterminate, before or at the follow-up 4 visit at 8 weeks after RBX2660 treatment.
d. If this occurs before the follow-up 4 visit at 8 weeks after RBX2660 treatment and the subject is not treated with antibiotics, the subject will remain in the trial and continue with the follow-up schedule for safety and effectiveness until the end-of-trial visit.
e. Treatment failure = CDI recurrence
f. Not tested or test result not available.
g. E.g., antibiotic treatment started before collection of stool sample for testing, test method failure (without re-testing), or a negative test result considered unreliable due to e.g., an excessive delay from collection of stool sample to testing.

7.1.1.4 Management of Possible CDI Recurrence

An illustration of the management of possible CDI recurrence is presented in Figure 7-1.

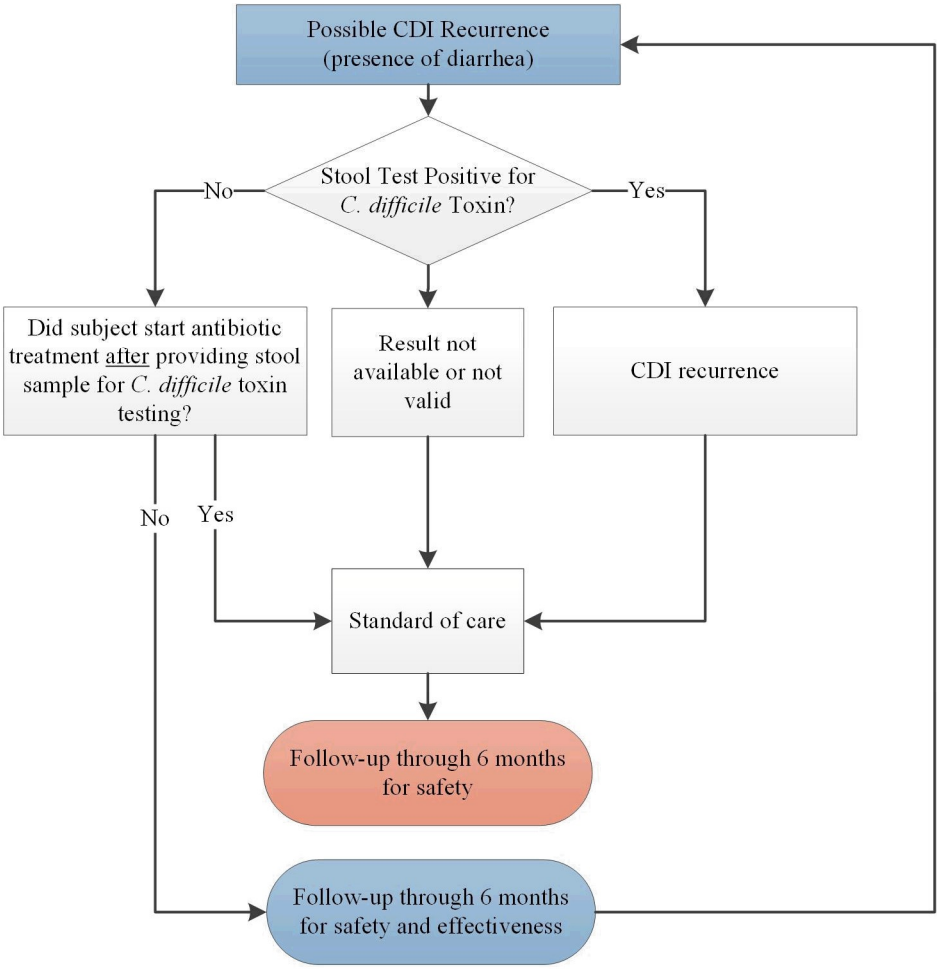
Subjects who are determined as treatment failures (CDI recurrence) (Section 7.1.1.3) should be treated according to SoC at the investigator's discretion, and will continue the follow-up assessments for safety through 6 months (end-of-trial) after administration of RBX2660.

If CDI recurrence is determined at the follow-up 4 visit at week 8, the subject should be treated according to SoC at the investigator's discretion and will continue the follow-up assessments for safety through 6 months (end-of-trial) after administration of RBX2660.

Subjects who are considered as having an indeterminate treatment outcome (Section 7.1.1.3), should be treated according to SoC at the investigator's discretion, and will continue the follow-up assessments for safety through 6 months (end-of-trial) after administration of RBX2660.

Subjects who present with diarrhea before the follow-up 4 visit at 8 weeks after RBX2660 treatment but have a negative toxin test and **who are not** treated with antibiotics, will remain in the trial and continue with the follow-up schedule as outlined in Table 6-1 until the end-of-trial visit.

Subjects who present with diarrhea before the follow-up 4 visit at 8 weeks after RBX2660 treatment but have a negative toxin test and **who are** treated with antibiotics, should be treated according to SoC at the investigator's discretion, and will continue the follow-up assessments for safety through 6 months (end-of-trial) after administration of RBX2660.



C. difficile: *Clostridioides difficile*; CDI: *Clostridioides difficile* infection

Figure 7-1 Management of Possible CDI Recurrence

Subjects who experience CDI after the follow-up 4 visit at week 8 should be diagnosed and treated according to SoC at the investigator's discretion. These subjects will continue the follow-up assessments for safety through 6 months after RBX2660 treatment according to the trial flow chart (Table 6-1).

7.1.2 Physician-experience Questionnaire

To capture each investigator's subjective experience on the usability of RBX2660 in clinical practice, all investigators in this trial will be required to complete a physician-experience questionnaire after each attempted colonoscopy, i.e., at the baseline visit. The questionnaire will capture each physician's opinion on various aspects of the usability of RBX2660 when delivered by colonoscopy, using closed-ended questions. The questions will cover aspects related to presentation of RBX2660, convenience of using RBX2660 by colonoscopy, and overall opinion of usability.

7.1.3 Clinician Global Impression of Improvement

At the follow-up 4 visit at week 8, or at a CDI recurrence visit for subjects who have been documented as treatment failures (recurrence of CDI), investigators will be asked to rate each subject's total improvement according to the CGI-I scale.

The CGI-I is a retrospective measure, referring back to when treatment started, i.e., the baseline visit. It will provide an investigator-rated summary of change in CDI since the start of trial treatment, reported as "very much improved", "much improved", "minimally improved", "no change", "minimally worse", "much worse", or "very much worse", coded as 1-7.[44]

7.1.4 Patient-experience Interview

Participation in the patient-experience interview is optional and signed informed consent must have been obtained through the ICF for the trial, as appropriate, before participation.

After RBX2660 treatment, subjects who have completed the follow-up 4 visit at week 8, or who have been documented as treatment failures (recurrence of CDI) at a CDI recurrence visit, will be contacted by a third-party CRO for an optional patient-experience interview by telephone within 14 days of attending the respective visit. The patient-experience interview is designed to examine the impact of living with CDI on a subject's health-related QoL, and to assess what a meaningful positive change after RBX2660 treatment would be from the subject's perspective.

The objective of the optional patient-experience interview is to capture the impact of the treatment in the subjects' own words. This is in line with patient-focused drug development, as per recommendations of the FDA.[45] In the interviews, subjects' ratings and perceptions of changes will be collected using qualitative and quantitative methods. These data will be used to aid in understanding what is, from a subject's perspective, a meaningful change threshold for indicating positive improvement in the impact on a subject's life. The results will be used for communication around the rCDI impact, primarily in peer-reviewed publications and in the Global Value Dossier (GVD) for payers and health technology agencies (HTAs).

A detailed description of the patient-experience interview is provided in the separate patient-experience interview protocol and the results will be reported separately.

All patient-experience interviews will be conducted over the telephone, in local language, by a trained interviewer from a third-party CRO.

7.1.5 Adverse Events

Adverse events, including SAEs, AESIs (i.e., septic shock, toxic megacolon, colonic perforation, and emergency colectomy, and new onsets of obesity, glucose intolerance, metabolic syndrome, or autoimmune conditions), and adverse events leading to death or ICU admission, will be recorded from the time of signed ICF for participation in the trial until the follow-up 6 visit (end-of-trial). Adverse events must be followed until resolved or the subject exits the trial. The procedures for collection and recording of adverse events are described in Section 8.

CDI diagnosis within 8 weeks after RBX2660 treatment, i.e., CDI recurrence (treatment failure), must only be reported as an adverse event in case it fulfills one or more of the SAE criteria (Section 8.5). CDI diagnosis after 8 weeks to 6 months after RBX2660 treatment, or after CDI recurrence (treatment failure), must be reported as adverse events.

Solicited events, identified through review of the solicited events diary (Section 7.2.9) at the follow-up 1 visit at week 1, should not also be reported as adverse events. However, solicited events that fulfill one or more of the SAE criteria should be reported as SAEs and followed-up by the investigator (Section 8.5).

7.2 Other Assessments

7.2.1 Demographics

Demographic data, including gender by birth, age, ethnicity, and race will be collected at screening.

7.2.2 Medical History

Information on relevant clinically significant previous and concomitant illnesses, signs or findings from assessments and examinations during screening, including surgical history, coronavirus disease 2019 (COVID-19) vaccination and past or ongoing occurrence of COVID-19 (Section 7.4), will be recorded as medical history. Previous CDI history must be recorded on the CDI history form.

7.2.3 Concomitant Medications

Information on concomitant medications will be collected at each visit throughout the trial, as described in Section 4.3.1. Concomitant medications are defined as any medication (prescription or non-prescription), nutritional supplement, or herbal preparation, other than the IMP, taken or used from screening until the follow-up 6 visit (end-of-trial). Vaccination against COVID-19 will be recorded throughout the trial as concomitant medication (Section 7.4).

The use of any concomitant medication within the last 30 days before screening will be recorded as prior medication. Exceptions are medications used for previous CDI episodes that must be recorded independent of when the CDI episode occurred, if possible. Prohibited medications/therapies are listed in Section 4.3.2.

7.2.4 Physical Examination

A modified physical examination will be performed at screening, follow-up 4, follow-up 6 (end-of-trial), and at the CDI recurrence visit as applicable.

The modified physical examination will include, at a minimum, assessment of:

- general appearance
- respiratory system
- cardiovascular system
- gastrointestinal system
- musculoskeletal system
- neurological system

No genitourinary examination is required unless medically indicated.

Findings from the modified physical examination assessed by the investigator as "clinically significant" qualifies as an adverse event and must be reported as described in Section 8.

7.2.5 Body Weight and Height

Body weight (without overcoat and shoes) will be measured at all mandatory site visits. The same scale should preferably be used on the subject during the course of the trial for consistency in readings.

Height (without shoes) will be measured at screening.

7.2.6 Vital Signs

Temperature, diastolic and systolic blood pressure (mmHg), pulse rate (beats per minute), and respiratory rate (breaths per minute) will be measured after resting for 5 minutes in a sitting position, according to usual clinical practice at all mandatory site visits.

Measurements outside normal ranges will be assessed by the investigator as "abnormal, not clinically significant" or "abnormal, clinically significant". If found "abnormal, clinically significant" this qualifies as an adverse event and must be reported as described in Section 8.

7.2.7 Pregnancy Test

A urine or serum pregnancy test will be performed in all female subjects of child bearing potential at screening, baseline, follow-up 4, and follow-up 6 (end-of-trial).

Female subjects of childbearing potential must, as applicable, have documentation of an acceptable effective method of contraception, as described in Section 4.1.1. For females ≥45 years, amenorrhea of ≥12 months duration based on the reported date of the last menstrual period without an alternative medical cause is sufficient documentation of post-menopausal status and do not require a pregnancy test.

7.2.8 Stool Diary

The electronic stool diary serves as a tool for the subject to record pre-treatment stool information and is explained to the subject at the screening visit. Subjects are required to start completing the stool diary immediately after the screening visit, on the same day, and until start of bowel preparation. This may require completion of the stool diary for up to 33 days. The diary will be reviewed on an ongoing basis by trial site staff and at the baseline visit before administration of RBX2660. The stool diary must demonstrate that the CDI symptoms were under control for the 2 consecutive days immediately before initiating the antibiotic washout period, and throughout the washout period up to start of bowel preparation, in order for the subject to receive treatment with RBX2660. Date and time of last antibiotic administration will also be recorded in the stool diary.

7.2.9 Solicited Events Diary

The electronic solicited events diary serves as a tool for the subject to report solicited events after RBX2660 treatment and is explained to the subject at the baseline visit. Subjects are required to start completing the solicited events diary immediately after receiving RBX2660 treatment, on the same day, and until the follow-up visit 1 at week 1, when it will be reviewed by the trial site staff. Through the completion of the solicited events diary, subjects are asked specific questions regarding frequency and severity of the following solicited events: fever, abdominal pain, vomiting, diarrhea, constipation, bloating, and flatulence.

Solicited events, identified through review of the solicited events diary at the follow-up 1 visit at week 1, should not also be reported as adverse events (Section 7.1.5). However, solicited events that fulfill one or more of the SAE criteria should be reported as SAEs and followed-up by the investigator (Section 8.5).

7.2.10 Safety Laboratory Variables

Blood samples for assessment of safety laboratory variables (hematology and clinical chemistry) will be collected at screening, baseline, follow-up 4, and at the follow-up 6 visit (end-of-trial), and at the CDI recurrence visit as presented in Table 6-1. The analyses will be conducted at the local laboratory at the trial site or another laboratory available to the trial site (Section 7.3).

The analytical variables are listed in Table 7-2.

Table 7-2 Safety Laboratory Variables

Clinical Chemistry	Hematology
Alanine aminotransferase	Hemoglobin
Albumin	Hematocrit
Alkaline phosphatase	Platelet count
Aspartate aminotransferase	Red blood cell count
Calcium	White blood cell count with differential (eosinophils, monocytes, basophils, neutrophils, lymphocytes, including absolute neutrophil count and CD4 lymphocytes)
Chloride	
C-reactive protein	
Creatinine	
Glucose	
HbA1c	
Potassium	
Sodium	
Total bilirubin	
Direct bilirubin	
Indirect bilirubin	
Total protein	
Blood urea nitrogen	

CD4: cluster of differentiation 4; HbA1c: hemoglobin A1c

Laboratory values will be reported to the investigator. Measurements outside normal ranges will be assessed by the investigator as “abnormal, not clinically significant” or “abnormal, clinically significant”. The laboratory reports will be signed and dated by the investigator.

Laboratory equipment in the local laboratory, or the laboratory available to the trial site, may provide standard analyses not requested in the protocol, but produced automatically in connection with the requested analyses according to specifications in the laboratory standard operating procedures. Such data will not be transferred to the trial database, but abnormal values will be reported to the investigator who must review all available laboratory results for concomitant illnesses and adverse events and report these according to this protocol.

7.2.11 Stool Samples for Exploratory Analyses of Potential Biomarkers

As part of the trial, subjects will be required to provide stool samples for exploratory research of potential biomarkers pertaining to the mechanism of action of microbiome restoration in patients with rCDI. The stool samples will be used for future analyses of potential biomarkers and will be analysed and reported separately from the current trial. Results of these exploratory analyses will not be provided to the sites.

Subjects are required to collect and ship stool samples in association with the trial visits as outlined below:

- baseline
- follow-up 1
- follow-up 3
- follow-up 4

- follow-up 5
- follow-up 6 (end-of-trial)
- CDI recurrence visit

Subjects will receive electronic reminders to collect the stool samples within the visit window of each visit. The baseline stool sample should be collected during the antibiotic washout period before the baseline visit and before bowel preparation. Stool samples collected in association with the CDI recurrence visit should be collected before starting any antibiotic treatment, if possible.

Subjects will collect the stool samples in pre-labelled containers provided by the trial site. Samples will be identified by the label on the container that contains only the subject number and date/time of the sample.

7.3 Handling of Biological Samples

All biological samples (blood and stool) will be analysed at the local laboratory at the trial site, or another laboratory available to the trial site, and will be handled in accordance with local procedures. Exceptions are the stool samples for exploratory research of potential biomarkers; these samples will be stored for a maximum of 5 years after approval of the clinical trial report. For all biological samples collected in the trial, it applies that analyses beyond those described in the protocol can only be performed after obtaining the required approvals. The processes related to handling of biological samples will be described in the ICF, and applicable Ferring policies and procedures, biobanking-related and personal data protection legislation, including local legislation, will be adhered to.

7.4 Implications of COVID-19

The following considerations must be taken into account due to COVID-19:

- Vaccination against COVID-19
  Vaccination against COVID-19 will be recorded at screening as part of the subject's medical history and/or throughout the trial as concomitant medication, and should be reported using the term "COVID-19 immunization". Based on currently available information, Ferring's risk assessment is that there will be no protocol-specific requirements on COVID-19 vaccination.
- Medical history related to COVID-19
  Past or ongoing occurrence of COVID-19 will be recorded at screening as part of the subject's medical history, and should be reported using the term "COVID-19".
- Adverse events related to COVID-19
  In case of confirmed COVID-19 by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive test, the term "COVID-19" should be used to report the adverse event irrespective of the type of test. In case of suspected COVID-19 infection, the term "Suspected COVID-19" should be used to report the adverse event. In case of recording concomitant medication against COVID-19, the indication should be "COVID-19" irrespective of the treatment.

- Missing visits / missing data
  In case subjects are prevented from attending scheduled visits due to COVID-19, the investigator (or designee) will attempt to contact the subject by telephone or other ways to inquire about potential adverse events and changes to concomitant medication. In case not attending scheduled visits due COVID-19 may have any implications in the drug accountability of IMP, it should be documented by the trial site staff.
- Protocol deviations due to COVID-19 illness and/or COVID-19 control measures
  Protocol deviations should be avoided whenever possible except when necessary to eliminate an immediate hazard to the subject. If deviations occur they will be documented as per standard practice, with additional specification whether they were related to COVID-19.

8 ADVERSE EVENTS

8.1 Adverse Event Definition

An adverse event is any untoward medical occurrence in a subject participating in a clinical trial. It includes:

- Any unfavorable and unintended sign, symptom or disease temporally associated with the use of the IMP, whether or not considered to be caused by the IMP.
- Adverse events commonly observed and adverse events anticipated based on the pharmacological effect of the IMP.
- Any laboratory abnormality, vital sign or finding from physical examination assessed as clinically significant by the investigator [note: Pre-existing conditions diagnosed through assessments and examinations at the screening visit or during the screening period are not adverse events, but are recorded as medical history.].
- Accidental injuries, reasons for any change in medication (drug and/or dose), reasons for any medical, nursing or pharmacy consultation, or reasons for admission to hospital or surgical procedures.

All adverse events will be coded using Medical Dictionary for Regulatory Activities (MedDRA, the MedDRA version will be documented).

8.2 Collection and Recording of Adverse Events

8.2.1 Collection of Adverse Events

The investigator must monitor the condition of the subject throughout the trial from the time of obtaining informed consent until the last visit.

The sources of adverse events cover:

- The subject’s response to questions about his/her health (a standard non-leading question such as “How have you been feeling since your last visit?” is asked at each visit).
- Symptoms spontaneously reported by the subject.
- Investigations and examinations where the findings are assessed by the investigator to be clinically significant changes or abnormalities.
- Other information relating to the subject’s health becoming known to the investigator (e.g., hospitalization).

8.2.2 Recording of Adverse Events

The investigator must record all adverse events in the Adverse Event Log provided for each subject in the EDC with information about:

- Adverse event description.

- Date and time of onset (time can be omitted, if applicable) [note: if date of onset of an event is the same as the date of informed consent or date of IMP administration, time is important and should not be omitted].
- Intensity.
- Causal relationship to IMP.
- Action taken to IMP.
- Other action taken.
- End date and time (time can be omitted, if applicable).
- Outcome.
- Seriousness.

Each of the items in the Adverse Event Log is described in detail in the following sections.

Adverse Event Description

Adverse events should be recorded as diagnoses, if available. If not, separate signs and symptoms should be recorded. One diagnosis/symptom should be entered per record.

If a subject suffers from the same adverse event more than once and the subject recovers in between the events, the adverse events should be recorded separately. If an adverse event changes in intensity, a worst-case approach should be used when recording the event, i.e. the highest intensity and the longest duration of the event.[d]

Note the following: A procedure is not an adverse event; the reason for conducting the procedure is. Hospitalization is not an adverse event; the reason for hospitalization is. Death is not an adverse event, but the cause of death is (an exception is sudden death of unknown cause, which is an adverse event).

Date and Time of Onset

The date of onset is the date when the first sign(s) or symptom(s) were first noted. If the adverse event is an abnormal clinically significant laboratory test or outcome of an examination, the onset date is the date the sample was taken or the examination was performed.

[d] Exception: if an adverse event with onset before the first IMP administration (i.e. a pre-treatment adverse event) worsens in intensity after IMP administration, this must be recorded as two separate events. The initial adverse event should be recorded with outcome “not recovered”, without recording end date and time. The second adverse event should be recorded with date and time of onset when the intensity changed.

Intensity

The intensity of an adverse event must be classified using the definitions in Table 8-1. In addition, all deaths related to an adverse event are to be classified as grade 5. If a specific event is not included in Table 8-1, the intensity of an adverse event should be classified using the Common Terminology Criteria for Adverse Events (CTCAE, the CTCAE version will be documented).[46]

Table 8-1 Intensity Grading Table

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Flatulence (gas)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	N/A	N/A
Belching (burping)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	N/A	N/A
Abdominal distension or bloating	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	N/A
Increased diarrhea	Increase of ≤ 3 stools over baseline per 24-hour period	Increase of 4–6 stools over baseline per 24-hour period	Bloody diarrhea if not present at baseline OR increase of ≥7 stools over baseline per 24-hour period OR IV fluid replacement indicated if not indicated at baseline	Life-threatening consequences, e.g., hypotensive shock
Abdominal cramping/pain	Discomfort/pain causing no or minimal interference with usual social and functional activities	Discomfort/pain causing greater than minimal interference with usual social and functional activities	Discomfort/pain causing inability to perform usual social and functional activities	Disabling pain causing inability to perform basic self-care OR inpatient hospitalization ≥24 hours
Constipation	Occasional or intermittent symptoms, occasional use of stool softeners, laxatives, dietary modifications or enema	Persistent symptoms with regular use of laxatives or enemas indicated	Symptoms causing inability to perform usual social and/or functional activities	Life-threatening consequences, e.g., obstruction, toxic megacolon

Colitis	No symptoms, regardless of pathologic or radiographic evidence of inflammation	Abdominal pain, mucus or blood in the stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences, e.g., perforation, bleeding, ischemia, necrosis, toxic megacolon
Fever	37.8 – 38.6°C (100.0 – 101.5° F)	38.7 – 39.3°C (101.6 – 102.8° F)	39.4 – 40.5°C (102.9 – 104.9° F)	> 40.5°C (104.9° F)
Fatigue/ malaise	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions
Chills	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	N/A
Rectal discomfort or irritation	No symptoms or symptoms not requiring medical intervention	Symptomatic with medical intervention (topical medications / treatments) indicated	Symptoms causing inability to perform usual social and functional activities or requiring medical intervention other than topical medications / treatments	N/A
Rectal bleeding	Mild or intermittent without transfusion	Persistent without transfusion	Requires transfusion	Life-threatening consequences
Nausea	Transient (≤24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased intake for 24-48 hours	Persistent nausea resulting in decreased intake > 48 hours OR aggressive rehydration indicated, e.g., IV fluids	Life-threatening consequence, e.g., hypotensive shock
Vomiting	Transient (≤24 hours) or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated, e.g., IV fluids	Life-threatening consequence, e.g., hypotensive shock

Hypotension	N/A	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Adverse event not identified elsewhere in this table	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

N/A: not applicable; IV: intravenous
Adapted from Division of AIDS Table for Grading of Severity of Adult and Pediatric Adverse Events and Addendum 3: Rectal Grading Table for Use in Microbicide Studies; May 2012.[47,48]

Causal Relationship to IMP or the Colonoscopy Procedure

The possibility of whether the IMP or the colonoscopy procedure caused the adverse event must be classified as one of the following:

Related:

There is evidence or argument to suggest a causal relationship between the IMP or the colonoscopy procedure and the adverse event. The adverse event may occur as part of the pharmacological action of the IMP or as part of the colonoscopy procedure, or may be unpredictable in its occurrence.

Relatedness to RBX2660 or the colonoscopy procedure is defined as:

- Possible: There is some temporal relationship between the event and the administration of the IMP or the colonoscopy procedure, though the event could also be explained by the subject's medical condition or other therapies.
- Probable: The temporal relationship between the event and administration of the IMP or the colonoscopy procedure is suggestive, and the event is unlikely to be explained by the subject's medical condition or other therapies alone.
- Definite: The event follows a reasonable temporal sequence from administration of the IMP or the colonoscopy procedure, follows a known or suspected response pattern to the IMP and/or colonoscopy procedure, improves upon stopping the IMP and/or colonoscopy procedure, and reappears upon repeated exposure, if that occurs.

Examples:

- Adverse events that are uncommon but are known to be strongly associated with IMP exposure or the colonoscopy procedure.

- Adverse events that are not commonly associated with IMP exposure or the colonoscopy procedure, but the event occurs in association with other factors strongly suggesting causation, such as a strong temporal association or the event recurs on rechallenge.

Unrelated:

There is no reasonable evidence or argument to suggest a causal relationship between the IMP or the colonoscopy procedure and the adverse event.

Unrelatedness to RBX2660 or the colonoscopy procedure is defined as:

- Unrelated: The event is due to an underlying or concurrent illness or effect of concomitant medication and is not related to the IMP or the colonoscopy procedure (e.g., has no temporal relationship to the IMP or the colonoscopy procedure, or has a much more likely alternative etiology).

Examples:

- Known consequences of the underlying disease or condition under investigation.
- Adverse events common in the trial population, which are also anticipated to occur with some frequency during the course of the trial, regardless of IMP exposure or the colonoscopy procedure.

For adverse events where the causality is classified as “unrelated”, the alternative causality should be recorded in the EDC as free text.

Action Taken to IMP

The action taken to the IMP in response to an adverse event must be classified as one of the following:

- No change (applicable for when the IMP regimen is maintained as well as for adverse events occurring before first dose of IMP or after last dose of IMP as per protocol).
- Discontinued.
- Interrupted.

Other Action Taken

Adverse events requiring therapy must be treated with recognized standards of medical care to protect the health and well-being of the subject. Appropriate resuscitation equipment and medicines must be available to ensure the best possible treatment of an emergency situation.

If medication is administered to treat the adverse event, this medication should be entered in the Concomitant Medication Log.

End Date and Time

The date and time (time can be deleted/omitted, if applicable) the subject recovered or died.

Outcome

The outcome of an adverse event must be classified as one of the following:

- Recovered (fully recovered or the condition has returned to the level observed at initiation of trial treatment)
- Recovered with sequelae (resulted in persistent or significant disability/incapacity)
- Recovering (the event is improving)
- Not recovered
- Fatal

8.3 Other Significant Adverse Events

8.3.1 Adverse Events Leading to Discontinuation

Adverse events leading to discontinuation from the trial, or discontinuation from treatment, will be considered as other significant adverse events.

Subject narratives will be prepared by Ferring for all adverse events leading to discontinuation from the trial and will be included in the clinical trial report.

8.3.2 Adverse Events of Special Interest

An AESI, whether serious or non-serious, is an adverse event of medical and safety concern for which ongoing monitoring and rapid communication by the investigator to Ferring is needed. Such events may require further investigation in order to characterize and understand them.

Ferring has defined AESIs for this trial. These are:

- septic shock
- toxic megacolon
- colonic perforation
- emergency colectomy
- new onsets of obesity, glucose intolerance, metabolic syndrome, or autoimmune conditions

All AESIs must be reported in the relevant section of the EDC. All AESIs, whether they fulfil the criteria for an SAE or not, should be reported in accordance with Section 8.5.2.

Subject narratives will be prepared by Ferring for all AESI cases and will be included in the clinical trial report.

8.4 Pregnancy and Pregnancy Outcome

If a pregnancy occurs after RBX2660 treatment, Global Safety at Ferring must be informed using the contact details in Section 8.5.2. The investigator must ensure follow-up of the mother and the fetus at least until the birth of the infant and approximately 4 weeks after the birth of the infant. In general, the follow-up will include the course; duration and the outcome of the pregnancy as well as neonatal health. Pregnancy should be reported as a non-serious adverse event (for tracking purposes only, pregnancy in itself is not an adverse event). If a pregnancy results in an abnormal outcome (birth defect/congenital anomaly) this must be reported as a serious adverse event to Global Safety at Ferring.

In cases in which a fetus may have been exposed through transmission of the IMP via semen following paternal exposure, it must be reported to Global Safety at Ferring, including the pregnancy outcome. If the pregnancy results in an abnormal outcome (birth defect/congenital anomaly) this must be reported as an SAE to Global Safety at Ferring.

8.5 Serious Adverse Events

8.5.1 Serious Adverse Event Definition

Serious Adverse Events during the Trial

An event is defined as a serious adverse event if it:	**Guidance**
results in **death**	-
is **life-threatening**	The subject was at immediate risk of death at the time of the event. The criterion does not refer to an event which might hypothetically have caused death if it were more severe.
requires **hospitalization**	Hospitalization is defined as requiring in-patient admission to hospital or as prolongation of existing hospitalization as a result of an event.
results in persistent or significant **disability/incapacity**	-
is a **congenital anomaly/birth defect**	Observed in any offspring of the subject conceived during treatment with the IMP.
is an **important medical event**	Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious such as important medical events that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

8.5.2 Collection, Recording and Reporting of Serious Adverse Events

SAE Reporting by the Investigator

All SAEs must be communicated **immediately** to Ferring Global Safety as soon as it becomes known to the investigator and not later than within 24 hours of their knowledge of the occurrence of an SAE.

The investigator is responsible for providing the completed and signed SAE Report Form with the fullest possible details **within 3 calendar days** of his/her knowledge of the SAE.

The same timelines and requirements apply for both the SAE Report Form and any follow-up with additional information regarding the SAE, i.e. 24 hours within obtaining knowledge of the follow-up information and 3 calendar days for providing the fullest possible details.

The SAE Report Form is included in the EDC, and must be completed and submitted according to the instructions provided on the form. In case the EDC cannot be accessed and hence the SAE Report Form cannot be filled in within the EDC, a paper SAE Report Form should be used and sent to Ferring Global Safety using the contact details below.

Global Safety, Ferring Pharmaceuticals A/S
E-mail: Safety.Mailbox@ferring.com
Fax: (+45) 88 38 01 47

Completion of the Demographics, Adverse Event Log, Medical History Log and Concomitant Medication Log are mandatory for initial reports and for follow-up reports if any relevant changes have been made since the initial report. Data entries must have been made in the EDC for Ferring Global Safety to access the information.

Additional information relevant to the SAE such as hospital records, results from investigations, e.g. laboratory parameters (that are not already uploaded in the EDC), invasive procedures, scans and x-rays, and autopsy results can be faxed or scanned and e-mailed to Ferring Global Safety using the contact details in the section above. In any case this information must be supplied by the investigator upon request from Ferring. On any copies provided, personal details such as subject's name, address, and hospital name should be concealed and instead subject number should be provided.

The investigator will supply Ferring (and the IRB, if applicable) with any additional requested information such as results of post-mortem examinations and hospital records.

Expedited Reporting by Ferring

Ferring will report all adverse events according to local regulations.

8.6 Follow-up of Adverse Events and Serious Adverse Events

8.6.1 Follow-up of Adverse Events with Onset during the Trial

During the trial, the investigator must follow-up on each adverse event until the subject's last visit. After the subject's last visit, the investigator must follow-up on any adverse event classified as serious or considered to have a "related" causality to the IMP until it is resolved or until the medical condition of the subject is stable. All such relevant follow-up information must be reported to Ferring. Follow-up should continue until the outcome of recovered, recovered with sequelae, or fatal, has been reached. If the event is considered stable or a chronic condition, the investigator and Ferring may agree that further follow-up is not required.

The Adverse Event Follow-Up Page in the EDC should be used for provision of follow-up information, and when no longer accessible, the site must report using paper forms.

8.6.2 Collection of Serious Adverse Events with Onset after Last Visit in the Trial

If an investigator becomes aware of an SAE after the subject's last visit, and he/she assesses the SAE to have a "related" causality to the IMP, the case will have to be reported to Ferring, regardless how long after the end of the trial this takes place.

Such reporting should be done using paper forms (submitted in paper form or electronically) using the same reporting contact details as given above.

8.7 Technical Complaints

A technical complaint can include but is not limited to problems with dose delivery (e.g., spike port difficulties), the packaging material (e.g., label text is not legible, evidence of EVA bag leakage, or damage to the EVA bag), or the physical appearance of a trial product.

If a technical complaint is identified, the investigator must assess whether it may be associated with an adverse event and/or an IMP medication error. Technical complaints are reported in accordance with the Trial Supply Manual.

9 STATISTICAL METHODS

The Global Biometrics department at Ferring will be responsible for the statistical analyses.

9.1 Determination of Sample Size

There are no pre-specified hypotheses in this trial, therefore there are no pre-planned significance tests or sample size calculations.

The outcomes of this trial will be reported by descriptive statistics as indicated below.

Subjects who have provided written informed consent, but do not meet all eligibility criteria will be considered screening failures.

9.2 Subject Disposition

A summary table will present the number of subjects in the population sets: screened, safety analysis set, full analysis set (FAS), the number of subjects that completed the trial, and the number of subjects that withdrew from the trial, with a breakdown of reasons/categories for trial withdrawals.

The number of subjects screened but not treated will be presented with the reason(s) for screening failure in a data listing. The subjects re-screened, including the screening number from the previous screening, and subjects treated after re-screening will also be listed.

9.3 Protocol Deviations

No per protocol analysis is planned, and thus protocol deviations will not be classified as major or minor. No protocol deviations will lead to exclusion of data from the analysis of effectiveness. In order to reflect general protocol adherence, protocol deviations will be classified as either “important” or “non-important”. Important protocol deviations will be summarized for all subjects in the FAS. All protocol deviations (“important” and “non-important”) will be listed in subject data listings. Protocol deviations related to COVID-19 will be identified and listed (Section 7.4).

9.4 Analysis Sets

9.4.1 Safety Analysis Set

The safety analysis set comprises all subjects treated with RBX2660.

9.4.2 Full Analysis Set (FAS)

The FAS comprises all subjects treated with RBX2660, and is identical to the safety analysis set.

9.5 Trial Population

9.5.1 Demographics and other Baseline Characteristics

Descriptive statistics of demographics and other baseline (i.e., before RBX2660 treatment) characteristics will be presented for the safety analysis set.

Categorical data will be summarized using numbers and percentages. The percentages are based on the total number of subjects with a corresponding assessment. Continuous data will be presented using the number of subjects, mean, standard deviation, median, interquartile range (IQR), minimum, and maximum. All baseline characteristics will be listed.

9.5.2 Medical History, Concomitant Medication and Other Safety Evaluations

Medical history will be coded using MedDRA. The MedDRA version will be documented.

Prior and concomitant medication will be summarized by Anatomical Therapeutic Chemical (ATC) classification 1st level (alphabetically), ATC classification 2nd level (in decreasing order of frequency), and treatment group.

Physical examination, vital signs, and safety laboratory variables will be summarized. Baseline values are defined as the last assessment before start of RBX2660 treatment.

9.6 Endpoint Assessments

9.6.1 General Considerations

The effectiveness endpoints will be analyzed for the FAS. Safety endpoints will be evaluated for the safety analysis set.

Categorical data will be summarized using counts and percentages, while continuous data will be presented using the number of subjects, mean, standard deviation, median, IQR, minimum, and maximum.

For estimated rates (e.g., the rate of no CDI recurrence within 8 weeks after treatment) the confidence interval will be calculated assuming a binomial distribution using the exact method (Clopper-Pearson).

All assessments will be listed in subject data listings.

9.6.2 Primary Endpoint

The analysis of the primary endpoint “RBX2660-related treatment-emergent adverse events (TEAEs) after RBX2660 treatment delivered by colonoscopy through 8 weeks, or treatment failure” is described in Section 9.8.

9.6.3 Secondary Endpoints

9.6.3.1 Recurrence of CDI within 8 Weeks after RBX2660 Treatment Delivered by Colonoscopy

This will be evaluated as the proportion of subjects with treatment success (absence of CDI diarrhea, i.e., no recurrence of CDI). The statistical treatment of various potential subject outcomes is summarized in Section 7.1.1.3.

The estimated success rate (rate of no CDI recurrence within 8 weeks after treatment) will be presented with 95% confidence intervals.

9.6.3.2 Time to CDI Recurrence from Baseline through 8 Weeks after RBX2660 Treatment Delivered by Colonoscopy

Time to CDI recurrence, i.e., time to start of CDI symptoms, will be estimated by the Kaplan-Meier method. Time to recurrence is defined as time to the start of CDI symptoms recorded at the CDI recurrence visit minus the date of treatment plus 1.

9.6.3.3 Physician-experience, as Determined by Questionnaire, Documenting Subjective Experience of Investigators on Usability of RBX2660 in Clinical Practice when Delivered by Colonoscopy

Physician-experience questionnaire scores will be reported quantitatively, as frequencies of different scores, where applicable.

9.6.3.4 Physician Perception of Patient Benefit, as Determined by Clinician Global Impression of Improvement (CGI-I) at 8 Weeks or at Treatment Failure after RBX2660 Treatment Delivered by Colonoscopy

CGI-I scores will be reported quantitatively, as frequencies of different scores.

9.6.3.5 Patient-experience Interview at 8 Weeks or at Treatment Failure after RBX2660 Treatment Delivered by Colonoscopy

All qualitative data analysis will be conducted by COS in NVivo v1.3, a software package which is designed to facilitate the systematic review, coding, and analysis of qualitative data. This program allows the researcher to code data at different levels of analysis and search for patterns and themes in the data across different variables of interest in a data set or population. Data will be thematically analyzed using a grounded theory approach with the aim to identify the key qualitative themes relating to the research objectives. To achieve this, discrete thematic codes will be grouped into concepts and then further collated into domains. For each theme, exemplary quotes will be highlighted for inclusion in the patient-experience interview report.

A preliminary codebook will be created based on the interview guide. Using the codebook, the researchers will code the interview transcripts and identify any new codes that emerge in the interviews. The COS study lead will monitor the revisions to the coding structure to ensure that all codes are clearly defined and consistently applied. Analysis will be conducted in the form of "reporting counts" for each of the themes identified from the qualitative analysis.

Quantitative analysis will be conducted in the form of "reporting counts" for each of the themes identified from the qualitative analysis. In addition, quantitative analysis of the patient-experience interview sample data will be conducted to describe the demographic and clinical background of the sample. These sample data would be retrieved from the EDC and provided via data pass-back. These analyses will follow general statistical methods, including:

- quantitative variables will be described by their frequency, mean, standard deviation, median, IQR, minimum and maximum values, and number of missing values, and

- categorical variables will be described by the frequency and percentage of each response choice, with missing data included in the calculation of percentage.

Quantitative data tables will be generated for each of the individual language groups, if applicable. All quantitative analyses will be conducted using Statistical Analysis System Version 9.3 or higher.

The results of the patient-experience interview will be reported separately.

9.7 Extent of Exposure and Treatment Compliance

Exposure and treatment compliance will be recorded by the investigator and summarized.

9.8 Safety

9.8.1 General Considerations

Safety parameters will be evaluated for the safety analysis set.

9.8.2 Adverse Events

The primary endpoint of the trial is "RBX2660-related treatment-emergent adverse events (TEAEs) after RBX2660 treatment delivered by colonoscopy through 8 weeks, or treatment failure". This is defined as a RBX2660-related TEAE fulfilling the 8 week criterion after RBX2660 treatment as outlined below.

A TEAE is any adverse event occurring after start of RBX2660 treatment and until the end-of-trial visit, or a pre-treatment adverse event or pre-existing medical condition that worsens in intensity after start of RBX2660 treatment and until the end-of-trial visit. A pre-treatment adverse event is any adverse event that occurs between signing the ICF and exposure to RBX2660. Serious adverse events and adverse events considered to have a "related" causality to RBX2660 and which are ongoing at the subject's last visit will be followed-up as described in Section 8.6.1.

SAEs include adverse events that fulfill one or more of the SAE criteria (Section 8.5).

AESIs include: septic shock, toxic megacolon, colonic perforation, and emergency colectomy, and new onsets of obesity, glucose intolerance, metabolic syndrome, or autoimmune conditions (Section8.3.2).

Two time-criteria will be used during the reporting of TEAEs as described below.

The following TEAEs are considered to fulfill the 8 week criterion: For subjects defined as treatment successes (Section 7.1.1.3), TEAEs with time of onset after treatment and before or at the follow-up 4 visit at 8 weeks after treatment should be included. For subjects defined as treatment failures or as indeterminate treatment outcomes (Section 7.1.1.3), TEAEs with time of onset after treatment and before time of onset of CDI symptoms should be included. If the time of onset of CDI symptoms is missing (e.g., due to an intercurring event) the date of the follow-up 4 visit at 8 weeks after treatment should be used, and if this is also missing, TEAEs with onset after treatment and before 56 days after treatment should be included.

TEAEs fulfilling the 8 week criterion after RBX2660 treatment will be summarized. The TEAEs will be tabulated by system organ class (SOC) and preferred term (PT). The total number of subjects reporting an adverse event, the percentage of subjects (%) with an adverse event, and the number of adverse events reported will be presented.

Summary tables will be prepared for:

- All TEAEs.
- TEAEs by causality (related/unrelated). The RBX2660-related TEAEs up to 8 weeks or treatment failure after RBX2660 treatment is the primary endpoint in this trial.
- Adverse events leading to death.
- Adverse events leading to ICU admission.
- TEAEs by intensity.
- Serious TEAEs.
- AESIs.
- TEAEs leading to discontinuation from IMP.
- TEAEs leading to discontinuation from the trial.
- TEAEs with an incidence of at least 5%.
- Non-serious TEAEs with an incidence of at least 5%.

Supporting data listings will be provided for:

- Pre-treatment adverse events by trial site and subject number.
- TEAEs by trial site and subject number, including causality and intensity.
- TEAEs sorted by MedDRA PT.
- All serious adverse events.
- All AESIs.
- Adverse events leading to death.
- Adverse events leading to ICU admission.
- Adverse events leading to discontinuation of IMP (related/unrelated).
- Adverse events leading to discontinuation from the trial (related/unrelated).

For TEAEs not fulfilling the 8 week criterion, i.e., TEAEs with an onset after 8 weeks, or after treatment failure, to 6 months after treatment, a similar set of tables and listings will be prepared.

Adverse events will be classified according to MedDRA. The MedDRA version will be documented.

9.8.3 Solicited Events

Solicited events collected through the solicited events diary will be summarized.

9.8.4 Safety Laboratory Variables

Safety laboratory variables will be grouped under “clinical chemistry” and “hematology”.

Baseline for all safety laboratory variables will be the values obtained at the last assessment before start of RBX2660 treatment. End of treatment period will include the last post-baseline observation during the trial.

Mean change and mean percentage change from baseline at end of treatment period will be presented for each laboratory variable. In addition, descriptive statistics, i.e., the number of subjects with data, mean, standard deviation, median, IQR, minimum, and maximum values, will be presented for observed values and change from baseline at each time-point for each laboratory variable.

Safety laboratory variables will be summarized.

Furthermore, summary tables will be presented that displays by time and laboratory variable, the number of subjects with clinically significant laboratory abnormalities.

9.9 Interim Analyses

No interim analyses are planned for the current trial.

9.10 Database Lock

There will be a database lock when the last subject has completed the 8-week follow-up visit (follow-up 4 visit) for analysis and reporting of data collected up to 8-weeks. This database lock will be referred to as the 8-week database lock. In addition, there will be a database lock at end-of-trial, referred to as final database lock, for final analysis and reporting (Section 13.1).

10 DATA HANDLING

10.1 Source Data and Source Documents

Source Data – ICH Definition

Source data are defined as all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).

Source Documents – ICH Definition

Source documents are defined as original documents, data, and records (e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).

Trial-specific Source Data Requirements – Ferring

No specific protocol data, apart from the physician-experience questionnaire and the CGI-I, can be recorded directly in the EDC without a prior written or electronic record. The data entered in the EDC that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained.

The investigator may need to request previous medical records or transfer records, and current medical records must also be available.

For each subject enrolled, the investigator must document in the subject's medical record that the subject participates in this trial and at least the following information must be recorded:

- Documentation of signed and dated ICF.
- Subject's name and date of birth.
- Confirmation of participation in the trial (trial identification, screening number).
- Eligibility for participation in the trial (inclusion/exclusion).
- Relevant medical history.
- Recurrent CDI medical history.
- Visit dates.
- Results of any examinations/tests and assessments performed.
- Details of any concomitant medication/therapy.
- Details of any adverse events/SAEs (including description and duration).

- Date of IMP administration.
- Reason for discontinuation/withdrawal, if applicable.

The following documents collected during the trial must be stored and archived together with the subject's hospital/medical records or in the investigator file as agreed upon prior to the trial start:

- Laboratory print-outs from local laboratory, or another laboratory available to the trial site (if used) – evaluated, signed and dated by the investigator.
- Relevant records for collection of laboratory samples.
- Drug Dispensing Logs.

Data entered by the subjects in the stool diary and the solicited events diary will be considered trial-specific source data.

10.2 Electronic Data Capture

An EDC provided by an independent third-party CRO will be used for data capture. The EDC is validated and access at all levels is granted/revoked following Ferring and vendor procedures, in accordance with regulatory and EDC requirements.

Trial data should be entered into the EDC in a timely manner, preferably within 3 working days after each trial visit (virtual or on-site).

The investigator will, in a timely manner, approve/authorize the EDC entries for each subject with an electronic signature which is equivalent to a handwritten signature.

The EDC system and the database will be hosted at the independent third party CRO. After the trial database is declared clean and locked at end-of-trial (final database lock [Section 3.4]), a final copy of the database will be stored at Ferring. The investigator will also receive a copy of the trial site's final and locked data (including audit trail, electronic signature and queries) as write-protected PDF-files produced by the independent third party CRO. The PDF-files will be stored in an electronic format and will be provided to the investigator before access to the EDC is revoked.

Entry errors occurring in the EDC will be corrected electronically. Such corrections/modifications will be automatically tracked by an audit trail detailing the date and time of the correction and the name of the person making the correction.

10.3 Use of Clinician Reported Outcomes

10.3.1 CGI-I

The CGI-I will be completed by the physician directly in the EDC.

10.3.2 Physician-experience Questionnaire

The physician-experience questionnaire will be completed by the physician directly in the EDC.

10.4 Use of Patient Reported Outcomes

10.4.1 Patient-experience Interview

All subjects in this trial will be invited to participate in an optional patient-experience interview by telephone. All patient-experience interviews will be conducted in local language and over the telephone by a trained interviewer from a third-party CRO.

10.4.2 Stool Diary

The stool diary will be completed electronically using a web browser on any device or computer. The diary will be reviewed by the trial site staff.

10.4.3 Solicited Events Diary

The solicited events diary will be completed electronically using a web browser on any device or computer. The diary will be reviewed by the trial site staff.

10.5 Data Management

A data management procedure will be created under the responsibility of the Global Biometrics department at Ferring. The data management plan will be issued before data collection begins and will describe all functions, processes, and specifications for data collection, and validation.

The data management plan will include information about the intended use of computerized systems and a description of the electronic data flow.

10.6 Provision of Additional Information

On request, the investigator will provide Ferring with additional data relating to the trial, duly pseudominized and protected in accordance with applicable requirements.

11 MONITORING PROCEDURES

11.1 Monitoring

The monitor will contact and visit the investigator periodically to ensure adherence to the protocol, ICH-GCP, standard operating procedures and applicable regulatory requirements, maintenance of trial-related source records, completeness, accuracy and verifiability of EDC entries compared to source data, verification of drug accountability, and compliance to safety reporting instructions.

The investigator will permit the monitor direct access to all source data, including electronic medical records, and/or documents in order to facilitate data verification. The investigator will co-operate with the monitor to ensure that any discrepancies that may be identified are resolved. The investigator is expected to be able to meet the monitor during these visits. In addition to on-site monitoring visits, remote monitoring is also an option. Remote monitoring is conducted off-site and may include video conference, telephone contact, and remote review of trial systems.

Details on monitoring will be described in the monitoring plan for the trial.

11.2 Audit and Inspection

The investigator will make all the trial-related source data and records available at any time to quality assurance auditor(s) mandated by Ferring, or to domestic/foreign regulatory inspectors or representatives from IRBs who may audit/inspect the trial.

The main purposes of an audit or inspection are to evaluate trial conduct and compliance with the trial protocol, ICH-GCP, the applicable regulatory requirements, and standard operating procedures. The subjects must be informed by the investigator and in the ICF that authorized Ferring representatives and representatives from regulatory authorities and IRBs may wish to inspect their medical records. During audits/inspections the auditors/inspectors may copy relevant parts of the medical records. No personal identification apart from the screening number will appear on these copies.

The investigator should notify Ferring without any delay of any inspection by a regulatory authority or IRB.

11.3 Confidentiality of Subject Data

The investigator will ensure that the confidentiality of the subjects' data will be preserved. In the EDC or any other documents submitted to Ferring, the subjects will not be identified by their names, but by an identification system, which consists of an assigned number in the trial. Documents that are not for submission to Ferring, e.g., the confidential subject identification code and the signed ICF, will be maintained by the investigator in strict confidence.

12 CHANGES IN THE CONDUCT OF THE TRIAL

12.1 Protocol Amendments

Any change to this protocol will be documented in a protocol amendment, issued by Ferring, and agreed upon by the investigator and Ferring, if applicable, prior to its implementation. Amendments may be submitted for consideration to the approving IRBs and regulatory authorities, in accordance with local regulations. Changes to the protocol to eliminate immediate hazard(s) to trial subjects may be implemented prior to regulatory authorities and/or IRBs approval/favorable opinion.

12.2 Deviations from the Protocol

Deviations from the protocol should not occur. If deviations occur, the investigator must inform the sponsor, and the implications of the deviation must be reviewed and discussed. All deviations must be documented, and a record of protocol deviations should be retained by the investigator and sponsor.

12.3 Premature Trial Termination

At any time, the investigator has the right to terminate his/her participation in the trial, and Ferring has the right to terminate the trial. Should termination become necessary, the procedures will be agreed upon after consultation between the two parties. In terminating the trial, Ferring and the investigator will ensure that adequate consideration is given to the protection of the best interests of the subjects. Regulatory authorities and IRBs will be informed.

In addition, Ferring reserves the right to terminate the participation of individual trial sites. Conditions that may warrant termination include, but are not limited to, insufficient adherence to protocol requirements and failure to enter subjects at an acceptable rate.

13 REPORTING AND PUBLICATION

13.1 Clinical Trial Report

The data and information collected during this trial will be reported in a clinical trial report prepared by Ferring and submitted for comments and signature to the signatory investigator.

The clinical trial report will be prepared when the last subject has completed the 8-week follow-up visit (follow-up 4 visit). At this timepoint there will be a database lock (8-week database lock, Section 3.4) for analysis and reporting of data collected up to 8 weeks after RBX2660 treatment. The data and information collected after the 8-week follow-up visit (follow-up 4 visit) will be reported in a clinical trial report addendum when the last subject has completed the last visit in the trial (end-of-trial) and after the final database lock.

13.2 Confidentiality and Ownership of Trial Data

Any confidential information relating to the IMP or the trial, including any data and results from the trial will be the exclusive property of Ferring. The investigator and any other persons involved in the trial will protect the confidentiality of this proprietary information belonging to Ferring.

13.3 Publications and Public Disclosure

13.3.1 Publication Policy

At the end of the trial, one or more manuscripts for joint publication may be prepared in collaboration between the investigator(s) offered authorship and Ferring. In a multi-site trial, any publication must acknowledge all sites, and the results must be reported in a responsible and coherent manner. Ferring requires that the results from multi-site trials are published only in their entirety, and not as individual site data.

In addition, the following requirements must be adhered to:

(i) a presentation or publication of the results must not take place until eighteen (18) months after locking of the trial database at end-of-trial, unless a) the results of the trial have already been published by Ferring, or b) specific written permission for publication is obtained in advance from Ferring;

(ii) at least sixty (60) days in advance of any publication or presentation (or submission hereof) related to the trial, a copy of the relevant publication or presentation must be furnished to Ferring for review and redaction of confidential information. At Ferring's request, investigator(s) must delay the publication or presentation for up to an additional ninety (90) days to enable Ferring to file patent applications or other intellectual property protection;

(iii) Ferring must be identified as the sponsor of the trial in any publication and presentation (including professional meetings and symposia).

Authorship is granted based on the International Committee of Medical Journal Editors (ICMJE) criteria (see current official version: http://www.ICMJE.org). The total number of authors is based on the guideline from the relevant journal or congress. In the event of any disagreement in the

content of a publication, both the investigator's and Ferring's opinion will be fairly and sufficiently represented in the publication.

No CRO or external laboratory involved in the conduct of this trial has any publication rights regarding this trial.

13.3.2 Public Disclosure Policy

ICMJE member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public, clinical trials registry. It is the responsibility of Ferring to register the trial in an appropriate public registry, e.g. www.clinicaltrials.gov; a website maintained by the National Library of Medicine (NLM) at the U.S. National Institutes of Health (NIH). Trial registration may occur in other registries in accordance with U.S. regulatory requirements. A summary of the trial results is made publicly available in accordance with US regulatory requirements.

14 ETHICAL AND REGULATORY ASPECTS

14.1 Institutional Review Board

An IRB will review the protocol and any amendments and advertisements used for recruitment. The IRB will review the ICF, its updates (if any), and any written materials given to the subjects. A list of all IRBs to which the protocol has been submitted and the name of the committee chairmen will be included in the clinical trial report.

14.2 Regulatory Authority Authorization / Approval / Notification

The regulatory permission to perform the trial will be obtained in accordance with applicable regulatory requirements. All ethical and regulatory approvals must be available before a subject is exposed to any trial-related procedure, including screening tests for eligibility.

14.3 End-of-Trial and End-of-Trial Notification

The end of this trial is defined as the date of the last visit that the last subject undergoes in the trial (follow-up 6). The primary completion date will be when the last subject has attended the follow-up 4 visit at week 8, or has been documented as a treatment failure, after RBX2660 treatment. The primary completion date can occur up to 4 months prior to the end-of-trial, when the last subject in the trial is examined or received an intervention for the primary endpoint.

The IRBs will be notified about the completion of the clinical trial according to local legislation.

In case of early termination for safety reasons, Ferring must notify the end of the trial to the relevant regulatory authorities and the concerned IRBs without delay, clearly explain the reasons, and describe follow-up measures, if any.

14.4 Ethical Conduct of the Trial

This trial will be conducted in accordance with the ethical principles that have their origins in the Declarations of Helsinki and Taipei,[49,50] in compliance with the approved protocol, ICH-GCP and applicable regulatory requirements.

14.5 Subject Information and Consent

The subject ICF in this trial will cover: general consent for trial participation, including consent for the stool samples for exploratory research of potential biomarkers, and specific consent for the patient-experience interview. Aspects relevant to the stool sampling and the patient-experience interview will be described in the ICF for the general trial. Participation in patient-experience interview is optional.

The investigator (or the person delegated by the investigator) will obtain a freely given written consent from each subject after an appropriate explanation of the aims, methods, sources of funding, any possible conflicts of interest, anticipated benefits, potential risks of the trial and the discomfort it may entail, post-study provisions and any other aspects of the trial which are relevant to the subject's decision to participate. The trial subject must be given ample time to consider

participation in the trial, before the consent is obtained. The ICF must be signed and dated by the subject and the investigator (or the person delegated by the investigator) who has provided information to the subject regarding the trial before the subject is exposed to any trial-related procedure, including screening tests for eligibility. Subjects must be given the option of being informed about the general outcome and the results of the trial.

The investigator (or the person delegated by the investigator) will explain that the subject is completely free to refuse to enter the trial or to withdraw from it at any time, without any consequences for his/her further care and without the need to justify his/her decision.

The subject should receive a copy of his/her signed and dated ICF before any trial-related procedure.

If new information becomes available that may be relevant to the trial subject's willingness to continue participation in the trial, a new ICF may be issued. In such case, the new ICF will be forwarded to the IRBs (and regulatory authorities, if required). The trial subjects will be informed about this new information and re-consent will be obtained.

Each subject will be informed that the monitor(s), quality assurance auditor(s) mandated by Ferring, IRB representatives or regulatory authority inspector(s), in accordance with applicable regulatory requirements, may review his/her source records and data. Data protection will be handled in compliance with applicable regulations.

14.6 Subject Participation Card

The subject will be provided with a Subject Participation Card bearing the following information:

- That he/she is participating in a clinical trial (including trial code).
- That he/she is treated with microbiota suspension.
- The name and telephone number of the investigator.
- The name, address, and telephone number of Ferring contact (as required by local regulations).

The subject will be asked to keep the Subject Participation Card in his/her possession at all times during the trial.

Additionally, each subject's primary care physician will be notified of their participation in the trial by the investigator, if the subject agrees and if applicable.

14.7 Compliance Reference Documents

The Declarations of Helsinki and Taipei, the consolidated ICH-GCP, and other national law(s) in the US shall constitute the main reference guidelines for ethical and regulatory conduct.

15 LIABILITIES AND INSURANCE

15.1 ICH-GCP Responsibilities

The responsibilities of Ferring, the monitor and the investigator are defined in the ICH-GCP consolidated guideline, and applicable regulatory requirements in the US. The investigator is responsible for adhering to the ICH-GCP responsibilities of investigators, including being responsible for all trial-related medical decisions, for dispensing the IMP in accordance with the approved protocol or an approved amendment, and for its secure storage and safe handling throughout the trial.

15.2 Liabilities and Insurance

Ferring is, as sponsor, responsible for ensuring appropriate general/product liability insurance and, as required in accordance with applicable laws and regulations, country-specific liability insurance coverage for claims made by a trial subject for injury arising from the subject's participation in the trial.

16 ARCHIVING

16.1 Investigator File

The investigator is responsible for maintaining all the records that enable the conduct of the trial at the site to be fully understood, and for maintaining a record of the location of their essential documents including source documents, in accordance with ICH-GCP. The trial documentation including all the relevant correspondence should be kept by the investigator for at least 15 years after the completion or discontinuation of the trial, if no further instructions are given by Ferring.

The investigator is responsible for the completion and maintenance of the confidential subject identification code which provides the sole link between named subject source records and pseudonymous EDC data for Ferring. The investigator must arrange for the retention of this Subject Identification Log and signed ICFs for at least 15 years after the completion or discontinuation of the trial.

No trial site document may be destroyed without prior written agreement between the investigator and Ferring. Should the investigator elect to assign the trial documents to another party, or move them to another location, Ferring must be notified. If the investigator retires and the documents can no longer be archived by the site, Ferring can arrange having the Investigator File archived at an external archive.

16.2 Trial Master File

Ferring will archive the Trial Master File in accordance with ICH-GCP and applicable regulatory requirements.

17 REFERENCES

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