A Phase 3 Open-Label Clinical Study to Evaluate the Safety and Tolerability of Rebiotix RBX2660 (microbiota suspension) in Subjects with Recurrent *Clostridium difficile* Infection

NCT03931941

Sponsor: Rebiotix Inc.
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Study Product: RBX2660 (microbiota suspension)

Protocol #: 2019-01

Version #: 7.0; 26MAY2022

1. Revision History and Sponsor Statement

Version	Date	Reason
1.0	14Jan2019	Initial Release
2.0	12Apr2019	Added 4-week assessment for second enema follow-up and clarified data collection elements.
3.0	22Jul2019	This protocol version is for submission to Health Canada and Canadian sites to address a request for incorporation of screening and testing procedures related to Health Canada's Safety Alert for Fecal Microbiota Therapy. This protocol version was not submitted to the FDA or US study sites.
4.0	06Sep2019	Aligned medical record documentation of recurrent CDI with current medical practice, and incorporation of the protocol clarification letter identifying Baseline stool sample testing of Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*.
5.0	15OCT2020	Increased the maximum number of subjects to be treated with RBX2660 to 500. This allows continued enrollment, open-label access, and prospective data collection in a broad patient population. Updated supporting clinical data to include final results from Phase 2 clinical studies of RBX2660.
6.0	08Dec2021	Increased the maximum number of subjects to be treated with RBX2660 to 600. Added final study results for Protocols 2017-01 and 2019-02
7.0	26MAY2022	Increased the maximum number of subjects to be treated with RBX2660 to 750. Clarified Other Endpoints analysis time points for Vancomycin Resistant Enterococcus

Sponsor Statement:

This protocol has been written in accordance with ICH E6 and the Rebiotix procedure governing clinical study protocol development.

[redacted]

26May2022

Date (DD/MMM/YYYY)

Rebiotix, Inc.

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2. Investigator's Acknowledgement of the Clinical Study Protocol

Study Name: A Phase 3 Open-Label Clinical Study to Evaluate the Safety and Tolerability of Rebiotix RBX2660 (microbiota suspension) in Subjects with Recurrent *Clostridium difficile* Infection	
Version Number: 7.0	**Version Date:** 26MAY2022
Investigational Site:	

I have read this protocol and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined herein and will complete the study within the time-frame designated.

I will provide copies of the protocol and all pertinent information to the individuals responsible to me who assist in the conduct of this study. I will discuss this material with them to ensure that they are fully informed regarding the conduct of the study and the study product prior to their conducting any study-specific activities.

Accepted by:

Principal Investigator's Signature *Date (DD/MMM/YYYY)*

Principal Investigator's Name

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3. List of Abbreviations and Acronyms

AE – adverse event
AIDS – Acquired Immunodeficiency Syndrome
ALT – alanine aminotransferase
AST – aspartate transaminase
BUN – blood urea nitrogen
CBC – complete blood count
CDAD – *Clostridium difficile*-associated diarrhea
CDI – Clostridium difficile infection
CFR – Code of Federal Regulations
CMP – comprehensive metabolic panel
CRE – Carbapenem-resistant *Enterobacteriaceae*
CRP – C-reactive protein
DSMB – Data and Safety Monitoring Board
EAC – Endpoint Adjudication Committee
eCRF – electronic case report form
ESBL – Extended spectrum beta-lactamase
FDA – Food and Drug Administration
FMT – Fecal microbiota transplant
FT – fecal transplant
GCPs – Good Clinical Practices
GDH – glutamate dehydrogenase
HIV – human immunodeficiency virus
IBD – inflammatory bowel disease
IBS – irritable bowel syndrome
ICF – informed consent form
ICH – International Council on Harmonisation
ICU – intensive care unit
IFU – Instructions for Use
IgG – Immunoglobulin
IP – Investigational product
IRB – Investigational Review Board
ISF – Investigator Site File
ITT – Intent-to-Treat
IV – intravenous
IVIG – intravenous immunoglobulin
MDRO – multi-drug resistant organism
mL – milliliter
MRSA – Methicillin-resistant *Staphylococcus aureus*
NA – not applicable
OTC – Over the counter
PAL – Product Accountability Log
PP – Per Protocol
rCDI – Recurrent *Clostridium diffcile* infection
REB – Research Ethics Board
SAE – serious adverse event

SAP – Statistical Analysis Plan
VRE – vancomycin-resistant *enterococci*

4. Study Synopsis

Title	A Phase 3 Open-Label Clinical Study to Evaluate the Safety and Tolerability of Rebiotix RBX2660 (microbiota suspension) in Subjects with Recurrent *Clostridium difficile* Infection.
Investigational Product	RBX2660 (microbiota suspension).
Indication for Use	RBX2660 is being studied for the prevention of recurrent *Clostridium difficile* infection (CDI) in individuals with prior recurrent CDI infection resolved following antibiotic treatment.
Study Purpose	The purpose of this study is to evaluate the safety and tolerability of RBX2660 in subjects with recurrent CDI who have had prior recurrent CDI that was resolved with antibiotic treatment.
Definition of Recurrent CDI for Study Entry	To be considered for enrollment, a subject must have medical record documentation of: a) a current diagnosis or history of recurrent CDI as determined by the treating physician, b) *or* has had at least two separate episodes of severe CDI resulting in hospitalization.
Definition of CDI Diarrhea On-Study	The definition of CDI diarrhea for use in enrolled subjects throughout the study (Baseline visit through month 6 visit) includes: 1. The passage of three or more unformed/loose stools (i.e., Bristol Stool Scale type 6-7) in 24 or fewer consecutive hours for at least two consecutive days; 2. *and* a positive stool test for the presence of *C. difficile* as determined by the central laboratory.
Objectives	Primary Objective: To evaluate the safety and tolerability of RBX2660 in subjects with recurrent CDI. Secondary Objectives: 1. To evaluate the efficacy of RBX2660 in preventing recurrent episodes of CDI through 8 weeks after treatment. 2. To evaluate the sustained clinical response rate of RBX2660 after treatment.

Endpoints	Primary Endpoint: Number of subjects with investigational product- and/or enema-related treatment-emergent adverse events (TEAEs). Secondary Endpoints: 1. Recurrence of CDI through 8 weeks after treatment. 2. Loss of sustained clinical response through 6 months after treatment. Safety Endpoints: 1. Number of adverse events per subject 2. Timing of attributable TEAEs 3. Duration of TEAEs 4. Relatedness of TEAEs 5. Severity of TEAEs 6. Causality of TEAEs to investigational product (IP), enema, *C. difficile* or prior condition 7. Number of each of the following CDI-related TEAEs through 8 weeks post-treatment: death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, and ICU admission. 8. Onset of new chronic conditions relative to RBX2660 treatment administration.
Study Design	This is an open-label Phase 3 study to evaluate the safety and tolerability of RBX2660 in subjects with recurrent CDI. Up to 750 subjects may be treated in this study. Consented subjects who meet all eligibility criteria are intended to be treated. Consented subjects who do not meet the eligibility criteria will be considered screen failures and will not count towards the total number of subjects treated. Potential subjects are expected to already be taking or have just been prescribed antibiotics to control recurrent CDI symptoms at the time of enrollment (Screening visit) per the investigator's standard of care. Patients who have already completed their prescribed course of antibiotics to treat recurrent CDI are not eligible for enrollment (Screening visit) unless they have another recurrence that is treated with antibiotics. Once the Screening visit is complete, in order to proceed to the Baseline Enema Administration: 1. Subject must meet all the inclusion and none of the exclusion criteria 2. Antibiotics will have been administered prior to the washout period

	3. CDI symptoms must be under control leading into the antibiotic washout period Control of CDI symptoms is defined as no longer meeting the symptomatic criteria for CDI diarrhea which is the passage of three or more unformed/loose (i.e., Bristol Stool Scale type 6-7) stools in 24 or fewer consecutive hours while taking antibiotics for the two consecutive days prior to the washout period. Thus, at a minimum the Subject Diary which is started at the Screening visit must show that symptoms are under control the two days prior to the washout period in order for the subject to receive the enema administration. The Baseline visit and administration of study treatment (enema administration) is to be completed as soon as possible, but no later than 21 days after the Screening visit. A minimum 24hr to a maximum of 72hr antibiotic washout period is required prior to administration of the study treatment. In-office study follow-up visits occur at weeks 1 and 8 after completing the Baseline visit. Telephone assessments occur at week 4 and at months 4 and 6 after the Baseline visit. Subjects are required to keep a detailed diary to assess for solicited events from the date of Screening visit to the 1-week follow-up visit. The diary will be collected and reviewed at the Baseline visit prior to enema administration. The subject will continue to complete the diary for one week following the Baseline visit (enema administration) and it will be collected and reviewed at the 1-week follow-up visit. Subjects may receive a second RBX2660 enema if they are deemed failures following treatment per the protocol-specified treatment failure definition. This second enema is to be administered within 21 calendar days of failure determination. If a subject receives a second RBX2660 enema, follow-up is completed via telephone assessments at 1-week, 4-weeks, 8-weeks, and 6-months after completing the second enema. A final analysis and report will be completed once the last subject has completed the 6-month follow-up visit for their study treatment, including 6-month follow-up after a second RBX2660 study treatment, if applicable.
Definition of Treatment Success	Treatment success is defined as: • The absence of CDI diarrhea for 8 weeks after completing a study treatment.

Definition of Sustained Clinical Response	Sustained Clinical Response is defined as: • Treatment success of the presenting CDI recurrence and no new CDI episodes for greater than 8 weeks through 6 months after completing a study treatment.
Definition of Treatment Failure (CDI recurrence)	Treatment failure (CDI recurrence) is defined as: • The presence of CDI diarrhea within 8 weeks of administration of a study enema, which includes a positive stool test for *C. difficile* as determined by the central laboratory. If a CDI recurrence is suspected, an in-office visit is required, and the subject must provide a fresh stool sample. A stool sample is to be sent to the central laboratory for analysis. In the case of suspected treatment failure where there is an immediate concern for subject safety, including the potential for serious adverse events, a test may be conducted at the site or other laboratory known to the site **in addition to** sending a sample to the central laboratory for testing. The central laboratory's testing result will be used for defining treatment failures, instead of the other laboratory's result.
Management of Treatment Failures	Following treatment, subjects meeting the protocol definition of treatment failure may be scheduled for administration of a second RBX2660 enema within 21 calendar days of failure determination. In order to receive the second RBX2660 enema, the site must provide documentation of meeting all treatment failure criteria as defined by the protocol. The use of antibiotics prior to a second RBX2660 enema is at the discretion of the investigator. If antibiotics are given to control symptoms, a 24-72 hour washout period prior to administration of a second RBX2660 enema is required. If elected, the second enema is to be administered within 21 calendar days of failure determination. If a subject receives a second RBX2660 enema, follow-up is completed via telephone assessments at 1-week, 4-weeks, 8-weeks and 6-months after the second RBX2660 treatment. Treatment failures who do not receive a second RBX2660 enema will continue to follow their original schedule of assessments through 6-month follow-up.
Concomitant Therapy	The subject must agree to not take any oral vancomycin, metronidazole, fidaxomicin, rifaximin, nitazoxanide, bezlotoxumab, or intravenous immunoglobulin (IVIG) through the 8-week follow-up assessment unless newly prescribed by a treating investigator during the course of the study

	as a result of a recurrent CDI diagnosis. Use of IVIG for treatment of a non-CDI indication is allowed.
Determination of Success/Failure: **(Investigator and EAC)**	The site investigator will make the initial determination of success or failure based on the pre-defined study definitions. The site investigator's assessment will then be provided to the Endpoint Adjudication Committee (EAC) for independent adjudication of treatment success or failure that will be utilized for study analysis and reporting purposes.
Medical Monitor	The Medical Monitor provides review of serious adverse events to assess accuracy of the reporting as related to seriousness and causality, and periodic review of adverse events for trends. The Medical Monitor's review will be provided to the Data and Safety Monitoring Board (DSMB) for analysis purposes and as applicable for the proper adjudication of potential study stopping rules.
Data and Safety Monitoring Board (DSMB)	The DSMB will review safety data for trends and may review safety analysis and the final analysis. Details regarding DSMB responsibilities related to analysis oversight, data reporting, and timing of meetings will be provided in the DSMB Charter.
Sample Size	The purpose of this study is to assess the safety and tolerability of RBX2660 in a recurrent CDI population that is broader and more inclusive than that included in prior studies using RBX2660. In order to continue to collect prospective safety information and allow open-label treatment access to a broad population of patients, up to 750 subjects will be treated with RBX2660. Subjects who have signed informed consent forms but do not meet all eligibility criteria will be considered screen failures. Subjects who withdraw for any reason prior to administration of the enema will be replaced without counting toward the sample size treatment cap of 750 subjects.
Statistical Considerations	The primary safety analysis will be performed on all subjects exposed to RBX2660 and will include descriptive statistics. Additional sub-analyses of safety data may be performed on sub-populations. Efficacy analyses will be performed on pre-defined analysis populations including Intent-to-Treat (ITT) and modified Intent-to-Treat (mITT). A final analysis of safety and efficacy results will be analyzed and reported in a final report after the last subject has completed the 6-month follow-up visit for their study treatment, including 6-month follow-up after a second RBX2660 study treatment, if applicable.

Study Stopping Rules	The DSMB will determine whether enrollment will be paused, the study terminated, or other action taken based on their assessment that: • There is probable cause that the IP or enema procedure (e.g., due to transfer from an RBX2660 donor) contributed to a new pathogenic intestinal infection in the stool of any subject; or • Any events of major significance such as death or other serious outcome for which a causal connection with the IP is plausible and represents an excess of the important adverse event(s). In the event that one of the preceding rules appears to be met based on site reported data and/or Medical Monitor review, the Rebiotix Operations Department will review the donor history and batch processing/release records for the product unit(s) administered in these cases. A report of the review will be forwarded to the DSMB Chair for review. If probable cause is suspected, the DSMB Chair will convene the entire DSMB for event review. Upon the DSMB's determination of action, the Chair will notify Rebiotix, who will notify the study sites. If enrollment is stopped, Rebiotix and the study sites will assess for the occurrence of similar events and evaluate IP and study records for possible root cause(s). Enrollment may be re-started upon further review and approval from the DSMB. As appropriate, the DSMB may recommend study termination or measures short of termination with the objective of reducing the risk of adverse events. Details regarding DSMB responsibilities will be provided in the DSMB Charter.
Inclusion Criteria	1. ≥ 18 years old. 2. Medical record documentation of either: a) a current diagnosis or history of recurrent CDI as determined by the treating physician, b) *or* has had at least two episodes of severe CDI resulting in hospitalization. 3. Is currently taking or was just prescribed antibiotics to control CDI related diarrhea at the time of enrollment. *Note: Subject's CDI diarrhea must be controlled (<3 unformed/loose stools/day, i.e., Bristol Stool Scale type 6-7, for the two consecutive days prior to the washout period) while taking antibiotics during screening.* 4. Willing and able to have an enema(s). 5. Willing and able to complete the stool and blood testing required for the study.

	6. Agrees to not take non-dietary probiotics from Screening through 8 weeks after receiving the last study enema (including OTC and prescription). 7. Agrees to not take any oral vancomycin, metronidazole, fidaxomicin, rifaximin, nitazoxanide, bezlotoxumab, or IVIG through the 8-week follow-up assessment unless newly prescribed by a treating investigator during the course of the study as a result of recurrent CDI diagnosis. *Note: Use of IVIG for treatment of a non-CDI indication is allowed.* 8. Agrees to practice a form of effective contraception during study participation; does not apply to persons with documented non-child-bearing potential. 9. Has a negative urine pregnancy test at the time of enrollment and on the day of each enema prior to administration (females of child-bearing potential only). 10. Willing and able to provide informed consent, and local privacy authorization as applicable. 11. Willing and able to complete the required Subject Diary. 12. Willing and able to meet all study requirements, including attending all assessment visits and telephone calls.
Exclusion Criteria	1. A known history of refractory CDI. 2. Has continued CDI diarrhea despite being on a course of antibiotics prescribed for CDI treatment. 3. Requires systemic antibiotic therapy for a condition other than CDI. 4. Patient previously participated in a Rebiotix clinical study receiving RBX2660. 5. Fecal microbiota transplant (FMT) within the past 6 months. 6. FMT with an associated serious adverse event related to the FMT product or procedure. 7. Bezlotoxumab (CDI monoclonal antibodies) if received within the last year. 8. Disease symptoms (e.g., diarrhea) caused by a confirmed intestinal pathogen other than *C. difficile*. 9. Have a current colostomy. 10. Intraabdominal surgery within the last 60 days. *Note: laparoscopic procedures that do not involve the GI tract are permitted*. 11. Planned surgery requiring perioperative antibiotics through the 8-week follow-up assessment. 12. Life expectancy of < 6 months. 13. CD4 count <200/mm^3 during Screening.

	14. An absolute neutrophil count of <1000 cells/μL during Screening. 15. Known or suspected current (< 90 days) illicit drug use. *Note: marijuana use is allowed*. 16. Pregnant, breastfeeding, or intends to become pregnant during study participation. 17. Participating in a clinical study of another investigational product (drug, device or other) and has not completed the required follow-up period. 18. Subject is eligible for another RBX2660 study. 19. Subject, in the opinion of the investigator, for whatever reason, should be excluded from the study.
Study Sites	Approximately 80 US and Canadian sites will participate in this study.
Study Sponsor	Rebiotix Inc. 2660 Patton Road Roseville MN 55113 USA www.rebiotix.com 651-705-8770
Contract Research Organization	Medpace, Inc. [redacted]
Central Laboratory	Medpace Central Labs LLC [redacted]

5. Background

RBX2660 (microbiota suspension) is an intestinal microbial suspension prepared from human stool obtained from carefully and thoroughly screened healthy human donors. It is being studied for the prevention of recurrent *Clostridium difficile* infection (CDI). RBX2660 is prepared from a standardized amount of stool mixed with saline and a cryoprotectant. It is stored at the manufacturer in a frozen state and is shipped frozen to the clinical site for administration via enema.

The donor selection process is multifaceted in its original screening of candidate donors as well as the ongoing assessment of donated samples prior to release for use in a clinical study. Some of these measures include the following:

1. Donor qualification screening includes questions that specifically address risk factors for colonization with multi-drug resistant organisms (MDROs), and individuals at higher risk of colonization with MDROs are excluded from donation. Examples of persons at higher risk for colonization with MDROs include:
    a. Health care workers
    b. Persons who have recently been hospitalized or discharged from long term care facilities
    c. Persons who regularly attend outpatient medical or surgical clinics
    d. Persons who have recently engaged in medical tourism
2. Donor stool testing includes MDRO testing to exclude use of stool that tests positive for MDRO. The MDRO tests include at minimum extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant *enterococci* (VRE), carbapenem-resistant *Enterobacteriaceae* (CRE), and methicillin-resistant *Staphylococcus aureus* (MRSA).
3. All drug product currently in storage has undergone screening and stool testing for MDROs as described above.
4. The informed consent process for subjects being treated with RBX2660 product as part of this study describes the risks of MDRO transmission and invasive infection as well as the measures implemented for donor screening and stool testing.

Rebiotix has concluded the first clinical study of RBX2660 (n=34): an open-label, non-controlled Phase 2 study (Protocol 2013-001) demonstrating the safety of the product. Rebiotix has also completed enrollment and interim reports for two additional Phase 2 clinical studies. Protocol 2014-01 is a prospective, multicenter, randomized, double-blinded, placebo-controlled, 3-arm Phase 2 study (n=127 treated) designed to demonstrate the efficacy and safety of RBX2660 for the prevention of recurrent CDI. Primarily, this Phase 2 study compared the efficacy of two enemas of RBX2660 to two enemas of placebo, and secondarily compared the efficacy of two enemas of RBX2660,

and one enema of RBX2660 + one enema of placebo to two enemas of placebo. The study has been completed. Protocol 2015-01 is an open label study comparing the efficacy and safety of two planned RBX2660 enemas (n=136 treated) to a historical control (n=110). This study is currently in long-term follow-up. Rebiotix is conducting a Phase 3 study (Protocol 2017-01) with expected treatment of up to 270 subjects. The purpose of Protocol 2017-01 is to confirm the efficacy and safety of RBX2660 for the prevention of recurrent CDI in subjects who have had prior recurrent CDI that was resolved with antibiotic treatment.

Finally, to complete the RBX2660 clinical development program, Rebiotix is initiating this Phase 3 study (Protocol 2019-01) to assess the safety and tolerability of RBX2660 in a recurrent CDI population that is broader and more inclusive than that included in prior studies evaluating RBX2660. In order to continue to collect prospective safety information and allow open-label treatment access to a broad patient population, up to 750 subjects will be treated with RBX2660.

The study will be conducted in compliance with this protocol as approved by the federal Food and Drug Administration and each site's governing Institutional Review Board or Research Ethics Board (Canada); in accordance with relevant regulations in 21 CFR Parts 11, 50, 54, 56 and 312; and the ICH E6 Good Clinical Practice: Consolidated Guidance.

5.1. Investigational Agent

RBX2660 (microbiota suspension) 50g/150mL is supplied in an enema bag within a brown opaque sleeve. Each bag contains one dose. Each dose contains a minimum of 10^7 microbes/mL of suspension in a saline/polyethylene glycol 3350 vehicle.

5.2. Preclinical Data

Fecal transplantation is being used today in many highly respected hospitals throughout the world with no significant reports of adverse events or other safety-related complications. Rebiotix conducted an extensive literature review beginning with the first published cases of fecal transplantation in 1958 (Eiseman, 1958). The literature presented significant evidence of the safe and effective use of fecal transplant (FT) for over 500 cases of recurrent CDI with no product-related adverse events reported to date and only a few procedure-related events, none of which occurred in subjects receiving FT via enema (Bakken, 2015). Based on the significant human clinical experience documented in the literature, it has been determined that preclinical studies are not warranted.

5.3. Clinical Data to Date

5.3.1. Phase 2 Study Protocol 2013-001 Final Data

On July 12, 2013, Rebiotix received permission from FDA to proceed with the Phase 2 clinical study entitled "A Phase 2 Open-label Clinical Trial Demonstrating the Safety of RBX2660 Microbiota Suspension for the Treatment of Recurrent *Clostridium difficile*-associated Diarrhea." This study ended with the last subject's 6-month assessment in July 2014.

It was a prospective, multi-center, open-label, non-controlled Phase 2 study designed to demonstrate the safety of RBX2660 (50 g/150 mL) for the treatment of recurrent CDI. The target population was adults (≥ 18 years old) with recurrent CDI who had either a) at least two recurrences after a primary episode (primary episode + ≥ two recurrences, i.e., at least three episodes) and had completed at least two rounds of standard-of-care oral antibiotic therapy or b) had at least two episodes of severe CDI resulting in hospitalization. Protocol 2013-001 was the first study assessing the safety of RBX2660, although similar fecal transplant products have been used in humans for many years, as discussed in the literature. The study's primary objective was to assess the safety of RBX2660, but additional information was gathered on efficacy and quality of life. Eligible subjects who proceeded to treatment received one treatment with RBX2660. If their CDAD returned before Day 56 after receiving one treatment, they were eligible to receive a second treatment if the re-treatment occurred within ten days of recurrence.

Follow-up visits occurred at Days 7, 30 and 60, and subjects remained in the study for telephone assessments for adverse events at 3 and 6 months. If a subject received a second treatment, the follow-up schedule was re-set, so that they came in for visits at 7, 30 and 60 days after the second treatment and receive 3- and 6-month telephone assessments. Subjects also kept a written diary for the first 60 days after each treatment with RBX2660, including the collection of detailed information on solicited adverse events for the first eight days after receiving an RBX2660 enema.

A total of 40 subjects were enrolled in this study. Of these, six did not proceed to receive a treatment with RBX2660 (screen failures). Of the 34 treated subjects, two subjects terminated their participation in the study around their respective 7-day visit. One subject died 35 days after receiving their second treatment with RBX2660 from respiratory failure, which was not related to RBX2660 or the administration procedure. All subjects completed the study (6-month telephone assessment after their last treatment with RBX2660).

Efficacy Results

The primary efficacy parameter of this study was treatment success, defined as the absence of CDAD (passage of three or more unformed stools in 24 or fewer consecutive

hours for at least two consecutive days) at 56 days after the last treatment with RBX2660. Of the 34 subjects who received at least one treatment with RBX2660, 31 subjects were presented in the efficacy analysis (31 had efficacy data available). Twenty-seven (27) out of 31 subjects, or 87.1%, were considered a treatment success. Sixteen (16) subjects or 50.0% (N=16/32) were considered a treatment success after their first treatment with RBX2660. Of the 16 subjects who failed their first treatment, 15 subjects proceeded to receive a second enema of RBX2660. Of those, 11 subjects or 78.6% (N=11/14) were considered a treatment success (one subject was not counted because she did not reach the 56-day endpoint). The reasons for the increased rate of success after the second enema were not fully understood. It may be due to a number of factors, including changes in the subject's microbiome after one treatment, complete elimination of vancomycin from the subject's stool over time, or other reasons not yet determined.

Safety Results

The subjects enrolled in Protocol 2013-001 had significant pre-existing comorbidities. Even with their recurrent CDI symptoms temporarily controlled by standard-of-care antibiotic therapy, they remained very sick people. The incidence, severity, and seriousness of the adverse events observed in this study represented the first attempt to systematically and rigorously collect, and consistently analyze and characterize them in a subject group suffering from recurrent CDI who were treated with an intestinal microbiota fecal transplant product. There were 188 adverse events reported by 28 subjects, which were gathered through the very rigorous solicitation of events. The adverse events reported were mostly mild-to-moderate in severity and were primarily related to gastrointestinal disorders as was expected in this population. Most resolved within the 7-day interval after RBX2660 administration. This was true whether the subject received one or two treatments with RBX2660.

Of the reported solicited events, which were collected during the 8-day period inclusive of the day of dosing and the subsequent 7-day follow-up period, the incidence and severity of these events declined over the 8-day period. The proportion of subjects reporting solicited AEs was lower on Day 7 in subjects who received two treatments (41.7%; N=5/12) with RBX2660 than in subjects who received one treatment (51.5%; N=17/33), yet the efficacy rate after two treatments rose from 50% to 78.6% after one treatment. There were seven subjects who experienced a total of 20 SAEs, only one of which had a possible relationship to RBX2660; most were probably or definitely related to a pre-existing condition, including CDI. Overall, adverse events declined over time; 66.5% of AEs occurred from Baseline through the 7-day visit. This was the first known study to prospectively, systematically, consistently and aggressively solicit, record, and classify adverse events in subjects who received an intestinal microbiota fecal transplant product. It was therefore expected that the incidence rate of reported AEs might be higher than that reported in the literature, but it represented the true rate and types of

events experienced by this population after treatment with an intestinal microbiota fecal transplant product.

Figure 5-1 shows the decline in the proportion of subjects reporting solicited adverse events over the 8 days from the day of dosing (Day 0) to the seventh day after dosing (Day 7).

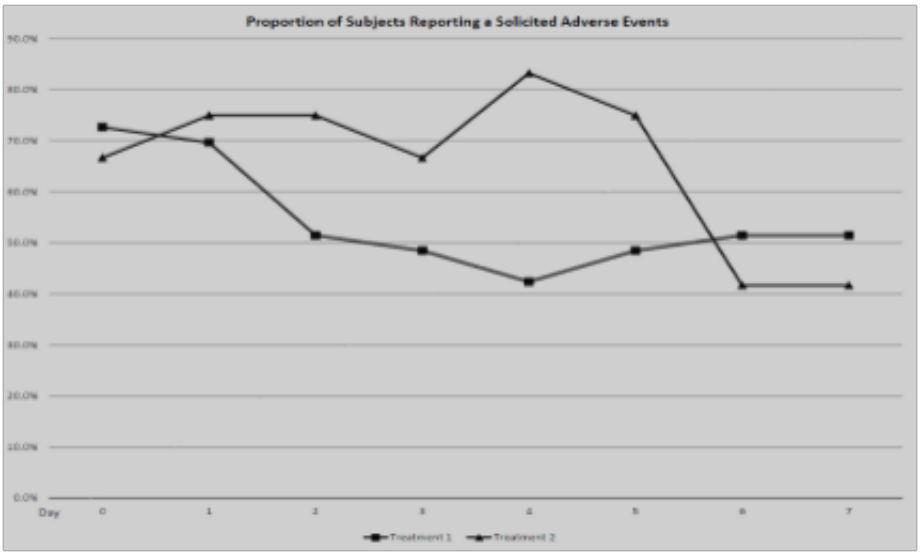

Figure 5-1: Proportion of Subjects Reporting Solicited AEs from Day 0-Day 7

Conclusions

The RBX2660 treatment success rate of 87.1% represented a significant improvement over reported success rates for oral antibiotics and was within the range of results reported in the literature for similar fecal microbiota products. This was the first known study to prospectively, systematically, consistently and aggressively solicit, record, and classify adverse events in subjects who received an intestinal microbiota fecal transplant product. It was therefore expected that the incidence rate of reported AEs (188 events in 28 subjects) might be higher than that reported in the literature, but it represented the true rate and types of events experienced by this population receiving this therapy. The incidence rate and type of adverse events were not unexpected, given the high number of comorbidities that existed in this very sick, primarily elderly population. None of the SAEs were determined to be probably or definitely related to RBX2660 and most were related to a pre-existing condition, primarily *C. difficile* infection. The success rate increased after two treatments with RBX2660, yet the proportion of subjects reporting solicited AEs was lower on Day 7 in subjects who received two treatments than in subjects who received one treatment. The efficacy benefit of RBX2660 outweighed the incidence and type of adverse events, especially the risk of recurrent CDI, thus providing a satisfactory benefit-to-risk ratio.

5.3.2. Phase 2 Study Protocol 2014-01 Final Study Data

Study Design

Study Protocol 2014-01 was a prospective, multicenter, randomized, double-blinded, placebo-controlled, 3-arm Phase 2B study demonstrating the efficacy and safety of RBX2660 for the treatment of recurrent CDI (rCDI). The target population was adults (≥ 18 years old) with rCDI who had either a) at least two recurrences after a primary episode (i.e., at least three episodes) and had completed at least two rounds of standard-of-care oral antibiotic therapy or b) had at least two episodes of severe CDI resulting in hospitalization. Randomization was at a 1:1:1 ratio to one of the three groups: Group A (2 enemas of RBX2660), Group B (2 enemas of placebo), and Group C (1 enema of RBX2660 and 1 enema of placebo). One complete study treatment consisted of two enemas administered 7 ± 2 days apart; the second enema could be administered sooner if CDI diarrhea (passage of ≥ 3 unformed stools in ≤ 24 consecutive hours for at least two consecutive days) recurred in less than 7 days.

Efficacy Results

A total of 150 subjects were enrolled in the study and 133 were randomized (Intent-to-Treat, ITT, N=45 in Group A, N=44 in Group B, and N=44 in Group C) of which five (5) subjects were withdrawn prior to treatment. In total, 128 randomized subjects were exposed to blinded enema (Safety Population, SP), with 127 subjects successfully completing at least one blinded enema.

The primary efficacy parameter of this study was Treatment Success, defined as the absence of CDAD (passage of three or more unformed stools in 24 or fewer consecutive hours for at least two consecutive days) at 56 days after the last treatment with RBX2660. The efficacy objectives of the study were analyzed using all three analysis populations: ITT, modified ITT, and Per Protocol (PP).

The primary efficacy objective of Group A as compared to Group B was not met, however, a sensitivity analysis of a secondary efficacy objective using the PP population comparing Group C to Group B did achieve statistical significance (p=0.017). A comparison of the active RBX2660 treatment Groups A and C demonstrated no statistical difference indicating that there was no efficacy difference between one or two enemas of RBX2660.

Safety Results

Adverse events were collected for each subject by the investigational sites and solicited via a subject diary from the point of enrollment (information consent) through study exit. Safety results were analyzed using the SP, which consisted of any subject who had an attempted study drug administration (N=128), whether placebo or RBX2660. There

were 907 AEs reported by 106 subjects. Of those AEs, 841 events in 105 subjects occurred after administration of study IP and are termed treatment emergent adverse events (TEAEs). The TEAEs reported were mostly mild-to-moderate in severity (87.6%) and were primarily related to gastrointestinal disorders (32.5%) as would be expected in this population.

Overall Results

These results indicate that one or two doses of RBX2660 is a safe and potentially effective therapy for rCDI.

5.3.3. Phase 2 Study Protocol 2015-01 Final Study Data

Study Design

Study protocol 2015-01 was a prospective, multicenter, open-label Phase 2 study that assessed the efficacy and safety of RBX2660 as an adjunct to antibiotics for the treatment of rCDI, as compared to a historical control population of subjects treated with standard-of-care antibiotic therapy. The study population was adults (≥ 18 years old) with rCDI who had either: a) at least two recurrences after a primary episode and had completed at least two rounds of standard-of-care oral antibiotic therapy, or b) at least two episodes of severe CDI resulting in hospitalization.

The primary efficacy objective of Treatment Success for the study was measured by the recurrence-free rate of CDI diarrhea without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant through 56 days after completion of study treatment with RBX2660. One treatment consisted of 2 enemas of RBX2660 7 ± 2 days apart. Subjects were expected to participate in the study for approximately 25-27 months: 1 month enrollment; 8 weeks efficacy follow-up; and an additional 22 months for safety follow-up.

A total of 272 subjects enrolled in the study, including 110 subjects in the Historical Control arm, 13 subjects enrolled but not treated, and 149 subjects received at least one (1) enema (Safety Population, SP). In total, 217 subjects were evaluable for treatment outcome and followed for at least 56 days (day 49 – 63) after the last treatment (Evaluable Population).

Efficacy Results

The efficacy objectives of the study were analyzed using RBX2660 treated subjects (n=142) who received at least one enema and were evaluable for a treatment outcome. This arm was compared to a closely matched Historical Control arm (n=75) chosen from a retrospective chart review of subjects treated with antibiotics for rCDI who matched key eligibility criteria and had an evaluable treatment outcome. The primary endpoint,

proportion of subjects with Treatment Success, was significantly greater in the RBX2660 arm (78.9%) as compared with the Historical Control arm (30.7%; p<0.0001; Pearson's chi-square test, Evaluable Population).

Safety Results

A total of 879 adverse events were reported in 124 subjects (83.2% of subjects) in the RBX2660 treatment arm. A similar total and percentage of adverse events were documented in the Historical Control arm (n=767 events in 71 subjects; 68.3% of subjects). In the RBX2660 arm, 805 treatment-emergent adverse events (TEAEs) were reported in 123 subjects (82.6% of subjects). The TEAEs reported were mostly mild-to-moderate in severity (84.1%), and the most common TEAEs overall were diarrhoea, abdominal pain and urinary tract infections in descending order. In the Historical Control arm, 665 TEAEs were reported in 67 subjects. Severity was not collected for events in the Historical Control arm. The most common TEAEs overall were urinary tract infections, back pain, diarrhoea, and oedema peripheral in descending order.

In the RBX2660 treated arm, 208 serious TEAEs were reported in 52 subjects (25.8% of all TEAEs). In the Historical Control arm, 83 serious TEAEs were reported for 30 subjects (12.5%). AEs resulting in Death were recorded for 15 RBX2660 treated subjects (10.1%), one of which was reported as possibly related to IP or enema procedure and definitely related to *C. difficile* disease and pre-existing condition(s). Death was reported for five of the subjects in the Historical Control arm (4.8%).

Overall Results

With this study, the efficacy results for RBX2660 are demonstrated to be repeatable, and the safety profile is acceptable for this patient population. The efficacy and safety observed in this study demonstrate that RBX2660 has an appropriate benefit-risk assessment as a treatment for rCDI.

5.3.4. Phase 3 Study Protocol 2017-01 Final Study Data

Study Design:

Study Protocol 2017-01 was a prospective, multi-center, double-blind, placebo-controlled Phase 3 study to evaluate the efficacy and safety of RBX2660 for the prevention of rCDI in 267 subjects randomized and treated in a 2:1 ratio to receive RBX2660 (Group B) or Placebo (Group A). The primary endpoint was the recurrence of CDI within 8 weeks of blinded treatment. The secondary endpoint was the loss of sustained clinical response through 6 months after blinded treatment. Safety endpoints included the number of AEs per subject, timing of attributable TEAEs, relatedness and severity of TEAEs.

Efficacy Results:

In total, 262 subjects had adjudicated outcomes for the primary endpoint in the mITT population. The primary statistical analysis used a Bayesian hierarchical model which formally incorporated data from a previous randomized Phase 2B study (Protocol 2014-01). RBX2660 demonstrated a 98.6% probability of superiority compared to Placebo in the prevention of CDI recurrence, with a success rate of 70.4% vs 58.1% through 8 weeks of blinded treatment. The posterior probability of superiority (0.986354) exceeded the threshold of 0.9750338 that controlled the Type I error rate at one-sided 0.025.

Sustained Clinical Response (Treatment Success at 8 weeks and no CDI recurrence for 6 months) was 90.6% (48/53) and 92.1% (116/126) in the Placebo and RBX2660 groups, respectively. Although there was no statistically significant difference between the two groups, the separation between RBX2660 and Placebo observed at 8 weeks was durable through 6 months.

Safety Results:

Of the 267 subjects in the safety population, 178 subjects (66.7%) reported a total of 708 TEAEs throughout the duration of the study; 478 TEAEs were reported by 154 (57.7%) subjects within 8-weeks of either treatment and 230 TEAEs were reported by 84 (31.5%) subjects more than 8-weeks after either treatment. More than a half (56.6%, 401/708) of TEAEs reported throughout the study occurred within 8-weeks of blinded treatment, which included solicited AEs collected during follow-up visits and telephone assessments and through Subject Diaries.

When assessed by maximum severity per subject, the majority of TEAEs (317/368, 86.1%), reported in 147 subjects (82.6%), were assessed as mild-to-moderate, 12.5% (46/368) TEAEs were severe, and 1.4 % (5/368) TEAEs were potentially life-threatening. The most frequently occurring TEAEs (256/708, 36.2%) were related to gastrointestinal disorders as would be expected in this population. Of the 54 serious TEAEs reported throughout the study, as assessed by investigator, none (0) were related to IP, none (0) were related to enema procedure, 16 (29.6%) events were related to *C. difficile* disease, and 40 (74.1%) events were related to a pre-existing condition. Of serious TEAEs that occurred within 8 weeks of the blinded period, 20 events occurred in 13 (4.9%) of subjects. Death was reported in 2 subjects, none (0) were reported as related to IP or enema procedure; both deaths were the result of AEs related to a pre-existing condition (one fatal event of multimorbidity; one fatal event of cardio-respiratory arrest).

Overall Conclusions:

Overall, the primary efficacy endpoint of study 2017-01 met the pre-specified success criteria and demonstrated clinically meaningful results. The efficacy outcomes demonstrated that RBX2660 was superior to Placebo at reducing recurrence of CDI through 8 weeks after completing blinded treatment, response was durable through 6 months, and the safety profile is acceptable for this patient population. In summary, RBX2660 has demonstrated a positive benefit-risk profile for the reduction of CDI recurrence in adult patients following antibiotic therapy for rCDI.

5.3.5. Retrospective Study Protocol 2019-02 Final Study Data

Study Design:

Study Protocol 2019-02 was a retrospective, multicenter, safety and tolerability study of RBX2660 administered under Enforcement Discretion for the prevention of rCDI. The study was intended to obtain safety data from up to 200 patients who received RBX2660 under Enforcement Discretion during a defined period. The primary endpoint was the number of subjects with IP- and/or enema-related TEAEs. The secondary objectives were to evaluate efficacy of RBX2660 in preventing recurrent episodes of CDI through 8 weeks after treatment and loss of Sustained Clinical Response rate of RBX2660 through 6 months after treatment.

Efficacy Results:

In the Primary Safety Set population, Treatment Success through 8 weeks after the last dose of RBX2660 was achieved by 53 (82.8%) subjects, and 47 (88.7%) subjects had a Sustained Clinical Response through 6 months after the last dose of RBX2660.

Safety Results:

TEAEs were considered related to RBX2660 for 11 (17.2%) subjects and related to the enema procedure for 3 (4.7%) subjects. In addition, there were 2 deaths reported during the study analysis period which were considered not related to RBX2660 or the enema procedure. SAEs were experienced by 8 (12.5%) subjects and all SAEs were considered not related to RBX2660 or the enema procedure.

Overall Results:

RBX2660 was well tolerated and efficacious in this patient population administered RBX2660 under Enforcement Discretion. As described, Treatment Success through 8 weeks after the last dose of RBX2660 was achieved by 53 (82.8%) subjects; of these subjects, 47 (88.7%) had a Sustained Clinical Response through 6 months after the last dose of RBX2660. These efficacy outcomes were comparable to the outcomes observed in previous, prospective, controlled clinical studies of RBX2660. RBX2660 was well

tolerated, with few TEAEs attributed to RBX2660 or the enema procedure. The safety profile was characteristic of subjects with recurrent CDI and comparable to the safety profiles observed in previous clinical studies of RBX2660, suggesting that treatment with RBX2660 for recurrent CDI is appropriate for the broader CDI population.

5.4. Treatment Rationale and Risk/Benefit

RBX2660 microbiota suspension was developed after careful, controlled component and process testing performed by Rebiotix using applicable scientific methods. RBX2660 (microbiota suspension) 50 g/150 mL is supplied in an ethylene vinyl acetate enema bag. Each bag of RBX2660 contains one enema; each enema of RBX2660 consists of a suspension of minimum 10^7 live organisms/mL in polyethylene glycol 3350/0.9% Sodium Chloride Irrigation, USP, solution to ensure a consistent product is delivered. The enema route was chosen over other potential routes of administration because the risk of procedural complications for the subject is low and the method is easy to perform and requires no special equipment. In addition, the enema route for fecal transplants is routinely used and has high efficacy rates reported in the literature with no reports of safety issues.

Recurrent CDI often results in severe and serious consequences. An assessment of the risk associated with recurrent CDI and the benefit of preventing recurrent episodes by administering multiple enemas of RBX2660 supports the conclusion that the risk of the predominately mild to moderate AEs experienced by subjects in the Phase 2 studies is outweighed by the potential benefit. This study will assess the safety and tolerability of RBX2660 in a recurrent CDI population that is broader and more inclusive than that included in prior studies using RBX2660. Data supporting the use of FMT procedures to prevent CDI in patient populations similar to those eligible for this study is limited. Kelly (2014) reported 12-week safety and efficacy of FMT in 80 immunocompromised patients: solid organ transplants (19), HIV/AIDS (3), cancer (7), inflammatory bowel disease (IBD, 36), and other chronic medical conditions (15). Of note, the IBD patients did not experience a higher incidence of AEs (14%) or SAEs (11%) compared to other immunocompromised patients (16% and 18%, respectively; $p \leq 0.3224$). Three patients with IBD (14%) experienced disease exacerbation post FMT, noting that their colonic disease activity leading up to FMT was severe. The rates of AEs and SAEs were comparable to those reported in the Rebiotix studies where subjects were followed well beyond 12-weeks. The treatment success rate, with 12 patients receiving two FMTs, was 89% demonstrating that the benefit of treatment outweighed the risk.

Though the risk profile for the broader eligible patient population has not been well characterized in multiple, controlled clinical studies, the body of safety and efficacy evidence for RBX2660 and other FMT procedures, along with the rigor of Rebiotix's donor screening process, and implementation of quality assurance testing at all stages of

manufacturing supports a favorable benefit-to-risk profile for the use of RBX2660 in the prevention of recurrent CDI.

6. Study Objectives and Endpoints

6.1. Primary Objective

To evaluate the safety and tolerability of RBX2660 in subjects with recurrent CDI.

6.2. Secondary Objectives

1. To evaluate the efficacy of RBX2660 in preventing recurrent episodes of CDI through 8 weeks after treatment.
2. To evaluate the sustained clinical response rate of RBX2660 after treatment.

6.3. Other Objectives

1. To evaluate health-related quality of life in subjects with CDI as measured by the Cdiff32 questionnaire.
2. To identify Baseline characteristics predictive of efficacy outcomes.
3. To characterize the changes from the Baseline fecal microbial composition at each timepoint.
4. To evaluate the efficacy of a second dose of RBX2660 in preventing CDI recurrence.
5. To assess the clearance of vancomycin resistant enterococcus in subjects who are carriers at Baseline.
6. To assess the clearance of *C. difficile* following enema treatment at 1-week, 4-weeks, 8-weeks, 4-months, and 6-months after study treatment in subjects receiving RBX2660.

6.4. Primary Endpoint

Number of subjects with investigational product- and/or enema-related treatment-emergent adverse events (TEAEs).

6.5. Secondary Endpoints

1. Recurrence of CDI within 8 weeks of treatment.
2. Loss of sustained clinical response through 6 months after treatment.

6.6. Safety Endpoints

1. Number of adverse events per subject

2. Timing of attributable TEAEs
3. Duration of TEAEs
4. Relatedness of TEAEs
5. Severity of TEAEs
6. Causality of TEAEs to IP, enema, *C. difficile* or prior condition
7. Number of each of the following CDI-related TEAEs through 8-weeks post treatment: death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, and ICU admission
8. Onset of new chronic conditions relative to treatment administration

6.7. Other Endpoints

1. Baseline characteristics: sex, age, number of prior CDI events, race, ethnicity
2. Subject fecal microbial composition at Screening, 1-week, 4-weeks, 8-weeks, 4 months, and 6 months after study treatment
3. Cdiff32 questionnaire at Screening, 1-week, 8-weeks, 4 months, and 6 months
4. Recurrence of CDI within 8 weeks of a second RBX2660 treatment
5. Concentration of vancomycin resistant enterococcus in stool samples at Screening, 1-week, 4-weeks, 8-weeks, 4 months, and 6 months after study treatment
6. Presence of *C. difficile* in stool samples at Screening, 1-week, 4-weeks, 8-weeks, 4 months, and 6 months after study treatment

7. Study Design

7.1. Study Purpose

The purpose of this study is to assess the safety and tolerability of RBX2660 in subjects with recurrent CDI who have had prior recurrent CDI that was resolved with antibiotic treatment.

7.2. General Study Design

This is an open-label, Phase 3 study to evaluate the safety and tolerability of RBX2660 in subjects with recurrent CDI.

Up to 750 subjects may be treated in this study. Consented subjects who meet all eligibility criteria are intended to be treated. Consented subjects who do not meet the eligibility criteria will be considered screen failures and will not count towards the total number of subjects treated.

Potential subjects are expected to already be taking or have just been prescribed antibiotics to control recurrent CDI symptoms at the time of enrollment (Screening visit)

per the investigator's standard of care. Patients who have already completed their prescribed course of antibiotics to treat recurrent CDI are not eligible for enrollment (Screening visit) unless they have another recurrence that is treated with antibiotics.

Once the Screening visit is complete, in order to proceed to the Baseline Enema Administration:

1. Subject must meet all the inclusion and none of the exclusion criteria
2. Antibiotics will have been administered prior to the washout period
3. CDI symptoms must be under control leading into the antibiotic washout period (**Section 7.3.1**)

Control of CDI symptoms is defined as no longer meeting the symptomatic criteria for CDI diarrhea which is the passage of three or more unformed/loose (i.e., Bristol Stool Scale type 6-7) stools in 24 or fewer consecutive hours while taking antibiotics for the two consecutive days prior to the washout period. Thus, at a minimum the Subject Diary which is started at the Screening visit must show that symptoms are under control the two days prior to the washout period in order for the subject to receive the enema administration.

Study treatment (enema administration) is to be completed as soon as possible, but no later than 21 days after the Screening visit. A minimum of 24hr to a maximum of 72hr antibiotic washout period is required prior to administration of the study treatment.

In-office study follow-up visits will occur at weeks 1 and 8 after completing the Baseline visit. Telephone assessments will occur at week 4 and at months 4 and 6 after the Baseline visit. Subjects are required to keep a detailed diary to assess for solicited events from the date of Screening visit to the 1-week follow-up visit. The diary will be collected and reviewed at the Baseline visit prior to enema administration. The subject will continue to complete the diary for one week following the Baseline visit (enema administration) and it will be collected and reviewed at the 1-week follow-up visit.

Subjects may receive a second RBX2660 enema if they are deemed failures following treatment per the protocol-specified treatment failure definition (**Section 7.3.6**). This second enema is to be administered within 21 calendar days of failure determination. If a subject receives a second RBX2660 enema, the follow-up is completed via telephone assessments at 1-week, 8-weeks, and 6-months after completing the second enema. A final analysis and report will be completed once the last subject has completed the 6-month follow-up visit for their study treatment, including 6-month follow-up after a second RBX2660 study treatment, if applicable.

See **Figure 7-1** for an illustration of the Study Design.

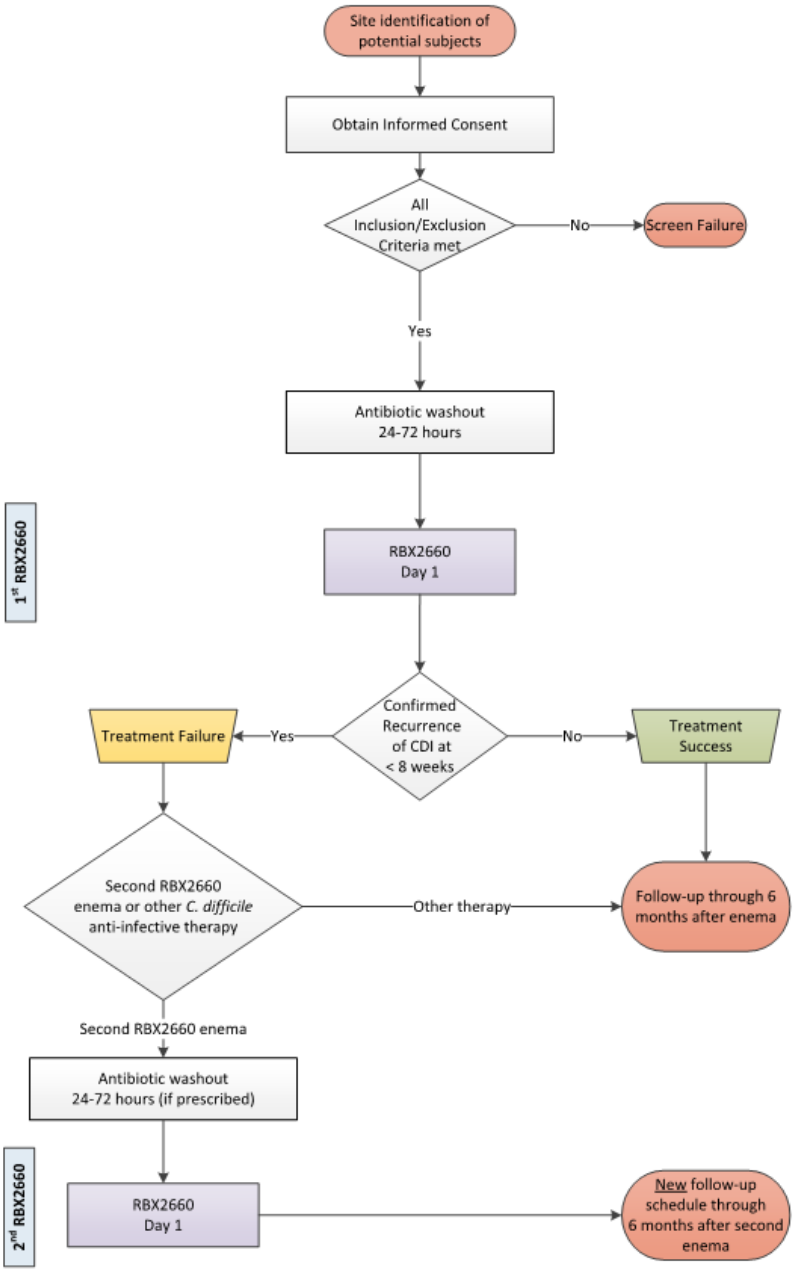

Figure 7-1: Open-Label Study Design.

7.3. Study Definitions

7.3.1. Control of symptoms during antibiotic use

Control of CDI symptoms during antibiotic use is defined as no longer meeting the symptomatic criteria for CDI diarrhea which is the passage of three or more unformed/loose (i.e., Bristol Stool Scale type 6-7) stools in 24 or fewer consecutive hours while taking antibiotics for the two consecutive days prior to the washout period.

Thus, at a minimum the Subject Diary which is started at the Screening visit must show that symptoms are under control the two days prior to the washout period in order for the subject to receive the enema administration.

7.3.2. Indeterminate Treatment Outcome

If neither the protocol-specified definition for Treatment Success nor Treatment Failure is met, the treatment outcome will be considered Indeterminate.

7.3.3. Medications

7.3.3.1. Prior Medications

Prior medications are defined as any medication (prescription or non-prescription), nutritional supplement, or herbal preparation taken or used within 30 days prior to the first dose of study drug.

7.3.3.2. Concomitant Medications

Concomitant medications are defined as any medication (prescription or non-prescription), nutritional supplement, or herbal preparation taken or used from first dose of study drug through 6-month follow-up assessment/end of study.

Concomitant medication information including changes to doses will be recorded in the study database from the Baseline visit through the 6-month follow-up assessment/end of study.

7.3.3.3. Prohibited Medications

Prohibited medications include any oral vancomycin, metronidazole, fidaxomicin, rifaximin, nitazoxanide, bezlotoxumab, or IVIG through the 8-week follow-up assessment unless newly prescribed by a treating investigator during the course of the study as a result of recurrent CDI diagnosis. Use of IVIG for treatment of a non-CDI indication is allowed. Use of non-dietary probiotics from Screening through 8 weeks after receiving the last study enema (including OTC and prescription) is also prohibited. Medication information will be collected at the Screening visit and changes in medications will be recorded throughout the study.

7.3.4. Recurrent CDI

The following are study definitions for recurrent CDI.

7.3.4.1. Recurrent CDI for Study Entry

To be considered for enrollment, a subject must have medical record documentation of:

a) a current diagnosis or history of recurrent CDI as determined by the treating physician,

b) *or* has had at least two episodes of severe CDI resulting in hospitalization.

7.3.4.2. Recurrent CDI On-study

1. The passage of three or more unformed/loose (i.e., Bristol Stool Scale type 6-7) stools in 24 or fewer consecutive hours for at least two consecutive days,
2. *and* a positive stool test for the presence of *C. difficile* as determined by the central laboratory.

7.3.5. Sustained Clinical Response

Sustained clinical response is defined as:

- Treatment success of the presenting CDI recurrence and no new CDI episodes through 6 months after completing a study treatment.

7.3.6. Treatment Failure

Treatment failure (CDI recurrence) is defined as:

- The presence of CDI diarrhea within 8 weeks of administration of a study enema, which includes a positive stool test for *C. difficile* as determined by the central laboratory.

7.3.7. Treatment Success

Treatment success is defined as:

- The absence of CDI diarrhea through 8 weeks after completing a study treatment.

7.4. Determination of Treatment Success/Failure

The site investigator makes the initial determination of treatment success or failure based on the pre-defined study definitions (**Section 7.3.1, 7.3.6, and 7.3.7**). The site investigator's assessment will then be provided to the Endpoint Adjudication Committee (EAC) for independent adjudication of treatment success or failure that will be utilized for study analysis and reporting purposes. This may be done on an on-going basis or a single meeting. Details of the EAC logistics, activity and responsibilities will be included in an EAC charter.

7.5. Management of Treatment Failure

Following treatment, subjects meeting the protocol definition of treatment failure (**Section 7.3.6**) may be scheduled for administration of a second RBX2660 enema within 21 calendar days of failure determination. To receive the second RBX2660 enema, the site must provide documentation of meeting all treatment failure criteria as defined in **Section 7.3.6**. If the definition of treatment failure is not met, a second study treatment of RBX2660 will not be provided.

Treatment failures are required to be entered in the study database shortly after determination of failure to allow for sponsor awareness and assurance that a second study treatment of RBX2660 can be provided if requested.

The use of antibiotics prior to a second RBX2660 enema is at the discretion of the investigator. If antibiotics are given to control symptoms, a 24-72 hour washout period prior to administration of a second RBX2660 enema is required. If the subject will have a second enema, it needs to be administered within 21 calendar days of failure determination.

If a subject receives a second RBX2660 enema, follow-up is completed via telephone assessments at 1-week, 4-weeks, 8-weeks, and 6 months after the second study treatment of RBX2660.

Treatment failures who do not receive a second RBX2660 enema will continue to follow their original schedule of assessments for the duration of the study based on the first study enema.

If a subject has a treatment failure after receiving a second study treatment of RBX2660, the subject should be treated per the investigator's discretion.

7.6. Medical Monitor

The Medical Monitor provides review of serious adverse events or events reported by the site as related to the investigational product or enema procedure to assess accuracy of reporting as related to seriousness and causality, and periodic review of adverse events for trends. Details of this review are documented in a Safety Management Plan. The Medical Monitor's review will be provided to the DSMB for analysis purposes and as applicable for the proper adjudication of potential study stopping rules.

7.7. Data and Safety Monitoring Board

The DSMB will review safety data for trends for the final analysis. Details regarding DSMB responsibilities related to analysis oversight, data reporting, and timing of meetings will be provided in the DSMB Charter.

7.8. Endpoint Adjudication Committee

The Endpoint Adjudication Committee (EAC) provides independent adjudication of treatment success or failure that will be used for study analysis and reporting purposes. Details of the EAC logistics, activity and responsibilities will be included in an EAC charter.

7.9. Bias

This is an open-label study with a single arm. The study is not blinded. Measures taken to minimize bias include selection of qualified investigators and study sites, adherence to the eligibility criteria (**Section 8**), and adherence to this protocol.

8. Subject Selection and Withdrawal

8.1. Inclusion Criteria

All responses must be "yes" to include a subject in the study:

1. ≥ 18 years old.
2. Medical record documentation of either a) a current diagnosis or history of recurrent CDI as determined by the treating physician, b) *or* has had at least two episodes of severe CDI resulting in hospitalization.
3. Is currently taking or was just prescribed antibiotics to control CDI related diarrhea at the time of enrollment. *Note: Subject's CDI diarrhea must be controlled (<3 unformed/loose, i.e., Bristol Stool Scale type 6-7, stools/day for the two consecutive days prior to the washout period) while taking antibiotics during screening*.
4. Willing and able to have an enema(s).
5. Willing and able to complete the stool and blood testing required for the study.
6. Agrees to not take non-dietary probiotics from Screening through 8 weeks after receiving the last study enema (including OTC and prescription).
7. Agrees to not take any oral vancomycin, metronidazole, fidaxomicin, rifaximin, nitazoxanide, bezlotoxumab, or IVIG through the 8-week follow-up assessment unless newly prescribed by a treating investigator during the course of the study as a result of recurrent CDI diagnosis. *Note: Use of IVIG for treatment of a non-CDI indication is allowed.*
8. Agrees to practice a form of effective contraception during study participation; does not apply to persons with documented non-child bearing potential.
9. Has a negative urine pregnancy test at the time of enrollment and on the day of each enema prior to administration (persons of child-bearing potential only).

10. Willing and able to provide informed consent, and local privacy authorization as applicable.
11. Willing and able to complete the required Subject Diary.
12. Willing and able to meet all study requirements, including attending all assessment visits and telephone calls.

8.2. Exclusion Criteria

All responses must be "no" to include a subject in the study:

1. A known history of refractory CDI.
2. Currently has continued CDI diarrhea despite being on a course of antibiotics prescribed for CDI treatment.
3. Requires systemic antibiotic therapy for a condition other than CDI.
4. Patient previously participated in a Rebiotix clinical study receiving RBX2660.
5. Fecal microbiota transplant (FMT) within the past 6 months.
6. FMT with an associated serious adverse event related to the FMT product or procedure.
7. Bezlotoxumab (CDI monoclonal antibodies) if received within the last year.
8. Disease symptoms (diarrhea) caused by a confirmed intestinal pathogen other than *C. difficile*.
9. Currently has a colostomy.
10. Intraabdominal surgery within the last 60 days. *Note: laparoscopic procedures that do not involve the GI tract are permitted*.
11. Planned surgery requiring perioperative antibiotics through the 8-week follow-up assessment.
12. Life expectancy of < 6 months.
13. CD4 count <200/mm^3 during Screening.
14. An absolute neutrophil count of <1000 cells/µL during Screening.
15. Known or suspected current (< 90 days) illicit drug use. *Note: marijuana use is allowed*.
16. Pregnant, breastfeeding, or intends to become pregnant during study participation.
17. Participating in a clinical study of another investigational product (drug, device or other) and has not completed the required follow-up period.
18. Subject is eligible for another RBX2660 study.
19. Subject, in the opinion of the investigator, for whatever reason, should be excluded from the study.

8.3. Subject Recruitment and Screening

Subjects are recruited by qualified and trained site personnel when they are identified as recently diagnosed with or experiencing recurrent CDI or are experiencing at least a

second hospitalization due to CDI. Potential subjects are to be fully informed as to this study's purpose, requirements, anticipated risks, etc., and are to be given the chance to review the informed consent form and receive satisfactory answers to all questions. Subjects sign the study-specific, IRB/REB-approved Informed Consent and local privacy authorization (as applicable), which is the point at which the potential subject is considered enrolled in the study.

Subjects are eligible to receive study treatment upon confirmation that they have met all eligibility criteria listed in **Sections 8.1** and **8.2**. Antibiotics being taken for their recurrent CDI symptoms must stopped for a 24-72 hour washout period prior to receiving the study enema.

9. Investigational Product

9.1. RBX2660 Description

RBX2660 microbiota suspension 50 g/150 mL is supplied in an enema bag. Each bag of RBX2660 provides one enema. Each enema of RBX2660 consists of a suspension of minimum 10^7 live organisms/mL in polyethylene glycol 3350/0.9% Sodium Chloride Irrigation, USP, solution.

9.2. Rebiotix Kit Description

Investigational product is supplied in a single-enema bag fitted with a spike port and a rectal tube assembly that is used to administer IP as an enema; these components are packaged together in the Rebiotix Kit. Rebiotix Kit components include:

- 1 bag of RBX2660 with a spike port contained in a brown opaque sleeve
- 1 rectal tube assembly
- 1 biohazard disposal bag
- 1 Instructions for Use (IFU)

9.3. Treatment Regimen

Study treatment needs to be administered within 21 days of the Screening visit. Product is to only be administered by the authorized IP Administrator who must be a qualified and trained health care professional following the Instructions for Use and standard site procedures. Subjects are to remain at the site under supervision for at least one-hour post-enema administration for vital sign assessment (temperature, heart rate, blood pressure, respiratory rate) and observation.

Subjects with a documented treatment failure following treatment may be scheduled for administration of a second RBX2660 enema. In order to receive a second RBX2660 enema, a subject must have documentation of meeting all treatment failure criteria as defined by the protocol (**Section 7.3.6**).

If, in the opinion of the investigator, antibiotics are warranted for the control of CDI symptoms prior to receiving the second RBX2660 enema, they can be administered but need to be stopped 24-72 hours before enema administration. The total time from a treatment failure (CDI recurrence) determination to administration of a second enema is within 21 calendar days.

See **Section 10** for the Schedule of Study Procedures and detailed description of the requirements.

9.4. Subject Follow-up Requirements

Subjects are assessed at office visits at 1- and 8-weeks and via telephone calls at 4-weeks and at 4 and 6 months after the study enema. See **Section 10** for follow-up assessments and phone calls, and **Section 10.16** for subject withdrawal/termination.

9.5. Concomitant Therapy

The subject must agree to not take any oral vancomycin, metronidazole, fidaxomicin, rifaximin, nitazoxanide, bezlotoxumab, or IVIG through the 8-week follow-up assessment unless newly prescribed by a treating investigator during the course of the study as a result of recurrent CDI diagnosis. Use of IVIG for treatment of a non-CDI indication is allowed. Use of non-dietary probiotics from Screening through 8 weeks after receiving the last study enema (including OTC and prescription) is also prohibited.

Medication information will be collected at the Screening visit and changes in medications will be recorded throughout the study. Subjects should be encouraged to notify their general practitioners of study participation and the requirements for concomitant therapy.

9.6. Preparation of Investigational Product

Instructions for Use with detailed directions for IP preparation and administration are included in each Rebiotix Kit.

9.7. Packaging

Investigational product is supplied in an enema bag fitted with a spike port and a rectal tube assembly that is used to administer the product via enema; these components are packaged together in the Rebiotix Kit.

9.8. Ordering, Receipt and Storage, Dispensing, Returning, and Tracking Investigational Product

9.8.1. Ordering Investigational Product

Authorized site personnel will order IP through the study database. If a subject is a confirmed treatment failure and will proceed to a second RBX2660 treatment, the site will document the failure in the database, and complete a re-order form in the database to trigger a shipment of IP for the second RBX2660 treatment.

9.8.2. Receipt and Storage of Investigational Product

Note: Each unit of IP is designated to be administered to a specific subject on a specific date; administration of IP designated for one subject to another subject is prohibited.

IP is shipped to the authorized person/department as specified on the Delegation of Authority log at the site in a temperature-controlled shipping package. The authorized person opens the shipping package, visually confirms that the unit of IP arrived intact, confirms that the temperature indicator has not been tripped and records receipt and inspection on the Product Accountability Log. IP is to be thawed and stored in the refrigerator until administration.

IP must be kept in a secure location under the control of the Investigator or authorized designee at all times; see **Section 9.8.5** for details. IP must be administered to the subject by the expiration date as specified on the label. If it is not administered by the expiration date, it must be destroyed by site staff per the institution's processes and be recorded on the Product Accountability Log.

9.8.3. Dispensing and Administering Investigational Product

Dispensing procedures vary from site to site, but each site's study investigator is responsible for delegating the administration of IP to a qualified and trained health care professional; this person(s) ("IP Administrator") must be identified on the Delegation of Authority Log and receive product-specific training on IP administration.

IP is to be administered by the IP Administrator after all inclusion/exclusion criteria have been met, the subject had a negative pregnancy test (if applicable) and the 24-72 hour antibiotic washout period has been completed. Administration is via enema

following the Instructions for Use in each product package and the site's standard procedures.

9.8.4. Returning IP

Do not return IP to Rebiotix. IP received but not administered to a subject must be destroyed by site staff per the institution's processes. Destruction must be recorded on the Product Accountability Log.

9.8.5. Product Accountability and Tracking

Each unit of IP is sent to the site for administration to an assigned subject and is labeled with a batch number to be recorded in the electronic case report form (eCRF) and on the Product Accountability Log. Each unit must be tracked on the Product Accountability Log by the person(s) authorized on the Delegation of Authority Log to perform that step. IP must be kept in a secure location under the control of the site investigator or authorized designee as recorded on the Delegation of Authority Log. The Product Accountability Log and Delegation of Authority Log are to be maintained by the site and confirmed during monitoring visits. The steps to be tracked include dates of receipt and dispensing, batch number, subject number, storage in refrigerator, and destruction if not administered to a subject. The IP Administrator must ensure the correct batch number is administered to the correct subject.

10. Study Procedures

10.1. General Information

Study information is collected on study-specific eCRFs by the site. Study monitoring will occur at regular intervals to ensure the protection of subject rights and safety, data integrity and accuracy, and proper study conduct in compliance to the protocol and applicable regulations including 21 CFR 312 and ICH E6 GCPs. Study visits and telephone assessments must occur with the window specified in **Table 10-1**; visits and calls out of window or missed are to be reported as protocol deviations. The study-required procedures are to be conducted as shown in **Table 10-1**.

Timely and complete follow-up visits will ensure the scientific integrity of the data is maintained and allow for continued oversight of subject safety.

Table 10-1 Schedule of Events

Activity	Screening (Enrollment)	Baseline / Enema Administration (≤ 21 days from Screening)	Follow-up Visits: 1- and 8-Week (± 3 days) Assessments[1]	Follow-up Phone Assessment: Week 4 (± 3 days)	Follow-up Phone Assessment: 4 and 6 months (± 14 days)	Unscheduled Possible Recurrence Visit	Second Enema Administration[1] (≤ 21 calendar days post Tx Failure)	Second Enema Follow-up Phone Assessment: 1-, 4-, and 8-weeks (± 3 days), and 6 months (± 14 days)
Informed consent obtained	X							
Demographics, medical history	X							
Prescribe/continue antibiotics for CDI symptom control	X							
Physical exam	X							
Stool sent to Rebiotix by subjects for testing and archiving (optional)	X		X	X	X	X		X
Central Lab CBC w/differential testing[3]	X[3]	X						
Central Lab CD4 testing	X[3]							
Central Lab CMP & CRP testing	X							
C. difficile testing[4]						X[4]		
Central Lab stool and blood testing		X						
Urine pregnancy testing performed at site (if applicable)	X	X					X	
Cdiff32 Questionnaire	X		X		X	X		
Inclusion/exclusion criteria confirmed	X	X						
24-72hr washout period confirmed		X					X[7]	
Enema administered		X					X	
Product complaint (if applicable)		X					X	
CDI symptoms assessed		X	X	X	X	X	X	X
Vital signs assessed	X	X				X	X	
Weight	X		X	X	X			X
Subject Diary discussed/reviewed	X	X	X[5]					
Employment status assessed	X		X[6]		X			
Concomitant medications	X	X	X	X	X	X	X	X
Adverse events assessed		X	X	X	X	X	X	X
Solicited events assessed[2]		X	X[2]					

[1] Documented treatment failures may receive a second RBX2660 enema.
[2] Solicited events are collected in the Subject Diary from the day of administration of the baseline enema until the day prior to the 1-week Follow-up visit. The Subject Diary is reviewed at the 1-week Follow-up visit. Solicited events that increase in severity from Screening should be assessed for a possible adverse event.
[3] Exclusion criteria for absolute neutrophil count and CD4 should be assessed based on the blood samples collected at the Screening visit.
[4] Tested at the central laboratory.
[5] Subject Diary is reviewed only at the 1-week Follow-up visit.
[6] Collected at the 8-week follow-up visit only.
[7] If antibiotics were administered to manage CDI recurrence.

10.2. Informed Consent

All subjects must sign the study-specific IRB/REB-approved Informed Consent/HIPAA Form; see **Section 8.3**. The original signed Form is to be placed in the subject's study file. A copy of the signed Form is also to be given to the subject for their records.

10.3. Subject Diary

The Subject Diary serves as a tool for the subject to record pre- and post-treatment health and information. The Subject Diary is reviewed for solicited event occurrence and severity, changes to medications, occurrence of adverse events, and possible recurrence of CDI. The subject also documents changes in medications.

At the Screening visit, subjects are provided a Subject Diary to complete each day until the day prior to the study enema (Baseline visit). This may be up to 21 days. The diary will be reviewed at the Baseline visit prior to enema administration.

Prior to the subject leaving at the conclusion of the Baseline visit, the subject is instructed to continue completing the Subject Diary for each day until the 1-week office visit.

The following information is entered into the study database:

- solicited event severity
- changes to medication
- occurrence of adverse events

10.4. Optional Stool Samples for Additional Testing by Rebiotix

Subjects may agree (consent) to provide additional, optional stool samples to Rebiotix for research development activities. Subjects consenting to the Optional Stool Sample collection are requested to provide stool samples directly to Rebiotix at the following time-points:

- Screening (between the time of enrollment and prior to treatment)
- at the time of the 1- and 8-week office visits
- at the time of the 4-week, and at 4- and 6-month phone calls
- at the time of possible treatment failure symptoms
- at the 1-, 4-, and 8-weeks and 6-month phone calls after a second enema, if applicable

Subjects collect the samples at home in pre-labeled containers given to them by the study team. Rebiotix provides the study sites with kits containing all the materials for collecting and shipping the samples, including subject directions, container labels, and prepaid, pre-addressed mailing labels. Samples are identified by the label on the container that contains only the subject's study identification number and date/time of the sample; samples will not contain any personal information that would identify the subject. Samples may be stored for approximately five years. The stool samples will be used for research about the microorganisms in the human gut and the effects of microbiota restoration therapy on the gut microbiome. Some of the research will include testing for microorganisms (*Clostridium difficile* and vancomycin-resistant enterococci) both before and after receiving the study enema. Failure to send in a sample does not result in a protocol deviation, however subjects should be reminded of this request.

10.5. Screening Visit

The following study activities/data will be collected/conducted at the Screening visit:

- Subjects are told about the study and informed consent is obtained (enrollment)
- Assign the subject number.
    - The format for subject number will include site code, followed by study-specific identifier, and 3-digit subject number assigned in sequential ascending order. For example: **AAA**OLS, RBX08-**###.** Where "AAA" is your study site's code, and "###" is the subject's number following a -001, -002, -003,… format.
- Standard demographics: gender, ethnicity, date of birth, race
- Relevant medical history, including comorbidity index variables (see **Section 16**)
- CDI medical history documentation will be collected
- Assess inclusion/exclusion criteria
- Measure vital signs: height, weight, blood pressure, pulse, breathing rate and temperature
- Physical exam (no genitourinary exam unless medically indicated) is performed to establish a study baseline health status (findings that can be attributed to the subject's medical history):
    - General
    - HEENT (head, eyes, ears, nose and throat)
    - Cardiovascular
    - Gastrointestinal

  - Musculoskeletal
  - Neurological
- Blood samples are collected and analyzed at the central laboratory for:
  - CD4 count
  - CBC w/differential
  - Comprehensive metabolic panel (CMP): Sodium, potassium, chloride, BUN, creatinine, albumin, AST, ALT, alkaline phosphatase, bilirubin (direct, indirect and total), glucose
  - C-reactive protein (CRP)

  NOTE: the results of the absolute neutrophil count (ANC) and CD4 tests must be confirmed ≥ 1000 cells/µL and ≥200/mm³, respectively
- Onsite urine pregnancy test for subjects of child bearing potential
- Complete the Cdiff32 health-related quality of life questionnaire completed by the subject (see **Section 17**)
- Document concomitant medications taken within the last 30 days
- Completion of the Subject Diary is explained and how to complete it is demonstrated; see **Section 10.3**
- Record employment status
- For subjects who consented to the Optional Stool Sample collection, remind them to obtain and ship a stool sample to Rebiotix anytime between their Screening visit and their Baseline visit dates

10.6. Randomization

There is no randomization in this study.

10.7. Order IP/Study Drug

Orders for IP/study drug are placed through the Medpace ClinTrak study database. IP should be ordered approximately four (4) business days in advance of the planned IP administration date, if not earlier. This timeframe is to accommodate the shipping schedule, and instructions for thawing which are located in the Instructions for Use.

10.8. Baseline Visit and Study Treatment Administration

IP/study drug should be ordered approximately four (4) business days in advance of the planned IP administration date, if not earlier.

The Baseline visit is performed on the day of the study treatment (IP) administration. IP administration must occur within 21 days of the Screening visit. Antibiotics for the control of CDI symptoms are continued until 24-72 hours before administration of the IP.

The following study activities/data will be collected at the Baseline visit prior to IP administration:

- Inclusion/exclusion criteria are confirmed including the following:
  - The control of symptoms during antibiotic use is confirmed by review of the subject diary (see **Section 7.3.1**)
  - Compliance to the washout period (discontinuation of the antibiotics for 24-72 hours prior to the Baseline visit) is confirmed
    - Stop date and time (AM/PM) of antibiotic
- Central laboratory blood sample collection; see **Table 10-2** for the list of tests. *NOTE: these results are <u>not</u> needed before proceeding to treatment per the protocol*
- Central laboratory stool sample collection; see **Table 10-2** for the list of tests. *NOTE: the results are <u>not</u> needed before proceeding to treatment per the protocol*
- Onsite urine pregnancy test for subjects of child bearing potential
- Assess vital signs: temperature, pulse, blood pressure, respiration
- Changes to concomitant medications since the last visit
- Review subject diary (see **Section 10.3**)
- Assess recurrence of CDI
- Assess for adverse events since enrollment
- Date and time of the last bowel movement prior to the enema
- Did the subject void his/her bladder prior to entering the administration room

Table 10-2: Baseline Testing Conducted by Central Laboratory

Test Name	Material Tested
Norovirus	Stool
Rotavirus	
Adenovirus	
Enteric pathogens (*Shigella, Salmonella*, *Campylobacter*, sorbitol-negative *E. coli.*, *Aeromonas, Plesiomonas, Yersinia*, and shiga toxins)	
Giardia antigen	
Cryptosporidium antigen	
Acid-fast staining (Cyclospora, Isospora)	
Ova and parasites	
Vancomycin-resistant *enterococci* (VRE)	
Methicillin-resistant *Staphylococcus aureus* (MRSA)	
Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*	
Vibrio	
Listeria	
Clostridium difficile	
Carbapenem-resistant *Enterobacteriaceae* Culture (CRE)	
CBC with Differential	Blood
HIV	
Hepatitis A (IgG)	
Hepatitis B Anti-Hepatitis B surface antigen	
Hepatitis C Antibody	
Treponema Antibody	

The following information is collected specific to the administration of the IP:

- Enema clinical batch number
- Enema administration start and end time
- Enema administration status (successful; attempted, not completed; opened, not administered)
- Subject position for enema
    - Length of time subject was in position after enema
- Did the Rebiotix kit components perform as expected
- Note whether the subject expelled or leaked any of the IP during the observation period

- How long was the enema retained before the expulsion or leakage (minutes)
- What was the estimated amount of enema that was expelled or leaked

Following successful completion of the above listed items, IP is administered via enema per the Instructions for Use and standard site procedure; see **Section 9.8.3**. Subjects remain at the site under supervision for at least one hour post-IP administration for vital sign assessment (temperature, heart rate, blood pressure, respiratory rate) about every 15 minutes and observation. If the subject's vital signs do not return to the pre-administration values ± 20%, assess the subject for a possible AE.

All safety data collected are to be recorded and reported as required in **Section 11**.

The subject is sent home after completion of study procedures including a review of the Subject Diary instructions, and is requested to call the study site if symptoms recur or at the onset of an adverse event.

10.9. 1-week In-Office Visit

The 1-week follow-up visit is to occur within the window of ±3 days from the Baseline visit. All safety data collected are to be recorded and reported as required in **Section 11**.

The following study activities/data is collected at the 1-week follow-up visit:

- Review of the subject diary for solicited events as defined in **Section 11.4**
- Assess recurrence of CDI
- Subject's weight
- Adverse event collection, including new onsets of obesity, metabolic syndrome, pre-diabetes, diabetes and/or autoimmune disorders
- Cdiff32 health-related quality of life questionnaire completed by the subject
- Changes to concomitant medications since the last visit
- For subjects who consented to the Optional Stool Sample collection, remind the subject to collect and ship a stool sample to Rebiotix

10.10. 4-week Telephone Assessment

This phone call must occur within the window of ±3 days (see **Table 10-1**); out of window or missed telephone assessments are to be reported as protocol deviations. All safety data collected are to be recorded and reported as required in **Section 11**.

The following study activities/data is collected at the telephone assessment calls:

- Assess recurrence of CDI
- Subject's weight, self-reported
- Adverse event collection, including new onsets of obesity, metabolic syndrome, pre-diabetes, diabetes and/or autoimmune disorders
- Changes to concomitant medications since the last visit
- For subjects who consented to the Optional Stool Sample collection, remind the subject to collect and ship a stool sample to Rebiotix

10.11. 8-week In-Office Visit

The 8-week follow-up visit is to occur within the window of ±3 days (see **Table 10-1**). All safety data collected are to be recorded and reported as required in **Section 11**.

The following study activities/data is collected at the 8-week follow-up visit:

- Assess Treatment Outcome and recurrence of CDI
- Subject's weight
- Adverse event collection, including new onsets of obesity, metabolic syndrome, pre-diabetes, diabetes and/or autoimmune disorders
- Changes to concomitant medications since the last visit
- Cdiff32 health-related quality of life questionnaire completed by the subject
- Record employment status
- For subjects who consented to the Optional Stool Sample collection, remind the subject to collect and ship a stool sample to Rebiotix

10.12. 4- and 6- Month Telephone Assessments

These phone calls must occur within the window of ±14 days (see **Table 10-1)**. All safety data collected are to be recorded and reported as required in **Section 11**. Prior to the call, mail out the Cdiff32 health-related quality of life questionnaire to the subject for completion, including the return, addressed and stamped envelope.

The following study activities/data will be collected at the telephone assessment calls:

- Assess recurrence of CDI
- Subject's weight, self-reported

- Adverse event collection, including new onsets of obesity, metabolic syndrome, pre-diabetes, diabetes and/or autoimmune disorders
- Changes to concomitant medications since the last visit
- Remind the subject to complete the Cdiff32 health-related quality of life questionnaire, if not received prior to the telephone visit.
- Record employment status
- For subjects who consented to the Optional Stool Sample collection, remind the subject to collect and ship a stool sample to Rebiotix

The last phone call will occur 6 months after the date of the last administration of IP.

10.13. Unscheduled Possible Recurrence Visit

If CDI recurrence is suspected any time within 8 weeks of the last study enema due to new onset documentation of three or more unformed/loose (i.e., Bristol Stool Scale type 6-7) stools within 24 or fewer consecutive hours for at least two consecutive days, possible treatment failure should be assessed. An in-office visit should occur if a recurrence of CDI is suspected. The subject must provide a fresh stool sample for shipment to the central laboratory to test for the presence of *C. difficile*.

The following study activities/data will be collected at the Unscheduled Possible Recurrence visit:

- Collect fresh stool sample to send to the central laboratory for analysis
- Assess recurrence of CDI
    - Start date of symptoms, current symptoms, central laboratory testing result, source of episode (*CDI recurrence, healthcare contagion, community contagion, or unknown*), treatment(s)
- Vital signs: temperature, pulse, blood pressure, respiration
- Adverse event collection, including the new onsets of obesity, metabolic syndrome, pre-diabetes, diabetes and/or autoimmune disorders
- Changes to concomitant medications since last visit
- Cdiff32 health-related quality of life questionnaire
- For subjects who consented to the Optional Stool Sample collection, remind the subject to collect and ship a stool sample to Rebiotix.

Treatment failure (CDI recurrence) is defined as:

- The presence of CDI diarrhea within 8 weeks of administration of a study enema, which includes a positive stool test for *C. difficile* as determined by the central laboratory.

To ensure consistency across clinical sites for the determination of study failure, the central laboratory will perform the required C. DIFF QUIK CHEK COMPLETE® Test (QCC Test) rapid enzyme immunoassay test for glutamate dehydrogenase (GDH) and toxins A and B.

In the case of suspected treatment failure where there is an immediate concern for subject safety, including the potential for serious adverse events, a test may be conducted at the site or other laboratory known to the site **in addition to** sending a sample to the central laboratory for testing. In the event the test results from the central laboratory differ from that of a local laboratory, the results from the central laboratory will be used in determination of treatment failure and eligibility to receive a second treatment with RBX2660, instead of the other laboratory's result.

10.14. Second RBX2660 Treatment

Subjects with a documented treatment failure meeting the protocol definition (**Section 7.3.6**) after receipt of the first RBX2660 enema may be scheduled for administration of a second RBX2660 enema. If antibiotics are given to control symptoms, a 24-72 hour washout period prior to administration of a second RBX2660 enema is required, however the second enema must be administered within 21 calendar days of failure determination.

In order to receive the second treatment with RBX2660, a subject must have documentation of the following:

- Meeting all treatment failure criteria as defined by the protocol (**Section 7.3.6**)
- Compliance to the washout period (discontinuation of antibiotics for 24-72 hours prior to administration visit) is confirmed, if applicable
- Negative urine-dipstick pregnancy test for subject of childbearing potential to confirm that they are not pregnant on the date of administration
- Enema administration is to occur within 21 calendar days of failure determination

The following study activities/data will be collected at this visit prior to IP administration:

- Onsite urine pregnancy test for subjects of child bearing potential
- Assess vital signs: temperature, pulse, blood pressure, respiration
- Changes to concomitant medications since the last visit
- Assess recurrence of CDI
- Assess for adverse events since enrollment
- Date and time of the last bowel movement prior to the enema
- Did the subject void his/her bladder prior to entering the administration room

Following successful completion of the above listed items, IP is administered via enema per the Instructions for Use and standard site procedure; see **Section 9.8.3**.

The following information is collected specific to the administration of the IP:

- Enema clinical batch number
- Enema administration start and end time
- Enema administration status (successful; attempted, not completed; opened, not administered)
- Subject position for enema
    - Length of time subject was in position after enema
- Did the Rebiotix kit components perform as expected
- Note whether the subject expelled or leaked any of the IP during the observation period
    - How long was the enema retained before the expulsion or leakage (minutes)
    - What was the estimated amount of enema that was expelled or leaked

Subjects remain at the site under supervision for at least one hour post-IP administration for vital sign assessment (temperature, heart rate, blood pressure, respiratory rate) about every 15 minutes and observation. If the subject's vital signs do not return to the Baseline visit's (**Section 10.8**) pre-administration values ± 20%, assess the subject for a possible AE.

All safety data collected are to be recorded and reported as required in **Section 11**.

If a subject receives a second RBX2660 enema, follow-up is completed via telephone assessment at 1-week, 4-weeks, 8-weeks, and 6 months per **Section 10.14.1** after the second RBX2660 treatment.

No additional treatments of RBX2660 beyond a second enema will be administered as part of this study for treatment failures.

The study will be considered complete when all subjects have reached their 6-month follow-up after the last study enema received, including second enemas, or have exited, whichever is earlier.

10.14.1. 1-week, 4-weeks, 8-weeks, 6-month Telephone Assessment

- Subject's weight, self-reported
- Adverse event collection, including new onsets of obesity, metabolic syndrome, pre-diabetes, diabetes and/or autoimmune disorders
- Changes to concomitant medications since the last visit
- For subjects who consented to the Optional Stool Sample collection, remind the subject to collect and ship a stool sample to Rebiotix

10.15. Study Exit

A Study Exit eCRF will be completed for each subject who is treated. Data to be collected at this visit includes exit date and reason for study exit. This visit may occur in conjunction with the 6-month Follow-Up visit for subjects who complete the study and do not have ongoing adverse events that have not stabilized.

The following information will be collected on the Study Exit eCRF:

- Exit date
- Reason for study exit
  - Completed Study
  - Screen Failure
  - Withdrawal by subject
  - Study Terminated by Sponsor
  - Investigator withdrawal
  - Adverse Event
  - Death
  - Lost to Follow-Up
  - Other (to be specified)

Additional data will be collected when the following occur:

- Medication changes
- Adverse Events

10.16. Subject Withdrawal or Termination

A subject's study participation is considered complete once the 6-month telephone assessment is conducted after receiving the last study enema or the subject exits the study, whichever comes first. A subject may withdraw at any time for any reason or be withdrawn from the study prematurely for the following reasons:

- Withdrawal of consent by subject
- Lost to follow-up
- Failure to comply to study requirements
- Termination of study by the sponsor
- Death
- Other (to be specified)

The reason for withdrawal is recorded on the Study Exit eCRF. In the event that a subject requests to withdraw from the study, every attempt should be made to have the subject provide an update on the current status of any adverse events and current medications. If the subject has an ongoing adverse event deemed related to IP or enema procedure, recommend following the subject until the adverse event has resolved or stabilized prior to exiting the study.

For a subject who is suspected of being lost-to-follow-up, a minimum of three attempts must be made to contact subjects using two different contact methods (e.g., telephone, email, text, and letter); one attempt must be a registered postal letter or traceable courier notice (e.g., FedEx Express) to the subject's last known address. The contact attempts and methods are to be recorded on the Study Exit eCRF. 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 phone call contact or visit.

Completion of a Study Exit eCRF is required for all treated subjects when they exit the study regardless of reason, including completion of all study requirements, visits and assessments. Subjects who were determined to be screen failures or otherwise exited prior to receiving a dose of the study drug will also have a Study Exit eCRF completed.

11. Assessment of Safety

11.1. Adverse Event Management

Adverse events (AE), which include clinical laboratory test variables, will be monitored and documented from the time of informed consent. Subjects should be instructed to report any AE that they experience to the investigator. Investigators should make an assessment for AEs at each visit and record the event on the appropriate eCRF.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified by the investigator and recorded on the eCRF. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the investigator, it should be recorded as a separate adverse event on the eCRF. Additionally, the condition that led to a medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, or transfusion) should be recorded as an AE, not the procedure.

Any medical condition already present at Screening should not be reported as an AE unless the medical condition or signs or symptoms present at Screening change in severity or seriousness at any time during the study. In this case, it should be reported as an AE.

Clinically significant abnormal laboratory or other examination (e.g., electrocardiogram) findings that are detected during the study or are present at Screening and significantly worsen during the study should be assessed for an AE. The investigator will exercise their medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant abnormal laboratory values occurring during the clinical study will be followed until repeat tests return to normal, stabilize, or are no longer clinically significant. Any abnormal test that is determined to be an error does not require reporting as an AE.

For all CDI recurrences, information is recorded on the Follow-up eCRF and applicable CDI eCRF. Recurrence of CDI is not to be reported as an AE unless hospitalization $\geq$ 24 hours is required as part of treatment. If hospitalization $\geq$ 24 hours is required, the event qualifies as an AE and is to be reported as an SAE (see **Section 11.14** for sponsor reporting requirements).

AEs should be followed until the event resolves or the subject exits the study. Chronic condition is defined as a condition lasting > 3 months.

11.2. AE Definitions

- Adverse event: an adverse event (AE) is any untoward medical occurrence in a clinical investigation subject associated with the use of IP, which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of IP.
    - All AEs, including observed or volunteered problems, complaints, or symptoms are to be recorded on the eCRF within **ten (10) business days** of site awareness.
- Adverse reaction: All noxious and unintended responses to IP should be considered an adverse reaction. "Responses" to IP means that a causal relationship between IP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out.
- Serious adverse event (SAE): An AE or adverse reaction is considered serious if, in the view of either the investigator, Medical Monitor or sponsor, it results in any of the following outcomes:
    - Death
    - Life-threatening adverse event

    NOTE: An adverse event or adverse reaction is considered "life-threatening" if, in view of either the investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death.

    - Hospitalization $\geq$ 24 hours or prolongation of an existing hospitalization

    NOTE: Any hospital admission with at least one overnight stay will be considered an inpatient hospitalization. An emergency room visit without hospital admission will not be recorded as a serious adverse event (SAE) under this criterion, nor will hospitalization for a procedure scheduled or planned before signing of informed consent. However, unexpected complications and/or prolongation of hospitalization that occur during elective surgery should be recorded as AEs and assessed for seriousness. Admission to the hospital for social or situational reasons (i.e., no place to stay, live

too far away to come for hospital visits) will not be considered inpatient hospitalizations.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.
- Important medical event

NOTE: Important medical events that may not result in death, be life-threatening, or do require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalizations, or the development of drug dependency.

- Unexpected Adverse Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information.
- Unexpected adverse event or unexpected suspected adverse reaction: An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

11.3. Anticipated Adverse Events

The following is list of anticipated AEs that may or may not be causally related to IP, the enema procedure, or CDI:

- gas (flatulence)
- belching
- abdominal distension or bloating
- increased diarrhea
- abdominal cramping or pain
- constipation
- colitis
- fever ≥ 37.8° C (100.0°F)
- fatigue
- chills

- transmission of disease from the donor to recipient
- rectal irritation or pain
- rectal bleeding
- nausea
- vomiting
- hypotension
- puncture of the intestine

11.4. Solicited Events

The following list of anticipated events are solicited from subjects via the Subject Diary:

- gas (flatulence)
- abdominal distension or bloating
- rectal irritation or pain
- chills/severe shivering
- abdominal pain or cramping
- increased diarrhea
- constipation
- rectal bleeding
- nausea
- vomiting
- fever ≥ 37.8° C (100.0°F)

Through the completion of the Subject Diary, subjects are asked specific questions regarding frequency and severity of the solicited AEs.

11.5. Preexisting Condition

A preexisting condition, including abnormal physical exam findings, is one that is present at the start of the study and is to be recorded at the time of the Screening visit. A preexisting condition is only to be recorded as an adverse event if the frequency, intensity or the character of the condition worsens during study participation.

11.6. Serious Adverse Event Reporting – Procedures for Investigators

11.6.1. Initial Reports

All SAEs occurring from the time of informed consent until 6 months after administration of the last study enema of IP must be reported to Medpace Clinical Safety **within 24 hours** of the knowledge of the occurrence (this refers to any AE that meets any of the aforementioned serious criteria).

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, Medpace Safety personnel will be notified electronically and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to Medpace Safety at **medpace-safetynotification@medpace.com** or call the Medpace SAE hotline (phone number listed below), and fax the completed paper SAE form to Medpace (fax number listed below) within 24 hours of awareness. When the EDC system becomes available, the SAE information must be entered within 24 hours of the system becoming available.

Safety Contact Information:
Medpace Clinical Safety

Medpace SAE hotline:
[redacted]

11.6.2. Follow-Up Reports

The investigator must continue to follow the subject until the SAE has subsided or until the condition becomes chronic in nature, stabilizes (in the case of persistent impairment) or the subject dies. Chronic is defined as a condition lasting >3 months.

Within 24 hours of receipt of follow-up information, the investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (e.g., subject discharge summary or autopsy reports) to Medpace Clinical Safety via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

11.7. Grading Event Severity

Identified, subject-reported and unsolicited AEs are to be graded, according to severity, by the site investigator. For this classification of events, severity is not the same as

seriousness, which is defined in **Section 11.1**. Severity is an indication of the *intensity* of a specific event (e.g., mild, moderate or severe). Classification of an event as serious relates to an event's outcome or intervention criteria and is usually associated with events that pose a threat to a subject's life or functioning. An event can be severe but not serious, such as a migraine.

Using the definitions in **Table 11-1**, the site investigator will categorize the severity of a solicited or unsolicited event on the AE eCRF. If a particular event is not listed on this table, the NIH/NCI Common Terminology Criteria for Adverse Events found at online at the following address may be utilized.
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

Table 11-1: Severity Grading Table*

Parameter	Grade1: 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	NA	NA
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	NA	NA
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	NA
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

Parameter	Grade1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
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
Ch0ills	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	NA
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	NA

Parameter	Grade1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
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	NA	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

**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.*

11.8. Causality

For all adverse events, the investigator must pursue and obtain adequate information to determine the outcome of the adverse event and to assess whether the AE meets the criteria for classification as an SAE, serious suspected adverse reaction, suspected adverse reaction, unexpected AE, or unexpected suspected adverse reaction. For all AEs, sufficient information should be obtained by the investigator to determine the causality of the AE. For AEs with a causal relationship to the IP or the enema procedure, follow-up by the investigator is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator.

The investigator will make a causality assessment for all AEs and decide whether there is a reasonable possibility that the AE may have been caused by the study product or procedure, including an assessment of biologic plausibility, presence or absence of alternative causal explanations (such as continuation or exacerbation of the subject's recurrent CDI symptoms), and temporal relationship to product administration and/or the procedure. Relatedness to RBX2660 or the enema procedure is defined as:

- Unrelated: The event is due to an underlying or concurrent illness or effect of concomitant therapy and is not related to the study product or procedure (*e.g.*, has no temporal relationship to study product or procedure, or has a much more likely alternative etiology)
- Possible: There is some temporal relationship between the event and the administration of the study product or 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 study product or procedure is suggestive, and the event is unlikely to be explained by the subjects' medical condition or other therapies alone.
- Definite: The event follows a reasonable temporal sequence from administration of the study product or procedure, follows a known or suspected response pattern to the study product and/or procedure, improves upon stopping the study product, and reappears upon repeated exposure, if that occurs.

If an investigator is unsure about whether to report a finding as an AE, they are encouraged to enter the finding as an AE into the study database.

11.9. Pregnancy Reporting

If the subject or partner of a subject participating in the study becomes pregnant during the study after IP administration, the investigator should report the pregnancy to Medpace Clinical Safety within 24 hours of being notified. Medpace Clinical Safety will then forward the Exposure In Utero form to the investigator for completion.

The subject or partner should be followed by the investigator until completion of the pregnancy. If the pregnancy ends for any reason before the anticipated date, the investigator should notify Medpace Clinical Safety. At the completion of the pregnancy, the investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), the investigator should follow the procedures for reporting an SAE.

11.10. Medical Monitor

An independent Medical Monitor reviews all SAEs and other events reported by the site as related to the investigational product or enema procedure to provide an objective, qualified judgment of the events. The Medical Monitor is a physician not participating as an investigator in this study. The Medical Monitor has the responsibility to review and evaluate the information relevant to product safety throughout the development and implementation of the protocol. The Medical Monitor performs the following functions:

- Reviews SAEs and other AEs as requested;
- Reviews the study protocol and Investigator's Brochure for adequacy of safety oversight;
- Confers with site investigators, DSMB and the Rebiotix Clinical Department as applicable regarding potential safety concerns;
- Provides medical surveillance and provides appropriate recommendations for the conduct of the study as needed.

Details of the Medical Monitor activities and expectations will be documented in a Safety Management Plan.

11.11. Data and Safety Monitoring Board (DSMB)

The DSMB will consist of at least two physicians specializing in infectious diseases or gastroenterology who have experience managing subjects with recurrent CDI and are not investigators in the study. A biostatistician who is not involved with study design or analyses may assist the DSMB. The DSMB will operate in accordance with applicable

sections of the FDA Guidance for Clinical Trial Sponsors *Establishment and Operation of Clinical Trial Data Monitoring Committees.*

Details of the DSMB logistics, activity and responsibilities will be included in a DSMB charter. General responsibilities include oversight of subject safety through data review and assessment of trends. This includes the ability to stop the study based on protocol-defined stopping rules. Additionally, the DSMB may provide an independent review of the efficacy outcome to ensure the benefits of providing this treatment to subjects outweighs the risks.

11.12. Endpoint Adjudication Committee (EAC)

The EAC will provide independent adjudication of treatment success or failure that will be used for study analysis and reporting purposes. The EAC will be comprised of three physicians specializing in infectious diseases or gastroenterology who have experience managing subjects with recurrent CDI and are not investigators in the study. Details of the EAC logistics, activity and responsibilities will be included in an EAC charter.

11.13. Study Stopping Rules for Safety

Rebiotix will oversee review of all AEs reported during the study to observe for trends and unanticipated events, either in severity, seriousness or incidence, and to assess if a study stopping rule may be triggered. AE review includes the event description, onset date in relation to the treatment date, and the investigator's determination of causal relatedness to IP, the enema procedure, and pre-existing condition, as well as rating the event's severity and seriousness. Additionally, the Medical Monitor assessment of seriousness and causality will be provided and reviewed as it may be different from the site investigator report of the event. Anticipated AEs may trigger the study stopping rules.

The DSMB will determine whether enrollment should be paused, the study terminated, or other actions taken based on their assessment that:

a) There is probable cause that IP or enema procedure (e.g., due to transfer from an RBX2660 donor) contributed to a pathogenic intestinal infection in the stool of any subject, or
b) Any events of major significance such as death or other serious outcome for which a causal connection with the IP is plausible and represents an excess of the important adverse event(s).

To support the DSMB assessment, the Rebiotix Operations Department may be asked to review the donor history and batch processing/release records for the product unit(s) administered in these cases, and forward its report to the DSMB Chair for review. The DSMB Chair will review the Operations Department report. If probable cause is suspected, the DSMB Chair will convene the entire DSMB for event review.

Upon the DSMB's determination of action, the Chair will notify Rebiotix, who will notify the study sites.

If enrollment is stopped, Rebiotix and the study sites will assess for the occurrence of similar events, and evaluate IP and study records for possible root cause(s). Enrollment may be re-started upon further review and approval from the DSMB. As appropriate, the DSMB may recommend study termination or measures short of termination with the objective of reducing the risk of AEs.

11.14. Sponsor Reporting of Adverse Events

The sponsor will report all relevant information about suspected unexpected serious adverse reactions that are fatal or life-threatening as soon as possible to the FDA & Health Canada, and in any case no later than seven (7) days after knowledge by the sponsor of such a case, and that relevant follow-up information will subsequently be communicated within an additional eight (8) days.

All other suspected unexpected serious adverse reactions will be reported to the FDA as soon as possible but within a maximum of 15 days of first knowledge by the sponsor.

The sponsor will also inform all investigators as required.

11.14.1. Timeline for Reporting Requirements

The following describes the expedited safety reporting requirements by timeline for reporting and associated type of event:

- Within 7 calendar days of event discovery by Rebiotix

Rebiotix will notify FDA and Health Canada of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than seven (7) calendar days after the sponsor's initial receipt of the information.

- Within 15 calendar days of event discovery by Rebiotix

The AE must meet all three of these criteria for expedited reporting. Any study event that is:

- suspected adverse reaction
- serious
- unexpected

11.14.2. Additional Reporting Requirements

The sponsor will identify in IND safety reports all previous reports concerning similar AEs and analyze the significance of the current event in light of the previous reports.

11.14.3. Reporting AEs to FDA and Health Canada

Rebiotix will report AEs to FDA and Health Canada as required by applicable regulations. All AEs will be reported in the annual and final clinical study reports.

12. Sample Size and Statistical Considerations

This study has a Statistical Analysis Plan which contains complete details of the statistical methodology and analyses that will be employed for this study. A final analysis of safety and efficacy results will be analyzed and reported in a final report after the last subject has completed the 6-month follow-up visit for their study treatment, including 6-month follow-up after a second RBX2660 study treatment, if applicable.

12.1. Sample Size

The purpose of this study is to assess the safety and tolerability of RBX2660 in a recurrent CDI population that is broader and more inclusive than that included in prior studies using RBX2660. In order to continue to collect prospective safety information and allow open-label treatment access to a broad population of patients, up to 750 subjects will be treated with RBX2660.

Subjects who have signed informed consent forms but do not meet all eligibility criteria will be considered screen failures. Subjects who withdraw for any reason prior to administration of the enema will be replaced without counting toward the sample size treatment cap of 750 subjects.

12.2. Assessment of Safety and Tolerability

Assessment of the primary safety objective and other safety objectives will be performed on all subjects exposed to RBX2660 (Safety Population) and will include descriptive

statistics. Additional sub-analyses of safety data may be performed on sub-populations (e.g., subjects with irritable bowel disease or irritable bowel syndrome).

12.3. Assessment of Efficacy, Secondary Objectives

Assessment of secondary objectives will include descriptive statistics and be performed on pre-defined analysis populations including Intent-to-Treat (ITT), modified Intent-to-Treat (mITT) and Per-Protocol (PP). Additional sub-analyses of safety data may be performed on sub-populations.

Secondary Efficacy Endpoints:

1. ***Recurrence of CDI within 8 weeks of treatment.***

Recurrence of CDI after 8 weeks from completion of the study treatment is considered a new CDI.

The efficacy parameter used to determine treatment success is defined as the absence of CDI diarrhea through 8 weeks after completing a study treatment. Efficacy assessments will occur at each scheduled contact: 1-, 4- and 8-weeks.

2. ***Loss of sustained clinical response through 6 months after treatment***

Sustained clinical response is defined as treatment success of the presenting CDI recurrence and no new CDI episodes through 6 months after completing a study treatment. Efficacy assessments will occur at each scheduled contact: 4 and 6 months.

12.3.1. Analysis Population definitions

12.3.1.1. Safety Population

The safety population (SP) is defined as the population of subjects who had any treatment attempted or completed. The safety population will be used in analysis of all safety endpoints including the primary endpoint.

12.3.1.2. Intent-to-Treat Population

The ITT population is defined as all subjects enrolled.

12.3.1.3. Modified Intent-to-Treat Population

Analysis of the efficacy endpoints will use the modified Intent-to-Treat (mITT) population as a primary analysis population. The mITT population is defined as all subjects who successfully received treatment but excluding;

- subjects in whom treatment was attempted but not completed and;
- subjects who discontinue from the study prior to evaluation of treatment failure/success for the primary endpoint if the reason for exit is not related to CDI symptoms. Reason for exit will be captured on the exit form to allow for identification of such subjects. Examples of reasons unrelated to CDI symptoms may include:
  - Withdrawal of consent
  - Death unrelated to CDI

12.3.1.4. Per-Protocol Population

The per-protocol (PP) population will consist of all subjects who successfully received treatment analyzed according to the treatment they received, excluding;

- Subjects who have documented protocol deviations to inclusion or exclusion criteria.
- Subjects who exited prior to the 8-week efficacy evaluation if the reason for exit was not related to CDI symptoms in the same manner as the mITT population.

13. Study Administration

13.1. Institutional Review Board / Research Ethics Board Approval

The protocol, Informed Consent Form/HIPAA Form must be reviewed and approved by the respective IRB/REB and Rebiotix before subject recruitment and enrollment begins.

Prior to subject enrollment, a signed copy of the IRB/REB approval letter addressed to the investigator and a full copy of the IRB/REB-approved Informed Consent Form/HIPAA Form must be submitted to Rebiotix, certifying study approval.

Investigators are responsible for submitting and obtaining initial approval and continuing approval from the IRB/REB and forwarding copies of the approval letters to Rebiotix. The approval letters are to be kept in the Trial Master File (TMF) designated for this study.

13.1.1. US Sites

Investigators are responsible for submitting and obtaining initial approval and continuing approval from the IRB and forwarding copies of the approval letters to Rebiotix. The approval letters are to be kept in the Trial Master File (TMF) designated for this study.

The investigator will notify the Rebiotix study manager within five (5) business days of withdrawal of IRB approval.

Institutional Review Boards will operate in accordance to 21 CFR 56 and their own standard operating procedures.

13.1.2. Canadian Sites

Prior to subject enrollment, a signed copy of the REB approval letter addressed to the investigator, a full copy of the REB-approved Informed Consent Form, and completed Research Ethics Board Attestation must be submitted to Mapi Group, the Agent for Rebiotix in Canada, and to Rebiotix. Investigators are responsible for submitting and obtaining initial approval and continuing approval from the REB and forwarding copies of the approval letters to Rebiotix. Rebiotix will forward copies to Mapi Group. The approval letters are to be kept in the Trial Master File (TMF) designated for this study.

The investigator will notify Rebiotix within five (5) business days of withdrawal of REB approval. Rebiotix will notify Mapi Group.

REBs will operate in compliance with Health Canada regulations and their own standard operating procedures.

13.2. Form 1572 and Financial Disclosure

The principal investigator at each site (US and Canada) will complete and return a study-specific Form 1572 to the sponsor before beginning the study, as required by federal regulations. The investigator agrees to be responsible for conducting the investigational study in accordance with the protocol, applicable FDA regulations including reporting and record-keeping requirements, GCPs, local IRB/REB

requirements, and controlling dispensation and administration of RBX2660. In addition, the investigator is responsible for ensuring that informed consent is obtained from each subject prior to participating in the study, as well as protecting the rights, safety and welfare of participating subjects.

Individuals listed in Section 6 of Form 1572 are required to sign a Financial Disclosure form that certifies the individual and their immediate family's financial interest in Rebiotix and study outcomes. Investigators must inform Rebiotix of any changes to the information documented on the Financial Disclosure form throughout the course of the study and for a period of one year following completion of the study. In the event of a change in study personnel, the site may need to complete and submit a new Form 1572 to Rebiotix within 60 days of the change.

13.2.1. Canadian Sites: Qualified Investigator Undertaking Form

The principal investigator(s) at each Canada site will complete and return the Qualified Investigator Undertaking (QIU) Form to Rebiotix before beginning the study, as required by Health Canada. Rebiotix will forward the QIU to Mapi Group. The investigator agrees to be responsible for conducting the investigational study in accordance with the protocol, applicable Health Canada regulations including reporting and record-keeping requirements, GCPs, local REB requirements, and controlling dispensation and administration of RBX2660. In addition, the investigator is responsible for ensuring that informed consent is obtained from each subject prior to participating in the study, as well as protecting the rights, safety and welfare of participating subjects.

13.3. Subject Confidentiality

All information and data sent to Rebiotix, and/or its designees concerning subjects and their participation in this study are considered confidential by Rebiotix and its designees (subcontractors or contract research organization). Only authorized Rebiotix personnel or approved contracted agents of Rebiotix have access to some portions of these confidential files and will act in accordance with applicable regulations. The IRBs, REBs, FDA and Health Canada also have the right to inspect and copy all records pertinent to this study. All data used in the reporting of the study will eliminate identifiable references to the subjects as much as possible.

13.4. Study Oversight

Clinical personnel at Rebiotix have knowledge of Good Clinical Practices, pertinent laws and regulations, and documented training in standard operating procedures

pertaining to study management and monitoring and will provide study oversight. Rebiotix can be contacted at:

Rebiotix Inc
2660 Patton Road
Roseville, MN 55113
Phone: 651-705-8770

13.5. Study Site Qualification

Investigational center qualification visits or phone calls will be conducted by Rebiotix prior to acceptance of a site into this study. The site qualification visit or phone call will be scheduled to include time with the study investigator and study coordinator. Areas of discussion include a review of personnel training, investigator qualifications, IP Administrator qualifications, adequacy of potential subject pool, FDA- or Health Canada-regulated study experience, this study's specific requirements for procedures and equipment, and a review of staffing availability and appropriateness. A written report of the qualification call/visit will be generated by the sponsor representative who conducts the call/visit. Resolution of any concerns and/or completion of any appropriate study activities identified during the qualification process will be documented and submitted to the study investigator.

13.6. Investigator / Site Training

The sponsor will provide appropriate training prior to study initiation to each investigator, an IP Administrator, and study coordinator(s) at each site; other site personnel who will be involved in the study may be trained by the sponsor staff or the trained investigator. Training will address topics including ordering, secure storage and administration procedures, Subject Diary instructions, follow-up visit and phone call requirements, adverse event reporting, and accurate data collection. Training will include a detailed review of the protocol, eCRF completion, study-specific procedures, monitoring logistics, and regulatory requirements as applicable for the study roles.

13.7. Data Management

Electronic case report forms specifically created for this study will be used to collect study data. The study investigator or designee at each site is responsible for recording all data onto the study eCRFs. The investigator must review and electronically sign all eCRFs as instructed; these responsibilities cannot be delegated to another person.

Ongoing data review will be performed according to the study-specific Data Management Plan.

13.8. Monitoring

This study is monitored according to the study-specific Clinical Monitoring Plan. The investigator must allocate adequate time for such monitoring activities. The investigator must also ensure that the monitor or other compliance or quality assurance reviewers are given access to all study-related documents and study-related facilities (e.g., pharmacy, diagnostic laboratory), and has adequate space to conduct the monitoring visit. Study monitors and their activities are managed by Clinical Management or qualified designee; see **Section 13.4**. Study monitors will follow requirements as documented in monitoring procedures and the study-specific Monitoring Plan.

13.9. Direct Access to Source Data/Documents

The investigator is expected to facilitate study-related monitoring, audits, and inspections by the IRB/REB, sponsor and sponsor representatives, FDA, and Health Canada of all study-related documents including direct access to original source documents such as medical records and lab results, regulatory documents, study data, etc. The investigator will ensure access for the inspection of applicable study-related facilities (e.g., pharmacy, laboratory, exam rooms).

Participation as a study investigator and study site in this study implies acceptance and support of inspections by FDA, Health Canada and/or Rebiotix or its designee(s).

13.10. Investigator Responsibilities

The investigator is responsible for ensuring that the study is conducted according to the protocol, applicable FDA or Health Canada regulations for investigational new drugs, HIPAA (US only), GCPs and local IRB/REB requirements. Specific responsibilities are listed in this protocol.

Site study records and reports are kept in the Investigator Study File (ISF). Records and reports will remain on file for a minimum of two (2) years (US sites) or 25 years (Canada sites) after either the completion/termination of this study or the date RBX2660 receives market approval for the indication being studied, whichever is later. The study investigator must contact Rebiotix before destroying any records and reports pertaining to the study to ensure that they no longer need to be retained. Rebiotix must be

contacted if the study investigator plans to leave the site to ensure that arrangements for a new investigator or records transfer are made prior to investigator departure.

The investigator will promptly report to the IRB/REB all changes in the research activity and all unanticipated problems involving risk to subjects or others, and that they will not make any changes in the research without prior written approval from the sponsor and IRB. The investigator will adhere to all IRB/REB requirements imposed on this study.

13.11. Investigator Records

Records to be maintained by the investigator in the ISF include:

- Protocol and all amendments
- Signed Form 1572, or equivalent
- Signed Financial Disclosure Form(s)
- IRB approval letter including consent and HIPAA (US sites)
- IRB Membership list or Letter of Assurance (US sites)
- Signed Qualified Investigator Undertaking Form (Canadian sites)
- REB approval letter including the Informed Consent Form (Canadian sites)
- REB Attestation (Canadian sites)
- REB membership list or Letter of Assurance (Canadian sites)
- All correspondence relating to the study between the site/investigator/coordinator and the IRB/REB, sponsor, and CRO, as applicable.
- CVs and professional licenses for all investigators and key study personnel, as applicable
- Delegation of Authority and Site Signature Log
- Product Accountability Log
- Study Site Visitor Log
- Study Site Training Log
- Reports submitted to Rebiotix and/or the IRB/REB

The following records must be maintained for each subject enrolled in the study:

- Signed Informed Consent/local privacy authorization as applicable
- Compete, accurate and current eCRFs
- Adverse event reports and any supporting documentation
- Protocol deviations

- Relevant source documents, including procedure reports, lab reports, professional notes, etc.
- Records pertain to subject death during the investigation including death records, death certificate, and autopsy report if performed.

Rebiotix reserves the right to secure data clarification and additional medical documentation on subjects enrolled in this study at any time.

13.12. Investigator Reports

Study investigators are required to submit the following reports at the times specified below:

- Adverse Events: report all AEs to the sponsor via eCRF within **ten (10) business days** of site awareness.
- Serious Adverse Events and suspected adverse reactions: report to the sponsor via eCRF within 24 hours of site awareness.
- Progress and Final reports: the investigator will submit annual study progress reports and a final study report when the study is terminated at the site to Rebiotix and the IRB/REB, as required.

13.13. Study Site Termination

Rebiotix reserves the right to terminate a study site for any of the following reasons:

- Failure to properly secure subject informed consent or HIPAA Authorization prior to study enrollment or the conduct of any study-required procedures/assessments
- Failure to report adverse events as required in **Sections 11.2 and 11.6**
- Protocol deviations
- Repeated failure to appropriately and accurately complete eCRFs
- Failure to enroll an adequate number of subjects
- Loss of or unaccounted for product inventory
- Administrative decision by the company

13.14. Protocol Amendments

Neither Rebiotix, its designees (subcontractors or CRO), nor the study investigators may modify this protocol without obtaining written approval of the FDA, Health Canada,

and/or IRB/REBs as required. No modifications may be made without prior written approval of Rebiotix.

13.15. Protocol Deviations

Any deviations from this protocol undertaken to protect the life or physical well-being of a subject in an emergency situation must be reported to Rebiotix within 48 hours of occurrence and to the respective IRB/REB as soon as possible, but in no event no later than five calendar days after the emergency occurs. Protocol deviations of any kind must be avoided as much as possible and those that do occur will be tracked in the clinical study database.

13.16. Inspection/Auditing

The investigator will permit study-related inspection/auditing by the IRB/REB, sponsor, FDA and/or Health Canada to ensure compliance to applicable regulations, GCPs, and the study protocol.

14. Publication Plan

Rebiotix has unrestricted publication rights of the study data. The study site and the investigator are not entitled to make any publication or release any information pertaining to this study or its results without the prior written consent of Rebiotix. The decision as to whether to provide such consent shall be made once Rebiotix has had an opportunity to review the contents of any proposed publication or release regarding the investigation and, if necessary, to delay any publication or release in order to protect the confidential or proprietary nature of any information contained therein. Merely the fact that the proposed publication or release contains statements unfavorable to Rebiotix shall not constitute grounds for prohibiting publication; however, all unfavorable statements must be based on adequate scientific evidence. Rebiotix reserves the right to give the data to third parties for publication or release and to name co-authors. Rebiotix retains the right to review and edit all proposed manuscripts, abstracts, publications, and presentations based upon this study or its results prior to submission to any organization, business, agency, person, publisher, society, or other entity.

15.References

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Kelly CR, Ihunnah C, Fischer M, et al. Fecal Microbiota Transplant for Treatment of *Clostridium difficile* Infection in Immunocompromised Patients. *Am J Gastroenterol*. 2014: 109(7): 1065-1071.

NIH/NCI Common Terminology Criteria for Adverse Events found at online at the following address: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

16. Comorbidity Index Variables

Myocardial Infarction: ❍ Yes ❍ No

Congestive Heart Failure: ❍ Yes ❍ No

Peripheral Vascular Disease: ❍ Yes ❍ No

Cerebrovascular Disease: ❍ Yes ❍ No

COPD: ❍ Yes ❍ No

Asthma: ❍ Yes ❍ No

Connective Tissue Disease: ❍ Yes ❍ No

Diabetes Mellitus: ❍ Yes ❍ No
Type: ❍ Type I ❍ Type II
End-organ damage: ❍ Yes ❍ No

Chronic Kidney Disease (Moderate to severe): ❍ Yes ❍ No

Liver Disease: ❍ Yes ❍ No
Severity: ❍ Mild ❍ Moderate ❍ Severe

Hypertension: ❍ Yes ❍ No

Hemiplegia: ❍ Yes ❍ No

Chronic Pain: ❍ Yes ❍ No

Dementia: ❍ Yes ❍ No

Migraines: ❍ Yes ❍ No

Depression: ❍ Yes ❍ No

Anxiety: ❍ Yes ❍ No

Anemia: ❍ Yes ❍ No

Leukemia: ❍ Yes ❍ No

Malignant Lymphoma: ❍ Yes ❍ No

Solid Tumor: ❍ Yes ❍ No
Metastatic: ❍ Yes ❍ No

Eczema: ❍ Yes ❍ No

Acne: ❍ Yes ❍ No

Psoriasis: ❍ Yes ❍ No

AIDS/HIV: ❍ Yes ❍ No

Parkinson's Disease: ❍ Yes ❍ No

Rheumatoid Arthritis: ❍ Yes ❍ No

Systemic Lupus Erythematosus: ❍ Yes ❍ No

Sjogren's Syndrome: ❍ Yes ❍ No

CREST Syndrome: ❍ Yes ❍ No

Scleroderma: ❍ Yes ❍ No

Chronic Urinary Tract Infections: ❍ Yes ❍ No

Irritable Bowel Syndrome: ❍ Yes ❍ No

Crohn's Disease: ❍ Yes ❍ No

Ulcerative Colitis: ❍ Yes ❍ No

Peptic Ulcer Disease: ❍ Yes ❍ No

History of Constipation: ❍ Yes ❍ No

Gastroparesis: ❍ Yes ❍ No

Gastroesophageal Reflux Disease (GERD): ❍ Yes ❍ No

Alopecia (hair loss): ❍ Yes ❍ No

17. Cdiff 32 Health-Related Quality of Life Questionnaire

To be completed by the subject

DAILY ACTIVITIES

Over the last 7 days, because of your *C. diff* infection,

1. Have you had any difficulties and/or disruption carrying out your daily activities?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

2. Have you had any difficulties carrying out your leisure activities like gardening, walking, etc?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

3. Has it taken you longer to perform certain tasks at work (including work in the home)?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

4. Has your *C. diff* infection prevented you from leaving your house?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

ANXIETY

5. Are you afraid that your *C. diff* infection could come back again?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

6. Are you afraid that your *C. diff* infection could get worse in the future?

☐1	☐2	☐3	☐4	☐5

Not at all	A little bit	Moderately	Quite a bit	Extremely

7. Are you afraid that the next time you'll need antibiotics, your *C. diff* infection will appear again?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

Over the last 7 days,

8. Have you been worried about not knowing when the next diarrhea would arise?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

DIET

9. Are you afraid that certain food will worsen your *C. diff* infection?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

Over the last 7 days, because of your *C. diff* infection,

10. Have you felt frustrated about what you can eat and when?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

SLEEP

Over the last 7 days,

11. Because of your *C. diff* infection, have you had trouble sleeping?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

12. Because of your *C. diff* infection have you been woken up from sleep?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

DISCOMFORT

Over the last 7 days,

13. Have you been bothered by abdominal pain?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

14. Have you been bothered by flatulence (wind)?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

15. Have you been bothered by a bloated stomach?

☐1	☐2	☐3	☐4	☐5
Not at all	A little bit	Moderately	Quite a bit	Extremely

16. Have you avoided wearing some clothes (tight clothes, dress, light-colored clothes ...)?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

17. Have you been bothered by the smell caused by your *C. diff* infection related diarrhea?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

18. Have you been bothered by how much time you spend on the toilet?

☐1	☐2	☐3	☐4	☐5
Never	Rarely	Sometimes	Often	Always

COPING WITH DISEASE/ HEALTH PERCEPTION

Note! The following sentences are statements. Please indicate whether you agree or disagree with these statements.

19. Despite my C diff infection I can live a normal life.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

CONTROL OF DISEASE

20. I feel that I am not in control of my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

21. I have no idea what I should do when I have my *C. Diff* infection?

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

IMPACT OF STRESS

22. I believe that any stress can worsen my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

DYSPHORIA

23. I feel irritable because of my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

24. I feel isolated from others because of my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

25. I feel depressed because of my *C. diff* infection

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

26. I feel my life is less enjoyable because of my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

27. I worry about transmitting my *C. diff* infection to my family and/or friends.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

28. I feel much stressed because of my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

RELATIONSHIPS

29. Because of my *C. diff* infection, I have difficulty being around people I do not know.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

30. My *C. diff* infection is affecting my closest relationships.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

SOCIAL REACTION

31. I feel like I irritate others because of my *C. diff* infection.

☐1	☐2	☐3	☐4	☐5
Totally disagree	Mostly disagree	Don't know	Mostly agree	Totally agree

32. How would you rate your overall quality of life during the past week (that is, how have things been going for you)?

☐1	☐2	☐3	☐4	☐5
Very bad: could hardly be worse	Pretty bad	Good and bad part about equals	Pretty good	Very well: could hardly be better