Statistical Analysis Plan

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Protocol 2019-01

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

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Revision History

Version	Date	Reason
1.0	15Jan2019	Initial Release
2.0	12Apr2019	Added 4-week assessment for second enema follow-up, clarified visit windows, analyses and analysis populations.
3.0	06Sep2019	Removed reference to a minimum number of days for antibiotic use, aligning requirement to the revised protocol v4.0.
4.0	24Mar2022	• Increased enrollment allotment to allow up to 600 treated subjects *Note:* the enrollment allotment was previously updated (15Oct20) to include up to 500 treated subjects. SAP was not revised at that time • 6.2.1: Table 3 added to include Analysis Windows for AE reporting • 7.6.2.5: specified 14 daily questions. • 8.8: Subgroup analysis test updated to include ITT and an appropriate 2-dimensional test (Chi-Square test).
5.0	27Jun2023	• General clarifying changes throughout the document • Increased enrollment allotment to allow up to 750 treated subjects • 6.2.1 Table 3 added additional AE analysis windows • Added 7.6.2.6 Subgroup Safety Analysis • Added 7.10 Subgroup Efficacy Analysis • 8.8 updated subgroup analysis to include immunocompromising conditions and inflammatory bowel disease (IBD)

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1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	adverse event
ATC	anatomical therapeutic chemical
CDC	Centers for Disease Control
CI	confidence interval
CDI	*Clostridium difficile* infection
Cdiff32	CDI-specific health-related quality of life survey instrument
CSR	Clinical Study Report
DSMB	Data Safety Monitoring Board
eCRF	electronic case report form
FDA	Food and Drug Administration
HIV	human immunodeficiency virus
HLGT	High level group term
HLT	High level term
IBD	inflammatory bowel disease
ITT	intent-to-treat
IP	investigational product
ICU	intensive care unit
IQR	Interquartile range
IVIG	intravenous immunoglobulin
LLT	Lower-level term
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
mL	milliliters
N	number of subjects
PP	per-protocol
PT	preferred term
RBX2660	investigational drug product being evaluated
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SMQ	Standardized MedDRA query
SOC	system organ class
TEAE	treatment-emergent adverse event
US	United States
WHO	World Health Organization

INTRODUCTION

RBX2660 (microbiota suspension) is being studied for the prevention of recurrent *Clostridium difficile* infection (CDI) in adult subjects. 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.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

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

2.2 Secondary Objective

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.

2.3 Other Objectives

1. To evaluate health-related quality of life in subjects with CDI as measured by the CDI-specific health-related quality of life survey instrument (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.

3 STUDY DESIGN

3.1 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 750subjects 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.

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 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, the follow-up is completed via telephone assessments at 1-week, 4-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.

3.1.1 *Treatment Success*

Treatment success is defined as:

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

3.1.2 *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.

3.1.3 *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.

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.

3.1.4 *Definition of Indeterminate Treatment Outcome*

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

3.1.5 *Determination of Treatment Outcome*

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

3.2 Blinding

This study is not blinded.

3.3 Determination of 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 broader population of patients, up to 750 subjects will be treated with RBX2660. As an open-label study, there are no statistical powering considerations for the revised sample size.

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.

3.4 Treatment

Active treatment consists of one dose of RBX2660 delivered via enema.

4 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

4.1 Changes in the Conduct of the Study

There have been no changes to the conduct of the study or planned analysis, however, ad hoc analyses may be performed as needed to support regulatory review.

5 BASELINE, SAFETY AND EFFICACY EVALUATIONS

5.1 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-week (± 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]					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 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.

5.2 Time Point Algorithms

5.2.1 *Relative Day*

The date of study enema administration will be considered relative day 1, and the day before study enema administration will be relative day -1. Relative days will be calculated as follows only when the full assessment date is known (i.e., partial dates will have missing relative days, unless imputation methods have been specified):

For days before first enema:

Date of Assessment – Date of first enema administration

For days on or after first enema:

Date of Assessment – Date of first enema administration +1

For the purpose of statistical analysis, the analysis visit windows will be calculated in terms of study days since the day of the study enema administration, as illustrated in the following tables:

Table 1. Analysis Windows for Clinical Visits

Visit	Scheduled Study Day	Visit Window in Protocol (Days)	Visit Window for Analysis (Days)
Screening Visit	-20 through -1	-20 through -1	-20 through -1
Baseline/1st Enema	1	1	1
1-week Office	8	5 - 11	5 - 11
8-weeks Office	57	54 - 60	54 - 60

Table 2. Analysis Windows for Phone Assessments

Visit	Scheduled Study Day	Visit Window in Protocol (Days)	Visit Window for Analysis (Days)
4-weeks - Phone	29	26 - 32	26 - 32
4 months - Phone	121	106 – 134	106 – 134
6 months - Phone	181	166 – 194	166 – 194

Table 3. Analysis Windows for Adverse Event Reporting by Onset Interval

Onset Interval Name	Onset Interval Analysis Window	Window for Analysis (Days)
Baseline	Screening - < Baseline	≤ -1
1-week	Baseline - 1-week	1 through 8
4-week	> 1-week - 4-week	9 through 29
8-week	> 4-week - 8-week	30 through 57
0-8-week	Baseline - 8-week	1 through 57
4-month	> 8-week - 4-month	58 through 121
6-month	> 4-month - 6-month	122 through 180+
8-week-6-month	> 8-week - 6-month	58 through 180+

If a subject has more than one assessment occurring in the same visit window, the data from the visit closest to the scheduled study day will be used for analysis. If two visits have the same distance from the scheduled study day, i.e., one visit occurs -1 day from study day, and the other visit occurs +1 day from study day, the data from the visit after the scheduled study day will be used for analysis.

5.3 Analysis Populations

5.3.1 *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.

5.3.2 *Intent-to-Treat Population*

The ITT population is defined as all subjects enrolled, excluding screen failures.

5.3.3 *Modified Intent-to-Treat Population*

Analysis of the efficacy endpoints will use the modified Intent-to-Treat (mITT) population as a primary efficacy 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 8-week efficacy 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

5.3.4 *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.

5.4 Safety Variables

Assessment of the primary safety objective and other safety objectives will be performed on all subjects exposed to RBX2660 (Safety Population, see Section 6.3.1), and will include descriptive statistics. Additional sub-analyses of safety data may be performed on sub-populations.

5.4.1 *Primary Safety Endpoint*

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

5.4.2 *Other 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 intensive care unit (ICU) admission
8. Onset of new chronic conditions relative to treatment administration

5.4.3 ***Adverse Events***

An adverse event (AE) is any untoward medical occurrence associated with the use of IP, whether or not the event is considered product-related. Preexisting conditions that worsen in frequency, intensity or character of the condition during study participation will be recorded as an AE. AEs will be recorded for each subject from the day of enrollment through the 6-month telephone assessment. Serious adverse events (SAEs) will be recorded for each subject from the day of enrollment through the duration of the study.

5.4.3.1 Adverse Event Dictionary

AEs and SAEs will be classified by the Medical Dictionary for Regulatory Activities (MedDRA) version 20.0. The verbatim term recorded by the investigator will be mapped in MedDRA and system organ class (SOC), high level group term (HLGT), high level term (HLT), preferred term (PT), and lower level term (LLT) will be attached to the clinical database. Tables and listings will present data at the SOC and PT level.

5.4.3.2 Adverse Event Severity

Severity of AEs will be graded as mild, moderate, severe, or potentially life-threatening. The severity grade of events for which the investigator did not record severity will be categorized as "Unknown" for tabular summaries and data listings and will be considered the least severe for the purposes of sorting for data presentation. For summaries by severity grade, if a subject has multiple events occurring in the same SOC or PT, then the most severe event will be selected.

5.4.3.3 Relationship of Adverse Events to Study Treatment

Relationship of an AE to the Investigational Product (IP), the enema procedure, *C. difficile* disease, and a preexisting condition will be classified by the investigator as definite, probable, possible, or unrelated. Related events will include AEs that are classified as definitely, probably, or possibly related.

5.4.3.4 Treatment-Emergent Adverse Events

Adverse events will be considered treatment-emergent adverse events (TEAE) according to the following algorithm:

- If the complete onset date of an AE is known, then:
    - If AE onset date is prior to initial treatment date, then the AE will not be considered treatment-emergent.

- If AE onset date occurs after IP exposure on or after initial treatment date, then the AE will be considered treatment-emergent.
    - If AE onset date is partially known, then:
    - If day is unknown, and month/year occurs on or after first enema month/year, then AE will be considered treatment-emergent. Otherwise, AE will not be considered treatment-emergent.
    - If month/day is unknown, and year occurs on or after first enema year, then AE will be considered treatment-emergent. Otherwise, AE will not be considered treatment-emergent.

5.4.4 ***Other Observations Related to Safety***

5.4.4.1 Solicited Adverse Events

The following list of anticipated adverse events are solicited from subjects via the Subject Diary from the date of enrollment through the 7th day after receiving the study treatment.:

- 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)

Frequency and severity of the solicited AEs will also be captured in the Subject Diary.

5.4.4.2 Major Complications of CDI Events

Frequencies of major complications of CDI including death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, or ICU admission will be collected through the 6-month telephone assessment after the last study enema.

5.4.4.3 Hospitalizations for Recurrent CDI

Details regarding hospitalizations due to CDI, including admission and discharge date/time, reason for admission, whether the subject was admitted to an ICU, and ICU admission and discharge date/time (if applicable), will be collected following both the first and second study enemas

5.5 Efficacy Variables

5.5.1 *Efficacy Endpoint*

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.

1. Efficacy Endpoint: Recurrence of CDI within 8 weeks of treatment.

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

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 office visit: 1-, 4- and 8-weeks.

2. Efficacy Endpoint: 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 phone assessment: 4 and 6 months.

5.6 Other Endpoints

Other endpoints include the following:

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 for subjects who were carriers at Baseline at 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

6 STATISTICAL ANALYSIS

6.1 Disposition of Subjects

A summary table will present subject disposition for all subjects enrolled. The table will show the number of subjects enrolled (consented), the number of enrolled subjects not treated, and the number of subjects treated. The proportion of subjects who complete the study and who discontinue the study will be presented. Separately, a table or listing will be created to document the subjects that are included in each analysis population.

Discontinuation will be categorized by reason as a percentage of the number of subjects in each analysis population and overall. Additionally, subjects who were successfully treated but withdrew prior to the 8-week efficacy endpoint evaluation will be identified along with the reason for withdrawal to document which analysis population(s) to which they contribute.

6.2 Protocol Deviations

Protocol deviations will be tracked. The information available will be summarized by deviation type and listed by individual site.

6.3 Baseline Assessments

Baseline assessments are collected within 20 days before or on the day of study treatment administration. Summaries for age by group (< 65 and ≥ 65), sex, ethnicity, and race will be provided. Baseline height, weight and employment status will also be summarized. CDI history will be summarized by total number of episodes experienced prior to treatment. Additionally, the following will be summarized for each episode of CDI: duration (days), treatment administered, hospitalization with duration (if applicable) and available *C. difficile* test results.

The following Baseline assessments will be provided in summary table and listings:

- Medical history

- CDI history
- Laboratory testing (hematology)
- Vital signs (systolic/diastolic blood pressure [mmHg], pulse rate [beats/min], respiration rate [breaths/min], temperature [°C])

6.4 Demographic and Other Baseline Characteristics

All Baseline summaries will include the SP, ITT, mITT, and PP populations. Descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, interquartile range [IQR], minimum, and maximum for continuous variables and number of subjects [N] and the percentage for categorical variables) will be provided for all Baseline measures.

6.5 Prior and Concomitant Therapy

The World Health Organization (WHO) Drug Dictionary version 2017E B2 will be used to classify medications by preferred term and WHO Anatomical Therapeutic Chemical (ATC) at the highest level of ATC. Listings of all concomitant medications will be made by WHO ATC classification of ingredients and by preferred term. All listings of usage of medications will be based on the SP.

6.6 Safety Analysis

The SP will be used to summarize all adverse event data, unless otherwise specified. Safety data will be summarized for events after the first and second enemas, as well as combined. Events occurring after the first enema will be counted up to the date of retreatment, or study exit. Events on, or after, the date of a second enema will be assigned to the second period. The combined analysis will consider all events, after first enema through study exit, even if a subject receives a second enema.

6.6.1 *Analyses Supporting the Primary Safety Endpoint*

Assessment of the primary safety objective will include descriptive statistics and be performed on the SP. Additional sub-analyses of safety data may be performed on sub-populations.

6.6.2 *Other Safety Analyses*

6.6.2.1 Adverse Events

A summary of adverse events, TEAEs, non-serious TEAEs, and serious TEAEs will be summarized using counts of the number of events and frequencies (counts and percentages) of

the number of subjects. The incidence of each preferred term (PT) within the primary system organ class (SOC) as well as overall primary SOC incidence will be presented. Summaries may also be presented by PT, irrespective of SOC. A subject with multiple events coded to the same PT within a primary SOC will be counted only once for the PT within the primary SOC. Likewise, a subject with multiple events coded to the same SOC will be counted only once within the SOC.

An overall summary of AEs will present the incidence of (1) all TEAEs, (2) TEAEs related to IP, (3) TEAEs related to enema procedure, (4) TEAEs related to *C. difficile* disease, (5) TEAEs related to a preexisting condition, (6) serious TEAEs, (7) serious TEAEs related to IP, (8) serious TEAEs related to the enema procedure, (9) serious TEAEs related to *C. difficile* disease, (10) serious TEAEs related to a preexisting condition, and (11) TEAEs leading to death.

Frequency of occurrence and number and percent of subjects experiencing an event through 6 months follow-up will be presented for both the first and second enemas as follows:

- All TEAEs
- TEAEs by maximum severity
- TEAEs by relatedness to IP
- TEAEs by relatedness to *C. difficile* disease
- TEAEs by relatedness to enema procedure
- TEAEs by relatedness to preexisting condition
- TEAEs resulting in subject withdrawal
- TEAEs resulting in death
- Serious TEAEs
- Serious TEAEs by maximum severity
- Serious TEAEs by relatedness to IP
- Serious TEAEs by relatedness to *C. difficile* disease
- Serious TEAEs by relatedness to enema procedure
- Serious TEAEs by relatedness to preexisting condition
- Serious TEAEs resulting in subject withdrawal
- Serious TEAEs resulting in death

For the summary of AEs by maximum severity, if a subject has multiple events occurring in the same SOC or same PT, then the event with the highest severity will be counted. For AEs reported by causality, if a subject has multiple events occurring in the same SOC or same PT, the event with the highest association will be summarized.

A summary of TEAE and serious TEAE characteristics (severity, relatedness to IP, relatedness to *C. difficile* disease, relatedness to enema procedure, and relatedness to preexisting condition by outcome) will also be presented by first and second enema and overall.

Incidence of AEs and serious AEs will also be presented by onset interval separately for the first and second enemas (as applicable), as well as a combined presentation for the following intervals:

- Baseline (onset date prior to date of first enema)
- IP administration (onset date on the date of the first and second enema, as applicable)
- Intervals relative to the enema date (first and second enemas, as applicable):
    - 1-Week (day 8), 4-Weeks (day 29), 8-Weeks (day 57), 4 Months (Day 120), and 6 Months (Day 180).

For data presentation, SOC will be ordered alphabetically, with PT sorted by decreasing total frequency. No inferential statistics will be provided for the AE data.

Subject listings will be presented for all AEs including SAEs.

6.6.2.2 Major Complications of CDI events

Major complications of CDI events include death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, or ICU admission collected through the 6-month telephone assessment after the last study enema. Incidence of major complications of CDI events will be summarized by the first and second enemas.

6.6.2.3 Hospitalizations for Recurrent CDI

The number of subjects requiring hospitalization due to recurrent CDI through 8 weeks following the completion of the study treatment will be summarized, along with the length of hospital stay, the number of subjects requiring ICU admission, and the length of ICU stay during hospitalization.

A listing of all hospitalizations due to CDI throughout the study will be generated.

6.6.2.4 Rate of onset of new chronic conditions for those treated with RBX2660

Documentation of physician diagnosed chronic conditions for all subjects will be completed at Baseline and compared to new physician diagnosed chronic conditions reported at 1-week, 4-weeks, 8-weeks, 4 months and 6 months. Chronic condition is defined as a condition lasting >3 months. This will be presented as a rate of chronic conditions onset for the combined first and second enemas, as well as separately for first and second enemas.

6.6.2.5 Subject Diary including Solicited Adverse Events and Completion Compliance

All solicited adverse events recorded in the Subject Diary will be summarized utilizing frequencies (number of events), proportions (number of subjects with the event) and 95% CIs for categorical variables and using the descriptive statistics as listed in **Section 8.1** for continuous variables.

For each solicited event, severity scores will be summarized as both categorical and continuous values. For continuous summaries, severity scores will be identified as either pre-treatment or post-treatment (all diary entries captured after the first enema), and the average for each period will be calculated across individual subjects. Baseline for continuous summaries will be considered the average during the pre-treatment period. For categorical summaries, if a subject reports more than one severity score on the same date, the maximum severity will be used. Baseline for categorical summaries will be considered the last non-missing assessment prior to the enema.

For each categorical summary of event frequency, a two-sided 95% CI will be calculated using the normal approximation of the binomial.

The following summaries will be produced for the first enema:

- Frequency of severity scores on day of enema administration
- Frequency of severity scores on day 1 through 1-week post-treatment
- Frequency of maximum post-treatment severity score

Compliance in returning completed Subject Diaries will be calculated by dividing the number of diaries turned in, whether complete or not, by the number of diaries expected to be turned in. Furthermore, compliance will be summarized by calculating the proportion of subjects who turned in “complete” diaries, where complete is defined as answering all 14 daily questions (e.g.

11 solicited AE, Bristol Stool Type, New illnesses/injuries, New medications) for at least 80% of the expected number of diaries.

6.6.2.6 Subgroup safety analysis

The overview of AEs and TEAEs by SOC and PT will be summarized by the following subgroups:

- Subjects with immunocompromising conditions;
- Subject without immunocompromising conditions;
- Subjects with inflammatory bowel disease at time of IP administration;
- Subject without inflammatory bowel disease at time of IP administration.

Note: Inflammatory bowel disease includes the medical history with PT term "Ulcerative colitis", "Crohn's disease", or "Inflammatory bowel disease".

6.7 **Efficacy Analysis**

6.7.1 ***Analyses Supporting the Efficacy Endpoints***

6.7.1.1 Treatment Success

Assessment of secondary efficacy objectives will include descriptive statistics and will be performed on the mITT population. Additional sub-analyses of safety data may be performed on sub-populations.

6.7.1.1.1 Recurrence of CDI within 8 weeks of treatment

Recurrence of CDI is defined as the presence of CDI diarrhea within 8 weeks (54-60 days) of administration of a study enema, which includes a positive stool test for *C. difficile* as determined by the central laboratory. A CDI with an onset after 8 weeks (> 60 days) from completion of the study treatment is considered a new CDI. Onset of CDI is the date that the symptoms first started.

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-week, 4-weeks, 8-weeks.

6.7.1.1.2 Sustained clinical response

The endpoint of interest is sustained clinical response (i.e., the rate of treatment success of the presenting CDI recurrence and no new CDI episodes for greater than 8 weeks after completing a study treatment). Efficacy assessments will occur at the 4- and 6-month phone assessments.

6.7.1.2 Sensitivity Analysis for the Efficacy Endpoints

The efficacy analyses will be repeated using the ITT and PP population to assess the sensitivity of the endpoints. The ITT analysis will serve as the conservative analysis as it considers all subjects who exit prior to the 8-week efficacy assessment as treatment failures regardless of treatment failure documentation.

Finally, the sensitivity of the efficacy endpoints will be assessed in a multivariate logistic regression adjusting for possible interactions of the following covariates: age (< 65 years, ≥ 65 years), sex (Female, Male), race group (White, Non-white), ethnicity (Hispanic-Latino, not Hispanic-Latino), site geography (outside the US, Eastern US*, Southern US*, Northern US*, Western US*), and categories of number of previous episodes of CDI recurrence at Baseline (≤ 3, > 3). Comorbidities will also be reviewed for possible interactions. This analysis will be performed on all populations where at least 20 subjects are available in each sub-group.

*States included in the identified US geographical regions are as follows:

- Eastern US: Connecticut, Delaware, District of Columbia, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, Virginia, West Virginia
- Southern US: Alabama, Arkansas, Georgia, Florida, Kansas, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas
- Northern US: Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Wisconsin
- Western US: Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming

6.8 **Other Endpoints Analyses**

Additionally, the other analyses will be conducted on other populations or subgroups as needed.

6.8.1 *Comparison of Baseline characteristics in subjects with treatment success with one dose of RBX2660 vs. those who were unsuccessful.*

A multivariate analysis or regression analysis will be used to identify predictors (if any) for subject success with one RBX2660 dose vs. those who were unsuccessful, for example, age or number of prior CDI events.

6.8.2 *Subject fecal microbial composition at Screening, 1-week, 4-weeks, 8-weeks, 4 months, and 6 months after study treatment.*

Descriptive statistics will be used to present the changes from Screening fecal microbial composition to those at 1-week, 4-weeks, 8-weeks, 4 months, and 6 months after study treatment for those subjects treated with RBX2660.

6.8.3 *Cdiff32 scores at Screening, 1-week, 8-weeks, 4 months, and 6 months.*

Changes from Screening in the Cdiff32 scores will be summarized using descriptive statistics. Last observation carried forward (LOCF) will be used to impute missing post-Baseline data. No inferential statistics will be performed.

6.8.4 *Recurrence of CDI within 8 weeks of second RBX2660 treatment in subjects.*

Determine the treatment success rate of those subjects who received two doses of RBX2660. This success rate will be calculated at 8 weeks following the second RBX2660 enema.

6.8.5 *Concentration of vancomycin-resistant enterococci in stool samples for subjects who were carriers at Baseline.*

Lab generated values for the presence of vancomycin resistant enterococcus based on submitted stool samples at Baseline and at 1-week, 4-weeks, 8-weeks and 4 and 6 months will be assessed for all subjects to identify any trends in the clearance of vancomycin-resistant enterococci following enema treatment.

6.8.6 *Presence of C. difficile in stool samples at Screening, 1-week, 4-weeks, 8-weeks, 4 months, and 6 months after study treatment.*

Lab generated values for the presence of *C. difficile* based on submitted stool samples at Baseline and at 1-week, 4-weeks, 8-weeks, 4 months, and 6 months will be assessed for all subjects to identify any trends in the clearance of *C. difficile* following enema treatment.

6.9 Extent of Exposure to Study Treatment

The number and proportion of enemas (RBX2660) administered for each subject will be summarized using descriptive statistics. Time between study enema administrations will be summarized using descriptive statistics for both first and second enemas.

6.10 Subgroup Efficacy Analysis

The Chi-Square test will be used to assess the difference between the following additional subgroups:

- Subjects with immunocompromising conditions vs. Subject without immunocompromising conditions;
- Subjects with inflammatory bowel disease vs. Subject without inflammatory bowel disease at time of IP administration.

Note: Inflammatory bowel disease includes a medical history with PT term “Ulcerative colitis”, “Crohn's disease”, or “Inflammatory bowel disease”.

7 STATISTICAL METHODS

7.1 General Methodology

Data will be summarized using descriptive statistics with SAS® Version 9.3 or higher. Confidence intervals (CI) will be constructed at the level of 95% unless specified otherwise. For continuous variables, descriptive statistics (number of subjects [N], mean, standard deviation [SD], median, inter-quartile range [IQR], minimum, and maximum) will be generated. For discrete/categorical variables, the number and percentage of non-missing subjects will be generated. Standard operating procedures (SOPs) will be followed in the creation, validation and quality control of all data displays and analyses.

Subject listings of all data from the eCRFs as well as any derived variables will be presented.

7.2 Adjustments for Covariates

No adjustments for covariates are planned as this is a single-arm study.

7.3 Handling of Dropouts or Missing Data

No imputations will be used in analyses/summaries. However, a LOCF analysis will be used for evaluation of the Cdiff32 scores. Missing safety data will not be imputed beyond the adjustments for adverse event data mentioned in **Section 6.4.3**.

7.4 Safety Oversight

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 (see **Section 8.5.1**).

7.5 Final Analyses and Data Monitoring

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.

7.5.1 Study Stopping Rules

Enrollment will be paused if any of the following events are identified and the DSMB determines that there is probable cause that the IP or enema procedure contributed to the event:

1. 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

2. Any series of 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).

7.6 Multi-center Studies and Pooling of Centers

The study will be conducted at up to 80 clinical trial sites in the US and Canada.

This is a multicenter study and before data from all sites are pooled, the effect of site will be assessed for the secondary efficacy endpoints. A sufficient number of subjects (≥ 7) will be

required within each investigative site to determine if there are any outlying sites. If an investigative site is unable to enroll a sufficient number of subjects, it may be necessary to combine smaller sites (where enrollment is too low to detect differences) to create "pseudo-sites". Sites with < 7 subjects will be considered eligible for pooling. Eligible sites will be pooled sequentially with other sites in the same region until a sufficient number of subjects are reached (at least seven).

In this process, sites with < 7 subjects will be ordered numerically in ascending order, based on the investigative site number. Sites with < 7 subjects will then be sequentially pooled with other sites in the same region in ascending order by site number until the resulting pseudo-site has at least 7 subjects meeting the PP population definition, thus creating a new "pseudo-site". The process will begin again with the next sequential small site until all small sites have been pooled with other sites in the same region into a pseudo-site.

7.7 Multiple Comparisons/Multiplicity

As this study is not powered for a primary objective, and as descriptive statistics will be used for analysis of the primary objective, no adjustments for multiplicity are required.

7.8 Examination of Subgroups

Subgroup analysis of age (< 65 years, ≥ 65 years), sex (Female, Male), race group (White, Non-white), ethnicity (Hispanic-Latino, not Hispanic-Latino), site geography (outside the US, Eastern US*, Southern US*, Northern US*, Western US*) and number of previous episodes of CDI recurrence at Baseline will be conducted on the efficacy analysis if a sufficient sample size exists for each subgroup. This analysis will include the ITT and mITT analysis populations to understand any differences.

*States included in the identified US geographical regions are as follows:

- Eastern US: Connecticut, Delaware, District of Columbia, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, Virginia, West Virginia
- Southern US: Alabama, Arkansas, Georgia, Florida, Kansas, Kentucky, Louisiana, Mississippi, North Carolina, Oklahoma, South Carolina, Tennessee, Texas
- Northern US: Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Wisconsin

- Western US: Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, Oregon, Utah, Washington, Wyoming

Other subgroups will include subjects with a, medical history of Ulcerative Colitis (UC), Crohn's Disease (CD), and/or Inflammatory Bowel Disease (IBD) which was ongoing at the time of study treatment, and medical history of immunocompromising condition(s) which was ongoing at time of IP administration.

Because immunocompromising conditions may result from medications, diseases, or a combination of both, the following algorithm will be used to identify participants in this subgroup:

Medical History Rules

Standardized MedDRA query (SMQ) of Malignant tumours
Search will require at least 2 Broad terms or at least1 narrow term for events which were ongoing at the time of IP administration.

Other Medical History
Preferred Terms (PT), High level Term (HLT), or High Level Group Term (HLGT) will be used to identify the following conditions, which were ongoing at the time of IP administration, based upon the Centers for Disease Control (CDC) guidelines for immunocompromised conditions listed under CDC Pneumococcal Vaccine Recommendations (Pneumococcal Vaccine Recommendations | CDC)

- PT: End Stage Renal Disease
- PT: Renal Failure
- PT: Asplenia
- PT: HIV
- PT: HIV infection
- HLT: Haemoglobinopathies congenital
- HLGT: Immunodeficiency syndromes

Concomitant Medications:

Anatomical Therapeutic Chemical (ATC) classification will be used to identify immunocompromising medications taken within the following window: Two weeks prior to the first IP administration to 8 weeks after administration. Only systemic routes of administration will be included such as those taken orally or by injection (intramuscular, subcutaneous, or intravenous); topical medications will be excluded.

- ATC classification H02AB or H02BX – Corticosteroids. These medications must have been taken for 5 consecutive days within the window specified above.
    - As described in the protocol, 20 mg prednisone-equivalent is considered to be an immunocompromising dose. Prednisone equivalent doses are listed in the table below where the dose of the medications below is multiplied by the applicable conversion factor listed in the table (e.g., 4 mgs of Dexamethasone is equivalent to 26.8 mg prednisone)
    - The dosing regimen will be incorporated into a calculation of total daily dose, with a total of 20mg prednisone-equivalent per day as the threshold for immunocompromising.

Preferred Term	Prednisone equivalent conversion factor (multiplication)
Cortisone	0.2
Dexamethasone	6.7
Hydrocortisone	0.25
Methylprednisolone	1.25
Methylprednisolone Sodium Succinate	1.25
Prednisolone	1
Prednisone	1
Triamcinolone	1.25

- ATC classification L01, L03, L04 includes many systemic immunosuppressive medications with varying doses, dosing regimens, routes of administration, and half-lives. Use of any of the medications used two weeks prior to IP administration to 8 weeks after administration will be considered as immunocompromising. **Note:** ACT classification L02 will be excluded since these hormone-based medications are not considered to be immunocompromising.

A final output will include a summary table summarizing the total number of subjects flagged as immunocompromised and a listing detailing the immunocompromised subjects as seen in example Tables 1 and 2.

Example Table 1. Summary information Inflammatory Bowel Disease:

	Ulcerative colitis (UC)	Crohns disease (CD)	Inflammatory bowel disease (IBD)	Total
Number of participants	[number]	[number]	[number]	[number]

Example Table 2. Listing of Immunocompromised Subjects
(Note: listing should include one entry per row to display subjects with multiple SMQ hits in multiple rows)

USUBJID	*SMQ/[B or N]/MHDECOD	CMCLAS/ CMDecod/ CMRoute/ CMDose/CMDoseU/CMDoseFRQ CMDoseEq**

*SMQ /[B or N]= Standardized MedDRA Query Broad (B) or Narrow (N)**CMDoseEq=concomitant medication dose equivalent

8 COMPUTER SOFTWARE

All analyses will be performed by Medpace using Version 9.3 or later of SAS® software. All summary tables and data listings will be prepared utilizing SAS® software.