Vaccines and Related Biological Products Advisory Committee Meeting
September 22, 2022

FDA Briefing Document

RBX2660 (fecal microbiota suspension for rectal enema)
Trade Name: Rebyota

Applicant: Rebiotix Inc.

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Glossary

AE	adverse event
AESI	adverse events of special interest
AML	acute myeloid leukemia
BLA	Biologics License Application
CBER	Center for Biologics and Evaluation
CDAD	*Clostridioides difficile*-associated diarrhea
CDC	Centers for Disease Control and Prevention
CDI	*Clostridioides difficile* infection
COPD	chronic obstructive pulmonary disease
FMT	fecal microbiota transplant
ISS	integrated summary of safety
MedDRA	Medical Dictionary for Regulatory Activities
rCDI	recurrent *Clostridioides difficile* infection
SAE	serious adverse event
SMQ	Standardised MedDRA Query
SoC	standard of care
SOC	System Organ Class
TEAE	treatment emergent adverse event

1. Executive Summary

Rebiotix Inc. (the Applicant) submitted a biologics license application (BLA) to the US Food and Drug Administration (FDA) to support licensure of RBX2660 (Rebyota), a fecal microbiota suspension prepared from human stool collected from pre-screened, qualified donors and administered rectally by enema. The proposed indication for RBX2660 is to "reduce the recurrence of *Clostridioides difficile* infection in adults following antibiotic treatment for *Clostridioides difficile* infection." *Clostridioides difficile* infection (CDI) is an urgent public health concern, associated with significant morbidity and mortality. Recurrent infection is common, treatment options for recurrence are limited, and the currently recommended treatment regimens can be complex and prolonged. Bezlotoxumab is the only currently approved product for prevention of recurrent *C. difficile* infection (rCDI), indicated for use in patients 18 years of age or older who are receiving antibacterial drug treatment of CDI and are at a high risk for CDI recurrence.

The BLA includes data from six clinical studies: three Phase 2 trials (2013-001, 2014-01 and 2015-01), two Phase 3 trials (2017-01 and 2019-01), and one retrospective study (2019-02).

Due to enrollment challenges that precluded the conduct of two placebo-controlled Phase 3 trials, the Applicant conducted a single placebo-controlled Phase 3 trial (study 2017-01) with a primary efficacy analysis that employed a Bayesian hierarchical model formally integrating treatment success rates from a placebo-controlled Phase 2 study (2014-01) into study 2017-01. Study 2014-01 was a Phase 2, double-blind, randomized, placebo-controlled trial in adults ≥18 years old with documented rCDI. A total of 133 subjects were randomized 1:1:1 to receive two doses of RBX2660, two doses of placebo, or one dose of RBX2660 and one dose of placebo, administered 7±2 days apart. Study 2017-01 was a Phase 3, double-blind, randomized, placebo-controlled study in adults ≥18 years old with documented rCDI; a total of 289 subjects were randomized 2:1 to receive one dose of RBX2660 or one dose of placebo.

In the Bayesian analysis, treatment success was defined as absence of CDI diarrhea (passage of 3 or more unformed stools in 24 or fewer consecutive hours for at least 2 consecutive days) for 56 days (8 weeks) after completing the assigned treatment. The modified intent-to-treat (mITT) population was pre-specified as the primary analysis population. The 2017-01 data were analyzed with integration of data from study 2014-01, and the extent of borrowing was dependent on the similarity of effect for both active and placebo group per the planned design. In order to better align the analysis population definitions between the two studies, the Bayesian analysis was conducted with the analysis population definition of study 2017-01 applied to study 2014-01. In both studies, treatment with open-label RBX2660 was an option in the event of treatment failure.

The primary efficacy endpoint analysis for the Phase 3 study 2017-01 (mITT population), performed with a Bayesian analysis borrowing information from Phase 2 study 2014-01, resulted in an estimated difference in treatment success rates of 0.13 (95% credible interval: 0.02 to 0.24). The posterior probability that RBX2660 was superior to placebo was 0.991. The efficacy results met the second success threshold (posterior probability of superiority 0.9750338) that is considered equivalent to positive statistical evidence from a single adequate and well-controlled trial. However, the efficacy results did not meet the first success threshold (posterior probability of superiority 0.9993275) that would have been considered a statistically very persuasive finding in a single trial that could substitute for positive evidence from two independent adequate and well-controlled trials.

Safety data from studies 2013-001, 2014-01, 2015-01, 2017-01 and 2019-01 were pooled in an integrated summary of safety (ISS), including 6 months of follow-up after the last dose of study treatment across all studies. Safety was assessed by examining the incidence of treatment emergent adverse events (TEAEs), serious TEAEs, discontinuations due to TEAEs, and deaths due to TEAEs that occurred through 6 months after treatment. The ISS population included any subject who received at least one dose of RBX2660 or placebo. The ISS included an analysis of data from subjects enrolled in double-blind, placebo-controlled studies (n=312 RBX2660 recipients and 83 placebo recipients) and an analysis of data from all studies (n=749 RBX2660 recipients and 83 placebo recipients), including non-randomized studies and subjects who received open-label RBX2660. The safety review in this briefing document specifically focuses on the subjects who received one dose of RBX2660 (dosing regimen proposed for licensure; n=429), subjects who received blinded RBX2660 (n=193), subjects who received any dose of RBX2660, regardless of blinding or regimen (Any RBX2660; n=749), and placebo recipients (n=83).

Solicited adverse events (AEs) (gas or flatulence, abdominal distension or bloating, rectal bleeding, irritation or pain, chills/severe shivering, abdominal pain or cramping, increased diarrhea, constipation, nausea, vomiting, and fever) were collected from subjects via subject diary from the date of enrollment through the seventh day after receiving the assigned treatment (studies 2013-001, 2017-01 and 2019-01) or through the seventh day after receiving the second assigned study treatment (studies 2014-01 and 2015-01). In Study 2017-01, the most frequently reported solicited AEs from day 1 through day 7 were gas (flatulence), abdominal distension or bloating, and abdominal pain or cramping. Most solicited AEs were mild or moderate in severity.

The proportions of participants reporting TEAEs were 61.8% in the one-dose RBX2660 group, 69.9% in the blinded RBX2660 group, and 69.6% in the Any RBX2660 group compared to 60.2% in the placebo group. In all groups, the most commonly reported events were gastrointestinal. The severity of TEAEs was mostly mild or moderate, and most were related to pre-existing conditions. For both the one-dose and blinded RBX2660 groups compared to placebo, numerical imbalances in events of abdominal pain, nausea, flatulence, and abdominal distention were observed. The proportion of participants reporting related TEAEs was 22.6% in the one-dose RBX2660 group, 26.4% in the blinded RBX2660 group, and 23.3% in the Any RBX2660 group compared to 19.3% in the placebo group. The proportion of participants reporting severe and life-threatening TEAEs was 9.3% and 2.1%, respectively, in the one-dose RBX2660 group, 9.8% and 3.1%, respectively, in the blinded RBX2660 group, and 12.7% and 2.9%, respectively, in the Any RBX2660 group compared to 8.4% and 1.2%, respectively, in the placebo group.

The proportions of participants reporting serious TEAEs were 8.4% in the one-dose RBX2660 group, 10.4% in the blinded RBX2660 group, and 14.2% in the Any RBX2660 group, compared to 7.2% in the placebo group. A high rate of serious TEAEs was observed in the multiple-dose populations (19%, 28.6%, and 83.3% of subjects in the two, three, and four-dose RBX2660 groups, respectively). The most frequently reported serious TEAEs were in the MedDRA System Organ Classes of *Infections and infestations*, *Gastrointestinal disorders*, and *Respiratory, thoracic and mediastinal disorders*. A total of five subjects who received one or two doses of RBX2660 reported serious TEAEs that were considered possibly related to RBX2660 by the investigator, including three subjects in study 2014-01 (acute myeloid leukemia relapse, abdominal pain and recurrent CDI, and worsening chronic constipation) and two subjects in study 2015-01 (recurrent CDI and diarrhea and ileus, leukocytosis, CDI, and pyrexia). Following review of all of the individual case narratives, FDA did not consider any serious TEAEs to be plausibly related to RBX2660.

The proportions of participants reporting fatal TEAEs were 1.2% in the one-dose RBX2660 group, 2.6% in the blinded RBX2660 group, and 2.4% in the Any RBX2660 group, compared to 0% in the placebo group. The proportion of subjects reporting any TEAEs leading to death increased as the number of treatment exposures increased, ranging from 3.4% in subjects who received two doses of RBX2660 to 16.7% of subjects who received 4 doses of RBX2660. Of the 18 fatal TEAEs observed in the RBX2660 clinical program, 17 were adjudicated as being unrelated to treatment. FDA agrees with the assessment of causality for these cases. One death due to relapsed CDI on Day 21 (study 2015-01) was considered possibly related to RBX2660 and the enema procedure and definitely related to CDI by the investigator. Following review of the narrative and case report form by FDA, the event was considered not to be causally related to RBX2660 and was considered definitely related to recurrent CDI.

This meeting of the Vaccines and Related Biological Products Advisory Committee (VRBPAC) is being convened to discuss whether the available data support the safety and effectiveness of RBX2660 to reduce the recurrence of CDI in adults following antibiotic treatment for CDI.

2. Background

2.1. General Product Information

RBX2660 (Rebyota) is a human fecal microbiota suspension for enema delivery. Human stool is mixed with polyethylene glycol and sodium chloride and processed to form the fecal microbiota suspension. Stool donors are pre-screened and qualified based on assessment of general health and potential risk factors as well as the results of regular testing of stool and blood for specific pathogens. In the placebo-controlled studies, the placebo enema consisted of 150 mL normal saline.

RBX2660 is supplied in a kit containing drug product (150 mL fecal microbiota suspension in an enema bag) and an administration tubing set. RBX2660 is stored at -80°C and shipped frozen to the clinical site where it can be stored at -80°C until the expiration date. Prior to use, the product is thawed in a refrigerator at 2 - 8°C (36 - 46°F) for a maximum of 24 hours and stored under refrigerated conditions for a maximum of 4 days. RBX2660 may not be heated or re-frozen after receipt and is for a single-dose rectal use only.

2.2. Epidemiology

Clostridioides difficile (formerly *Clostridium difficile*), also known as *C. difficile* or *C. diff*, is a Gram-positive, spore forming, anaerobic rod-shaped bacterium that colonizes through the fecal-oral route and causes *C. difficile* infection (CDI). In the United States, CDI is associated with 15,000-30,000 deaths annually, with acute inpatient costs exceeding $4.8 billion (Fu et al. 2021). There is a growing trend of community-associated CDI while the rate of healthcare-associated CDI is generally decreasing (Fu et al. 2021). Population-based surveillance of CDI in ten U.S. sites identified 15,512 cases in 2017, including 7,973 healthcare–associated and 7,539 community-associated cases (Guh et al. 2020). The Centers for Disease Control and Prevention (CDC) consider CDI to be an urgent antibiotic resistance threat (CDC 2019). Globally, CDI incidence rate ranges from 1.1 to 631.8 per 100,000 population per year (Balsells et al. 2019).

Approximately 10%-30% of patients will develop recurrent CDI (rCDI) after a first CDI, and each recurrence increases the risk for subsequent recurrence, with reported recurrence rates of 65% after three episodes of CDI (McDonald et al. 2018, Hopkins and Wilson 2018, Fu, et al. 2021).

rCDI is defined as an episode of CDI occurring within 8 weeks of a previous episode (Surawicz et al 2013). rCDI may be due to relapse of previous CDI by the same strain or reinfection by a different strain (Tang-Feldman et al 2003). The high recurrence rate of CDI contributes to burden of disease and increased healthcare cost (Ghantoji et al 2010).

The most frequently reported risk factors for rCDI include age >65 years (Deshpande et al. 2015), antibiotic use for non-*C. difficile* infection after CDI diagnosis leading to disruption of the native intestinal microbiome, gastric acid suppression, infection with a hypervirulent strain (e.g., NAP1/BI/027, which produces a larger amount of toxins A and B), severe underlying disease, renal insufficiency, immunosuppression, inflammatory bowel disease, history of previous CDI, previous CDI severity, prolonged hospital stays, and lack of adaptive immune responses to toxins A and B (Song et al. 2019; Ma et al. 2017, McDonald et al. 2018).

2.3. Clinical Manifestations, Diagnosis, and Treatment

CDI symptoms may range from mild diarrhea to significant colitis. The most common signs and symptoms of moderate CDI are watery diarrhea >3 times a day for more than one day, mild abdominal cramping and tenderness. Symptoms are often associated with fever and leukocytosis. Severe infection can be associated with significant colitis, with signs and symptoms of more voluminous watery diarrhea as often as 10-15 times a day, mild to severe abdominal cramping/pain, fever, nausea, and leukocytosis. CDI complications include dehydration and kidney failure from significant loss of fluids and electrolytes due to severe diarrhea, which can result in hypotension. Although rare, toxic megacolon can occur when the colon is unable to expel stool and gas; and it can lead to colonic rupture, septicemia, and death if left untreated. Other complications include bowel perforation or peritonitis, and death from mild to moderate infection if not treated promptly. Surgical intervention with colectomy may be required when aggressive medical management is unsuccessful.

Diagnostic criteria for CDI include new-onset diarrhea (≥3 unformed stools in 24 hours without an alternative etiology) and positive testing for toxicogenic *C. difficile* or toxins. An algorithmic approach to testing is recommended, including highly sensitive tests, such as glutamate dehydrogenase (GDH) followed by confirmation with more specific tests, including enzyme immunoassays (EIAs) to detect toxins A and B and nucleic acid amplification testing (Kelly et al. 2021; McDonald et al. 2018).

An initial episode of CDI is often successfully managed by fluid replacement, discontinuation of antibiotics if possible, and initiation of first-line antimicrobial therapy with oral vancomycin or fidaxomicin (and occasionally IV metronidazole or rectally delivered metronidazole or vancomycin). Second-line agents include metronidazole, nitazoxanide, rifamycin and cytotoxin-binding agents such as cholestyramine or colestipol.

Options for the treatment of rCDI depend on the initial course of therapy and may include a 10-day course of fidaxomicin or vancomycin or a tapered and pulsed fidaxomicin or vancomycin regimen. Adjunctive bezlotoxumab (see Section 2.4), is indicated to reduce recurrence of CDI in patients 18 years of age or older who are receiving antibacterial drug treatment of CDI and are at a high risk for CDI recurrence. Treatment options are similar for patients with more than one recurrence, although they also include a subsequent course of rifaximin if a standard course of vancomycin is used (Johnson et al. 2021). While no fecal microbiota transplant (FMT) product is yet FDA-approved as safe and effective for prevention of rCDI, FMT has been recommended by various infectious diseases and gastroenterology practice guidelines and used widely, especially in the past ~10 years, as an unapproved product for this purpose.

Quality-of-life scores in patients with rCDI are lower compared to patients with a first episode of CDI, and consistently decrease with increasing number of CDI episodes (Garey et al. 2016). In considering the benefits and harms of treatment for rCDI, the expert panel contributing to the development of the Clinical Practice Guidelines by the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America judged, based on clinical experience, that patients experiencing rCDI will invariably put a high value on avoidance of a subsequent CDI episode (Johnson et al. 2021). Prevention of rCDI represents an unmet clinical need, as the only currently approved product for prevention, bezlotoxumab, is indicated only in conjunction with antibacterial drug treatment for CDI.

2.4. Currently Approved Therapies for Prevention

Bezlotoxumab (Zinplava) is a human monoclonal antibody that binds to *C. difficile* toxin B and neutralizes its effects and is indicated to reduce recurrence of CDI in patients 18 years of age or older who are receiving antibacterial drug treatment of CDI and are at a high risk for CDI recurrence. Bezlotoxumab is not indicated for the treatment of CDI and should only be used in conjunction with antibacterial drug treatment of CDI.

2.5. Clinical Development and Regulatory History

2.5.1. Bayesian Approach to Efficacy Assessments

Originally, the Applicant planned to conduct two independent placebo-controlled Phase 3 trials of approximately 300 subjects each to support licensure. The original primary efficacy analysis in each of the Phase 3 studies was a comparison of the efficacy of RBX2660 versus placebo in a planned target population of 300 subjects, allowing for a 20% loss-to-follow-up rate. In July 2013, the Agency released draft guidance on the decision to exercise enforcement discretion regarding the requirement of an investigational new drug (IND) application for use of FMT to treat CDI not responsive to standard therapies. Due to FDA's decision to exercise enforcement discretion, the Applicant reported enrollment challenges in the first Phase 3 study (2017-01). In the face of these enrollment challenges, the Applicant anticipated similar challenges in enrolling a second placebo-controlled Phase 3 study and proposed using a single placebo-controlled Phase 3 study as the basis for approval.

The Applicant proposed use of a Bayesian model, the goal of which was to demonstrate a clinically meaningful treatment effect with persuasive statistical evidence, by integrating the data from two double-blinded, randomized, placebo-controlled studies of participants ≥18 years old with documented rCDI and who received the same single dose treatment regimen intended for licensure. The two studies included in this approach are the Phase 3 study, 2017-01, and the Phase 2 study, 2014-01. Use of an integrated Bayesian efficacy analysis is supported by similarity of the studies, including in study design (both randomized, placebo-controlled, and blinded), study population, product formulation and dosing regimen, and treatment success definitions. Therefore, FDA agreed that the studies are generally exchangeable. However, because the two studies are not identical, an approach based on Bayesian hierarchical modeling with dynamic borrowing was considered acceptable. Consequently, the statistical success criteria were established to reflect the levels of statistical persuasiveness as part of the support for demonstrating substantial evidence of clinical effectiveness.

2.5.2. Safety Communications

Over the course of the clinical development program, multiple safety communications have been issued by FDA for FMT products, which resulted in changes in donor screening and stool testing practices for FMT products developed under IND and some products being used under enforcement discretion as follows:

- June 13, 2019: risk of serious or life-threatening infections due to transmission of multi-drug resistant organisms (FDA 2019).
- March 12, 2020: risk of serious or life-threatening infections due to infections caused by enteropathogenic *Escherichia coli* and Shiga toxin-producing *Escherichia coli*, including events that occurred following investigational use of FMT, suspected to be due to transmission of these pathogenic organisms from the FMT product (FDA 2020a).
- March 23, 2020: potential risk of transmission of SARS-CoV-2 and COVID-19 due to the documented presence of SARS-CoV-2 ribonucleic acid and/or SARS-CoV-2 virus in stool of infected individuals (FDA 2020b).
- August 22, 2022: potential risk of transmission of monkeypox virus due to the documented presence of monkeypox virus DNA in rectal swabs and/or stool samples from infected individuals (FDA 2022).

3. Overview of Clinical Studies

The clinical development program included six studies that were conducted in the United States and Canada, all of which enrolled adults ≥18 years of age with documented rCDI. The totality of evidence submitted to support licensure includes two placebo-controlled studies (a Phase 2 study 2014-01 and a Phase 3 study 2017-01), three open-label studies (Phase 2 studies 2013-001 and 2015-01, and the ongoing Phase 3 study 2019-01), and one retrospective study (2019-02). All of the prospective studies required subjects to have completed standard-of-care (SoC) oral antibiotic therapy with resolution of symptoms prior to initial treatment with RBX2660. In all studies except 2015-01, an open-label course of RBX2660 was allowed if a subject experienced a CDI recurrence after the first course of blinded treatment with either placebo or RBX2660. Features of each study design are presented in Table 1 and Table 2 below.

Table 1. Double-Blinded and Randomized Clinical Studies Submitted to the RBX2660 BLA

Design Feature	2014-01	2017-01
NCT number	02299570	03244644
Number of RBX2660 exposures	1-4	1-2
Phase	2	3
Study design	Randomized, Double-Blind, Placebo-Controlled	Randomized, Double-Blind, Placebo-Controlled
Sites, countries	21 sites US/Canada	44 sites US/Canada
Initiation date	10 Dec 2014	31 Jul 2017
Completion date	13 Nov 2015	03 Aug 2020
Enrolled	150	320
Treated	128	267
Completed study	91	234

Design Feature	2014-01	2017-01
Number of previous CDIs, including qualifying events	At least 2 recurrences after a primary episode (i.e., at least 3 episodes, completed at least 2 rounds of SoC antibiotics therapy OR at least 2 severe CDI resulting in hospitalization	At least 1 recurrence after a primary episode (i.e., at least 2 episodes, completed at least 1 round of SoC antibiotics therapy OR at least 2 severe CDI resulting in hospitalization
Primary efficacy endpoint: treatment success	The absence of CDAD[a] without the need for retreatment with *C. difficile* anti-infective therapy or fecal transplant (FT) at 56 days after administration of the last assigned study enema.	The recurrence of CDI diarrhea within 8 weeks of blinded treatment.
Antibiotic washout (hours)	24-48	24-72
Efficacy endpoint adjudication	Data safety monitoring board	EAC
Treatment received	Placebo or RBX2660	Placebo or RBX2660
Randomization: treatment groups (treatment dose) treatment regimen	1:1:1 ratio Group A: 2 doses RBX2660 Group B: 2 doses Placebo Group C: 1 RBX2660 dose/ 1 placebo dose 7 ± 2 days apart	2:1 ratio 1 dose RBX2660 1 dose placebo
Optional second treatment course	Yes, up to 2 doses	Yes, 1 dose
Efficacy analysis	8 weeks[b]	8 weeks and 6 months
Safety follow-up (months)	24	6
Key contribution to clinical development program	Dose-finding Integrated data for efficacy, Historical data for 2017-01 analysis using Bayesian hierarchical model Safety	Primary evidence of efficacy Sustained clinical response through 6 months Safety

Source: Reviewer's Table, Adapted from STN 125739/0, Clinical Overview
Abbreviations: CDI = *Clostridioides difficile* infection, rCDI = recurrent *Clostridioides difficile* infection, SoC = Standard of care, EAC = Endpoint Adjudication Committee, IBD = Inflammatory Bowel Disease (include ulcerative colitis, Crohn's Disease), IBS = Irritable Bowel Syndrome (includes microscopic colitis, celiac disease and immunocompromised conditions), US = United States.
a. *Clostridioides difficile*-associated diarrhea (CDAD) is defined as the passage of three or more unformed stools in 24 or fewer consecutive hours for at least two consecutive days.
b. After second enema

Table 2. Non-Randomized Clinical Studies Submitted to the RBX2660 BLA

Design Feature	Study 2013-001	Study 2015-01	Study 2019-01	Study 2019-02
NCT number	01925417	02589847	03931941	Not applicable
Number of RBX2660 exposures	1-2	1-2	1-2	1-2
Phase	2	2	3	Not Applicable
Study design	Open-label, uncontrolled	Open-label, historical controlled	Open-label, uncontrolled	Retrospective, open-label, uncontrolled, Enforcement Discretion
Sites, countries	11 sites US	29 sites US/Canada	29 sites US/Canada	5 sites US
Initiation date	15 Aug 2013	15 Oct 2015	30 Jul 2019	11 Nov 2015
Completion date	16 Dec 2013	03 Mar 2017	Ongoing; Data cutoff: 20 Apr 2021	01 Mar 2020
Enrolled	40	162	293	94
Treated	34	149	254	94
Completed study	31	107	123 (data cutoff April 2021)	64
Number of previous CDI/CDADs, including qualifying events	At least 2 recurrences after a primary episode (i.e., at least 3 episodes, completed at least 2 rounds of SoC antibiotics therapy OR at least 2 severe CDAD[a] resulting in hospitalization	At least 2 recurrences after a primary episode (i.e., at least 3 episodes, completed at least 2 rounds of SoC antibiotics therapy OR at least 2 severe CDI resulting in hospitalization	rCDI not defined, relied on investigator opinion. Broad population including IBS, IBD, immunocompromised conditions to reflect clinical practice	CDI event that prompted first RBX2660 under Enforcement Discretion defined as “qualifying CDI event”. rCDI defined as “on study CDI event” identified in the subject’s medical record by a positive laboratory stool test for CDI, microbiota therapy or anti-infective therapy for CDI treatment or suspected CDI diarrhea

Design Feature	Study 2013-001	Study 2015-01	Study 2019-01	Study 2019-02
Primary efficacy endpoint: treatment success	The resolution of subject's symptoms of CDAD[a] 56 days after receipt of RBX2660	The recurrence-free rate of CDI diarrhea without the need for retreatment with C. difficile anti-infective therapy or FT through 56 days after completion of study treatment with RBX2660 compared to the recurrence-free rate observed in the study population to the recurrence-free rate from antibiotic-treated historical controls	The absence of CDI through 8 weeks after treatment	The absence of CDI through 8 weeks after treatment
Antibiotic washout (hours)	24-48	24-48	24-72	Not applicable
Efficacy endpoint adjudication	None	None	EAC	None
Treatment received	1 dose RBX2660	2 doses RBX26607 ± 2 days apart	1 dose RBX2660	1 or 2 doses RBX2660
Optional second treatment course?	Yes	No	Yes	Investigator discretion
Efficacy analysis	8 weeks	8 weeks[b]	8 weeks and 6 months	8 weeks and 6 months
Safety follow-up (months)	6	24	6	6
Key contribution to clinical development program	Clinical proof of concept and safety	Supportive evidence of efficacy and safety	Supportive efficacy, persistence of efficacy, and safety Expanded rCDI patient population (e.g., IBD, IBS, and immunocompromised)	Supportive efficacy, persistence of efficacy Expanded rCDI patient population

Source: Reviewer's Table, Adapted from STN 125739/0, Clinical Overview
Abbreviations: CDI = *Clostridioides difficile* infection, CDAD = *Clostridioides difficile* associated diarrhea, rCDI = recurrent *Clostridioides difficile* infection, SoC = Standard of care, EAC = Endpoint Adjudication Committee, IBD = Inflammatory Bowel Disease (include ulcerative colitis, Crohn's Disease), IBS = Irritable Bowel Syndrome (includes microscopic colitis, celiac disease and immunocompromised conditions), US = United States.
a. CDAD defined as the presence of diarrhea, defined as passage of 3 or more unformed stools in 24 or fewer consecutive hours for at least two consecutive days and at least one positive stool test for the presence of toxigenic *C. difficile* or its toxins, or colonoscopic or histopathologic findings demonstrating pseudomembranous colitis.
b. Efficacy outcomes were only evaluated up to 8 weeks after the last enema

4. Studies Intended to Support Efficacy (2014-01 and 2017-01)

4.1. Study 2014-01

Study Title: A Phase 2b Prospective, Randomized, Double-blinded, Placebo-controlled Clinical Study Demonstrating the Efficacy and Safety of Rebiotix RBX2660 (microbiota suspension) for the Treatment of Recurrent *Clostridium difficile* Infection (CDI)

4.1.1. Study Design

Study 2014-01 was a Phase 2, prospective, multicenter, randomized, double-blind, placebo-controlled, 3-arm study designed to assess the efficacy and safety of RBX2660 for the prevention of rCDI when two enemas are administered 7±2 days apart in adults (≥18 years old) with rCDI who either a) had at least two recurrences after a primary episode and had completed at least two rounds of SoC oral antibiotic therapy, or b) had at least two episodes of severe CDI resulting in hospitalization.

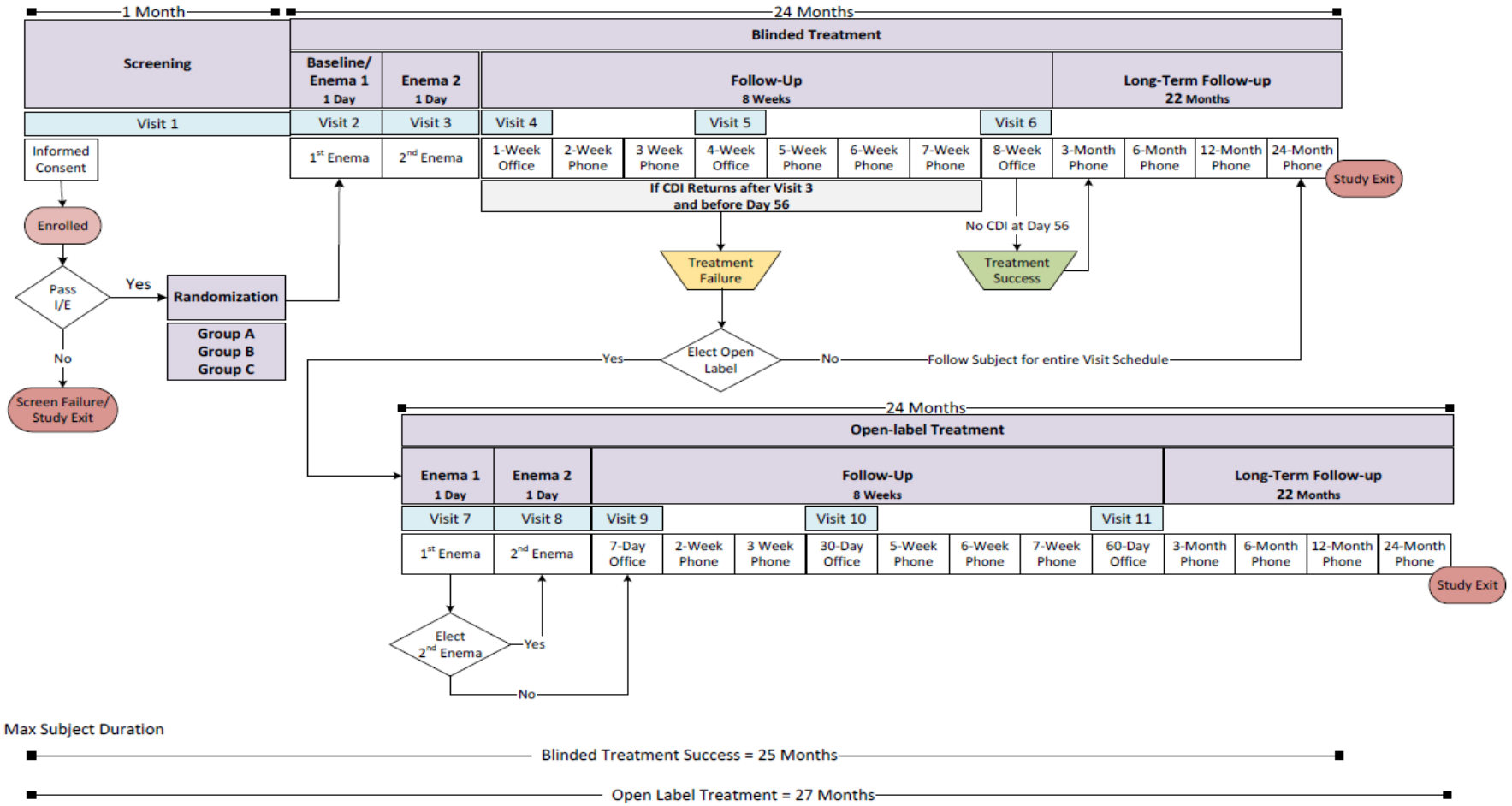
Subjects were on antibiotics to control rCDI symptoms at the time of enrollment, followed by a 24-48-hour washout period prior to receiving the first assigned study treatment. Symptom control, defined as the absence of CDI diarrhea, was required in order to be randomized to treatment. Subjects and site personnel who performed study follow-up procedures were blinded to the randomization assignment and delivered therapy.

Subjects were randomized 1:1:1 to one of the following groups:

- Group A: 2 enemas of RBX2660
- Group B: 2 enemas of placebo (150 mL of normal saline and cryoprotectant in the same formulation as RBX2660)
- Group C: 1 enema of RBX2660 and 1 enema of placebo

One complete assigned 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.

Figure 1. 2014-01 Study Design



Source: STN 125739/0, Clinical Study Report 2014-01

Enrolled subjects completed a diary to document self-reported events from enrollment to one week after completing the assigned study treatment (second enema). In-office study visits occurred at 1 week, 4 weeks, and 8 weeks after completing the assigned treatment. Telephone assessments for adverse events occurred at weeks 2, 3, 5, 6, and 7 and at months 3, 6, and 12. Assessments of serious adverse events (SAEs) occurred via phone call at 24 months after completing the assigned study treatment.

Subjects who experienced treatment failure (as determined by the site investigator at the time of CDI recurrence) in any study group were eligible to receive open-label treatment with RBX2660. These subjects could elect to receive up to two RBX2660 enemas 7±2 days apart or another therapy deemed most appropriate by their study investigator. If a subject received treatment with RBX2660 during the open-label portion of the study, the follow-up visits, phone calls and completion of a new posttreatment subject diary occurred according to the same schedule of the blinded portion of the study.

4.1.2. Study Endpoints

The primary efficacy study endpoint was treatment success, defined as the absence of *C. difficile* associated diarrhea (CDAD, defined as the passage of three or more unformed stools in 24 or fewer consecutive hours for at least two consecutive days) without need for retreatment with *C. difficile* anti-infective therapy or fecal transplant at 56 days after administration of the last assigned study enema, of Group A (two enemas of RBX2660) vs. Group B (two enemas of placebo) during the blinded period. Treatment outcome was initially determined by the site investigator. The 3-member independent data safety monitoring board, which consisted of two physicians with experience managing patients with rCDI and a biostatistician, reviewed each case of investigator-declared outcome (blinded to individual treatment assignment) and was the final adjudicator of treatment outcome for the efficacy analyses.

Treatment failure (CDI recurrence) was defined as:

- The presence of CDI diarrhea, with or without other CDI symptoms, at <8 weeks after administration of the last assigned study dose;
- A positive stool test for *C. difficile*;
- Need for re-treatment for CDI; AND
- No other cause for CDI symptoms had been determined.

Secondary and other efficacy endpoints:

1. Treatment success between Group C (1 enema of RBX2660 and 1 enema of placebo) vs. Group B (2 enemas of placebo) during the blinded period
2. Treatment success between Group A (2 enemas of RBX2660) vs. Group C (1 enema of RBX2660 and 1 enema of placebo) during the blinded period
3. SF-36 scores obtained at the 1-, 4- and 8-week assessment visits during the blinded period as compared to baseline
4. Time to CDAD recurrence after completion of the assigned study treatment for Group A vs. Group B
5. Time to CDAD recurrence after completion of the assigned study treatment for Group C vs. Group B
6. Time to CDAD recurrence after completion of the assigned study treatment for Group A vs. Group C
7. Treatment success during the open-label period

8. Time to CDAD recurrence during the open-label period

Safety assessments included:

1. Treatment emergent adverse events: Adverse events including SAEs and the onset of new chronic diseases were assessed through the 12-month phone calls. The 24-month call assessed for SAEs and the new onset of chronic disease.
2. Solicited adverse events were collected daily via subject diary through 7 days after a treatment with the assigned study enema (blinded portion) or after a treatment with RBX2660 (open-label portion). Solicited events included gas (flatulence), abdominal distension or bloating, increased diarrhea, abdominal pain or cramping, constipation, rectal bleeding, irritation or pain, nausea, vomiting, fever ≥38.0° C (100.4°F), and chills.

Adverse events were categorized by severity, seriousness and relatedness by site investigator.

Safety data analysis included the use of the following Standardised MedDRA Queries (SMQs): Gastrointestinal and nonspecific inflammation and dysfunctional conditions; Gastrointestinal perforation, ulceration, hemorrhage or obstruction; Hyperglycemia/new onset diabetes mellitus; Noninfectious diarrhea; Immune-mediated/autoimmune disorders; Shock; Systemic lupus erythematosus; Sepsis; Vasculitis; and Medication errors.

Specific Preferred Terms were not pre-specified as adverse events of special interest (AESIs) in the protocols. However, the Applicant designated events identified by two of the pre-specified SMQs (Hyperglycemia/new onset diabetes mellitus and Immune-mediated/autoimmune disorders) as AESIs to enhance detection of any potential safety signals. See Section 5.3.6 for details.

4.1.3. Analysis Populations

Analysis populations were defined as follows:

- Intent to treat (ITT): The ITT population consisted of all randomized subjects, regardless of whether they completed their assigned study treatment. Subjects were analyzed according to the randomized treatment rather than the actual treatment received should any treatment misallocations or discontinuations occur.
- Modified intent to treat (mITT): The ITT population who completed at least one dose of study treatment, regardless of which treatment received, excluding subjects who discontinued from the study during the blinded period prior to evaluation of treatment failure or success, for any reason and excluding deviations from any inclusion/exclusion criteria.
- Per protocol (PP): All ITT subjects who received the treatment to which they were randomized (both blinded enemas) and were evaluable for treatment success/failure 56 days after the last assigned treatment, excluding:
    - Subjects who withdrew consent or were lost to follow-up during the double-blind period, prior to evaluation of treatment success
    - Subjects who expelled a moderate or large amount of dose
    - Subjects who had major protocol deviations that might affect outcome as determined by a clinical review of subject data prior to database lock
    - Subjects who had eligibility criteria deviations.
- Safety population: The population of randomized subjects who received any study treatment. Subjects were analyzed according to the treatment they actually received.

4.1.4. Demographics and Disposition

Table 3 shows the demographic characteristics of subjects across the three treatment arms in the safety population. There was no difference in the baseline characteristics between treatment arms. Slightly more females than males participated in the study. Most of the subjects were white and not Hispanic. These trends were noted in all three arms of the studies, and no major imbalances between the treatment groups were identified.

Table 3. Demographic and Baseline Characteristics, Study 2014-01, Safety Population

Characteristic	Group A 2 Dose RBX2660 N=42	Group B 2 Dose Placebo N=44	Group C 1 Dose RBX2660 1 Dose Placebo N=42
Age	--	--	--
Mean years [range]	62.8 [24 – 89]	58.8 [19 – 92]	61.4 [18 – 88]
<65, n (%)	19 (45.2)	25 (56.8)	24 (57.1)
≥65, n (%)	23 (54.8)	19 (43.2)	18 (42.9)
Sex, n (%)	--	--	--
Male	17 (40.5)	14 (31.8)	18 (42.9)
Female	25 (59.5)	30 (68.2)	24 (57.1)
Ethnicity, n (%)	--	--	--
Hispanic or Latino	1 (2.4)	2 (4.5)	1 (2.4)
Not Hispanic or Latino	40 (95.2)	42 (95.5)	40 (95.2)
Not Reported	1 (2.4)	0	1 (2.4)
Race, n (%)	--	--	--
Black/African American	0	1(2.3)	2 (4.8)
White	42 (100)	43 (97.7)	40 (95.2)
Other	0	0 (0)	0
Antibiotic use at screening, n (%)	--	--	--
Vancomycin	39 (92.9)	40 (90.9)	36 (85.7)
Fidaxomicin	1 (2.4)	3 (6.8)	2 (4.8)
Other	2 (4.8)	1 (2.3)	4 (9.5)

Adapted from STN 125739/0, Clinical Study Report 2014-01, Table 8

Table 4 displays the subject disposition and protocol-specified analysis populations used to evaluate the primary and secondary efficacy endpoints.

A total of 150 subjects were enrolled at 21 clinical sites in the US and Canada. Of the enrolled subjects, 11.3% (n=17) did not proceed to randomization (screen failures) and exited from the study. Of the 133 randomized subjects, five subjects withdrew prior to treatment for the following reasons: “withdrawal by subject or investigator” (n=4) and death (n=1). One of the subjects who withdrew prior to treatment was re-enrolled, randomized, treated, and analyzed according to the second randomized assignment. In total, 128 subjects received blinded treatment.

Table 4. Subject Disposition, Study 2014-01

Subject Population	Group A 2 Dose RBX2660 N=45 n (%)	Group B 2 Dose Placebo N=44 n (%)	Group C 1 Dose RBX2660 1 Dose Placebo N=44 n (%)	Total Number of Subjects N=133 n (%)
Randomized	45 (100)	44 (100)	44 (100)	133 (100)
Intent to treat (ITT)	45 (100)	44 (100)	44 (100)	133 (100)
Safety population (SP)	42 (93.3)	44 (100)	42 (95.5)	128 (96.2)
Modified intent to treat (mITT)	40 (88.9)	43 (97.7)	38 (86.4)	121 (91.0)
Per protocol (PP)	28 (62.2)	31 (70.5)	24 (54.5)	83 (62.4)
Subjects who completed study	31 (68.9)	35 (79.5)	25 (56.8)	91 (68.4)
Subjects who entered open-label period	16 (35.6)	24 (54.5)	14 (31.8)	54 (40.6)

Source: STN 125739/0, Clinical Study Report 2014-01

4.1.5. Efficacy Analysis

Table 5 provides the efficacy results of the Phase 2 study 2014-01. The primary efficacy analysis compared the treatment success rate in the RBX2660 2-dose group (n/N=25/45; 55.6%) with that in the placebo group (n/N=19/44; 43.2%) in the ITT population. The difference was not statistically significant (p=0.243). Similar findings were observed in the secondary efficacy analysis comparing the treatment success rate in the 1-dose RBX2660 group (n/N=25/44; 56.8%) with that in the placebo group (p=0.201). The differences between either of the RBX2660 groups and the placebo group were numerically higher in the mITT population; however, statistical significance was not reached.

Table 5. Efficacy Analysis Results, Study 2014-01, ITT and mITT Populations

Endpoint	ITT 2 Dose RBX2660 N=45	ITT 1 Dose RBX2660 N=44	ITT 2 Dose Placebo N=44	mITT 2 Dose RBX2660 N=40	mITT 1 Dose RBX2660 N=38	mITT 2 Dose Placebo N=43
Treatment success, n (%)	25 (55.6)	25 (56.8)	19 (43.2)	25 (62.5)	25 (65.8)	19 (44.2)
Treatment failure, n (%)	20 (44.4)	19 (43.2)	25 (56.8)	15 (37.5)	13 (34.2)	24 (55.8)
Difference in success rate (compared with placebo) 95% CI	12.4 (-8.2, 33.0)	13.6 (-7.1, 34.3)	--	18.3 (-2.8, 39.4)	21.6 (0.4, 42.8)	--
p-value	0.243	0.201	--	0.095	0.051	--

Source: STN 125739/0, Clinical Study Report 2014-01
mITT= modified intent to treat; ITT= intent to treat
Note: Randomized subjects who did not complete the assigned study treatment were considered Treatment Failures. Subjects who discontinued the study prior to 56 days after administration of the last assigned study enema during the blinded period for any reason were considered Treatment Failures. Subjects who were declared Treatment Failures without meeting all four criteria for Failure, as adjudicated by the data safety monitoring board, were included under the category Indeterminate and counted as Treatment Failures for purposes of efficacy analysis.

4.2. Study 2017-01

Study Title: A Phase 3 prospective, randomized, double-blinded, placebo-controlled clinical study to evaluate the efficacy and safety of RBX2660 (microbiota suspension) for the prevention of recurrent *Clostridium difficile* Infection

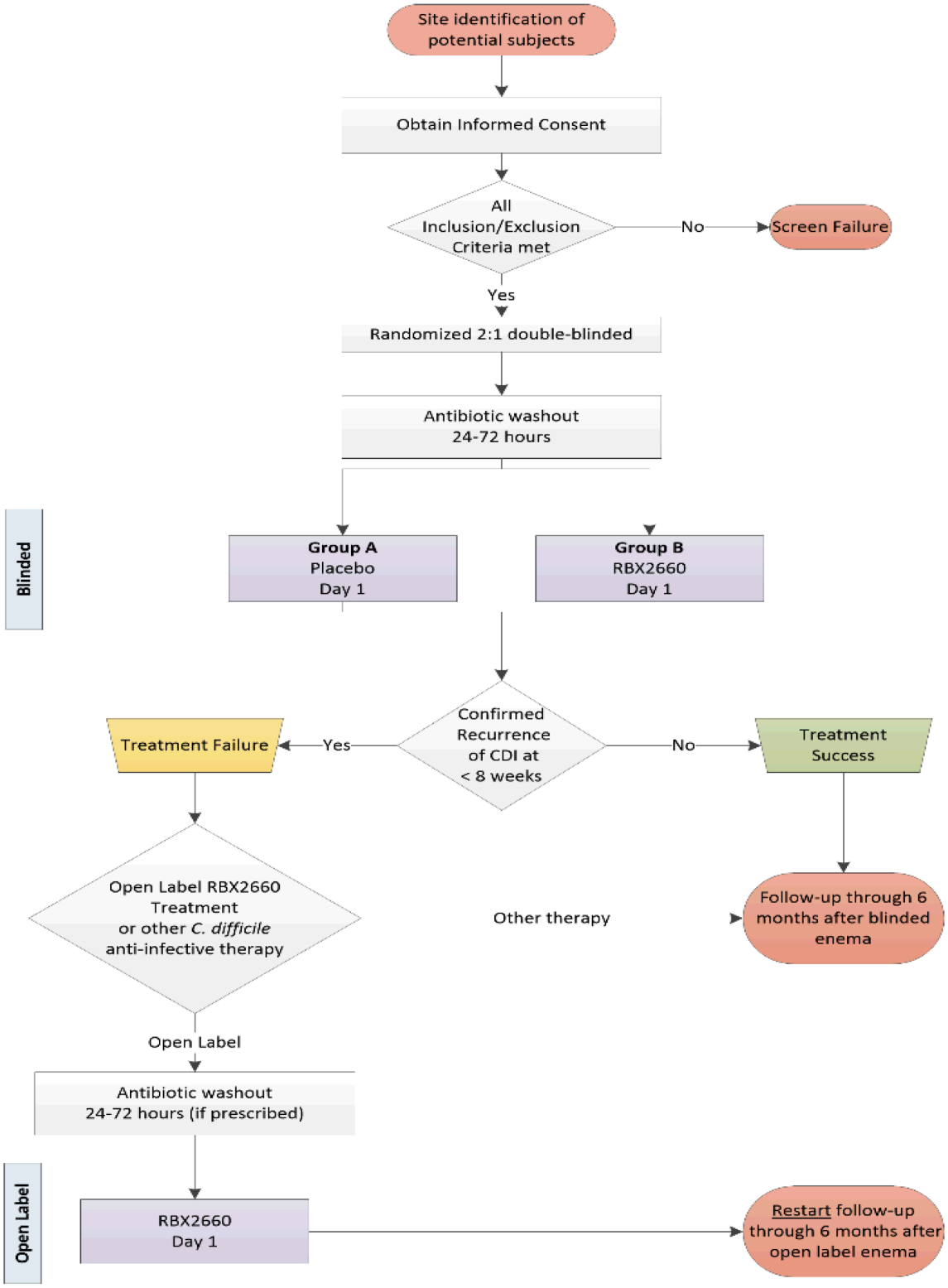
4.2.1. Study Design

Study 2017-01 was a prospective, multicenter, randomized, double-blinded, placebo-controlled Phase 3 study to evaluate the efficacy and safety of RBX2660 for the prevention of rCDI. A total of 289 subjects were randomized in a 2:1 ratio to receive RBX2660 or placebo. Randomized subjects were required to be on antibiotics to control rCDI symptoms at the time of study enrollment. Study treatment was administered within 21 days of the screening visit. Eligible subjects received a single blinded study enema following an antibiotic washout period of 24 to 72 hours and within 14 calendar days of randomization.

In-office study follow-up visits occurred at weeks 1, 4, and 8 after completing the blinded study treatment. Telephone assessments for adverse events occurred during weeks 2, 3, and 6 after the study enema and at months 3 and 6. Subjects were required to keep a detailed diary to assess for solicited events from the date of enrollment (informed consent) to the 1-week follow-up visit. The diary was collected and reviewed at the baseline visit prior to blinded enema administration. Subjects continued to complete the diary following enema administration and it was collected and reviewed at the 1-week follow-up visit.

Open-label RBX2660 enema treatment was an option for subjects who were deemed to have failed treatment per the pre-specified treatment failure definition. This open-label enema was administered within 21 calendar days of failure determination. If a subject received an open-label RBX2660 enema, the follow-up requirements started over from the day of the open-label RBX2660 enema administration according to the same schedule as required for the blinded portion of the study. The study design is described in Figure 2.

Figure 2. 2017-01 Study Design



Adapted from STN 125739/0, Clinical Study Report 2017-01

4.2.2. Study Endpoints

The primary efficacy endpoint was recurrence of CDI within 8 weeks of blinded treatment. Definitions for the efficacy analysis included the following:

- CDI diarrhea was defined as 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 and a positive stool test for the presence of *C. difficile* toxin documented at the time of the diarrhea.
- Treatment success was defined as the absence of CDI diarrhea through 8 weeks after completing the blinded study treatment.
- Treatment failure (CDI recurrence) was 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* toxin at the time of the diarrhea.

Treatment outcome was initially determined by the site investigator. The Endpoint Adjudication Committee, which consisted of 3 physicians (specialists in infectious disease or gastroenterology with experience managing subjects with rCDI who were not study investigators), performed a review of each case of investigator-declared outcome (blinded to treatment assignment) and was the final adjudicator of treatment outcome for the efficacy analyses.

The secondary efficacy endpoint was loss of sustained clinical response through 6 months after blinded treatment.

Safety assessments included:

1. Treatment emergent adverse events: Adverse events including SAEs were assessed through the 6-month phone call.
2. Solicited adverse events were collected daily via subject diary through 7 days after a treatment with the assigned study enema (blinded portion) or after a treatment with RBX2660 (open-label portion). Solicited events included gas (flatulence), abdominal distension or bloating, rectal irritation or pain, chills/severe shivering, abdominal pain or cramping, increased diarrhea, constipation, rectal bleeding, nausea, vomiting, and fever ≥37.8° C (100°F).

Adverse events were categorized by severity, seriousness and relatedness by the site investigator.

Specific Preferred Terms were not pre-specified as AESIs in the protocols. However, the Applicant designated events identified by two of the pre-specified SMQs (Hyperglycaemia/new onset diabetes mellitus and Immune-mediated/autoimmune disorders) as AESIs to enhance detection of any potential safety signals. See Section 5.3.6 for details.

4.2.3. Analysis Populations

Analysis populations were defined as follows:

- Intent to treat (ITT): All randomized subjects. Subjects were analyzed according to the randomized treatment received regardless of treatment misallocations. Randomized

subjects who exited prior to receiving blinded treatment were not included in the analysis.

- Modified intent to treat (mITT): All randomized subjects who successfully received blinded treatment but excluding:
  - Subjects who withdrew prior to treatment
  - Subjects for whom treatment was attempted but not completed; and
  - Subjects who discontinued from the study prior to evaluation of treatment success for the primary endpoint if the reason for the exit was not related to CDI symptoms.
- Per protocol (PP): All subjects who successfully received blinded treatment and were analyzed according to the treatment they received, excluding:
  - Subjects who had documented deviations from inclusion or exclusion criteria; and
  - Subjects who exited the study prior to the 8-week efficacy evaluation if the reason for exit was not related to CDI symptoms.
- Safety population: the population of randomized subjects who had any blinded treatment attempted or completed. Subjects were analyzed according to the treatment they actually received in case misallocations occurred.

4.2.4. Demographics and Disposition

In Study 2017-01, subjects were predominantly white, female, and not Hispanic or Latino. The median age was 63 years and 122 (45.7%) subjects were ≥65 years of age (Table 6).

Table 6. Demographic and Baseline Characteristics, Study 2017-01, Safety Population

Characteristic	Placebo N=87 n (%)	RBX2660 N=180 n (%)	Total N=267 n (%)
Age	--	--	--
Mean years [range]	57.7 [26 -86]	61.3 [19 – 93]	60.1 [19 – 93]
<65, n (%)	54 (62.1)	91 (50.6)	145 (54.3)
≥65, n (%)	33 (37.9)	89 (49.4)	122 (45.7)
Sex, n (%)	--	--	--
Male	27 (31.0)	57 (31.7)	84 (31.5)
Female	60 (69.0)	123 (68.3)	183 (68.5)
Ethnicity, n (%)	--	--	--
Hispanic or Latino	4 (4.6)	2 (1.1)	6 (2.2)
Not Hispanic or Latino	80 (92.0)	168 (93.3)	248 (92.9)
Not Reported	0 (0.0)	5 (2.8)	5 (1.9)
Unknown	3 (3.4)	5 (2.8)	8 (3.0)
Race, n (%)	--	--	--
Black/African American	6 (6.9)	8 (4.4)	14 (5.2)
White	78 (89.7)	168 (93.3)	246 (92.1)
Other	3 (3.4)	0 (0.0)	3 (1.1)
American Indian or Alaska Native	0 (0.0)	2 (1.1)	2 (0.7)
Asian	0 (0.0)	1 (0.6)	1 (0.4)
Multiple	0 (0.0)	1 (0.6)	1 (0.4)

Source: Adapted from STN 125739/0, Clinical Study Report 2017-01, Table 7

Table 7 summarizes subject disposition in the analysis groups used to evaluate the primary and secondary efficacy endpoints.

Table 7. Subject Disposition, Study 2017-01

Population	Placebo N=96 n (%)	RBX2660 N=193 n (%)	Total N=289 n (%)
Randomized	96 (100)	193 (100)	289 (100)
Intent to treat (ITT)	96 (100)	193 (100)	289 (100)
Safety population (SP)	87 (90.6)	180 (93.3)	267 (92.4)
Modified intent to treat (mITT)	85 (88.5)	177 (91.7)	262 (90.7)
Per protocol (PP)	78 (81.3)	167 (86.5)	245 (84.8)

Source: STN 125739/0, Clinical study report study 2017-01, Table 6

There were 40 screen failures out of the 320 enrolled subjects. Of the 40 subjects reported as screen failures, 31 exited the study and nine were re-screened and randomized. The most common reason for subject screen failure was “did not meet protocol inclusion or exclusion criteria.”

Of the 267 subjects randomized and treated, 33 subjects discontinued the study (RBX2660: 21 subjects, placebo: 12 subjects), 20 of whom withdrew during the blinded period and 13 of whom withdrew during the open-label period. The most common reason for withdrawal was “withdrawal by subject.” Two subjects in the RBX2660 arm discontinued from the study due to fatal TEAEs that were not related to RBX2660.

4.2.5. Efficacy Analyses

4.2.5.1. Primary efficacy endpoint analysis

The primary efficacy analysis was performed with a Bayesian hierarchical model formally integrating treatment success rates from study 2014-01 into study 2017-01. The extent of borrowing was dependent on the similarity of effect for both active and placebo groups per the planned design. Please refer to the relevant sections of the Applicant’s statistical analysis plan, attached to their briefing document, for more details. The mITT population was pre-specified as the primary analysis population for reporting purposes, supported by the ITT analysis as another important analysis for decision-making.

The statistical evidence for the treatment effect was evaluated based on the posterior probability of superiority for the RBX2660 group vs. the placebo group. Two thresholds for success were established: 1) a first, more stringent, success criterion that would be considered sufficiently persuasive to substitute for positive evidence from two adequate and well-controlled trials and 2) a second, less stringent, success criterion that would be considered sufficiently persuasive to constitute positive evidence from a single adequate and well-controlled trial.

The success thresholds were selected as analogues to frequentist one-sided type 1 error rates of 0.00125 and 0.025 without borrowing, but utilizing the Bayesian posterior probabilities of superiority. Two interim analyses were also considered in the design to allow early stopping due to futility or evidence of outstanding efficacy. An analogue to the Pocock error spending function was planned to address the increased chance of an erroneous conclusion due to the interim analyses. Accordingly, the success criteria for the interim and final analyses (first threshold) were initially set at posterior probability of superiority 0.99943; the second threshold for final analysis was set at 0.97706. At the end of the study, the Applicant adjusted the success thresholds based on the actual information fraction. As a result, the first threshold was set at posterior probability of superiority 0.9993275, and the second at posterior probability of superiority 0.9750338.

Refined analysis: Study 2017-01 Data

Table 8 shows the count data on the primary endpoint for each of the mITT, ITT, and PP analysis populations. The mITT population included 262 subjects with adjudicated outcomes for the primary efficacy analysis in study 2017-01.

Table 8. Primary Endpoint Outcomes by Treatment Arm and Analysis Population, Study 2017-01, mITT, ITT, and PP Populations

Endpoint	mITT Placebo N=85 n (%)	mITT RBX2660 N=177 n (%)	ITT[a] Placebo N=96 n (%)	ITT[a] RBX2660 N=193 n (%)	PP Placebo N=78 n (%)	PP RBX2660 N=167 n (%)
Not treated	0	0	9	13	0	0
Number with adjudicated outcome	85	177	87	180	78	167
Treatment successes	53 (62.4)	126 (71.2)	53 (60.9)	126 (70.0)	48 (61.5)	120 (71.9)
Treatment failures	32 (37.6)	49 (27.7)	32 (36.8)	49 (27.2)	30 (38.5)	46 (27.5)
Indeterminate	0 (0.0)	2 (1.1)	2 (2.3)	5 (2.8)	0 (0.0)	1 (0.60)
Imputed as failures[b]	0 (0.0)	2 (1.1)	0 (0.0)	2 (1.1)	0 (0.0)	1 (0.60)

Source: STN 125739/0, Clinical Study Report 2017-01
mITT= Modified intent to treat, ITT= intent to treat, PP= Per protocol
a. For the ITT population, percentage is calculated using the numbers of ITT subjects in each treatment arm excluding those who exited prior to receiving blinded treatment (N=87 Placebo and N=180 RBX2660) as the denominator.
b. Subjects that exited the study prior to 8 weeks due to CDI-related symptoms are imputed as failure

Study 2014-01 Data Borrowed for Study 2017-01 Primary Efficacy Analysis

Studies 2014-01 and 2017-01 investigated the same product in dosage, route, and formulation (for a single dose) and were generally similar in study design and study population. However, there were differences in treatment success definition, endpoint assessment period, and analysis population definition between the two studies. In an effort to improve exchangeability between Studies 2014-01 and 2017-01 and therefore provide more interpretable information for regulatory decision making, FDA requested a refined analysis with the following alignments between the two studies during the BLA review:

- Alignment of the primary endpoint definitions for treatment success: The Applicant indicated that the two definitions are identical although the language varies slightly.
- Alignment of the primary efficacy endpoint assessment period: Since there was 1 week between the two enemas in Study 2014-01, there were 9 weeks of assessment period compared to 8 weeks in Study 2017-01 after the single enema. The Applicant indicated that no treatment failures occurred during Week 9 and suggested that the number of treatment successes and failures in Study 2014-01 would not be changed if the primary endpoint assessment period were set to 8 weeks in Study 2014-01, in line with Study 2017-01.
- Alignment of analysis population (ITT, mITT, and PP) definitions between the two studies: Study 2014-01 and study 2017-01 have notable differences in their analysis population definitions:
    - mITT: the mITT population for study 2014-01 was defined as all subjects who completed at least one dose of study treatment excluding subjects who discontinued for any reason, and subjects with protocol deviations. In study 2017-01, the mITT population excluded subjects who withdrew prior to treatment, subjects in whom treatment was attempted but not completed, and subjects who discontinued from the study prior to evaluation of treatment failure/success for the primary endpoint if the reason for the exit was not related to CDI symptoms.
    - ITT: the ITT population of study 2014-01 included all randomized subjects, regardless of whether they completed their assigned study treatment. In comparison,

the ITT population of study 2017-01 included all randomized subjects but excluded subjects who exited prior to receiving blinded treatment.

- PP: in the PP population for study 2014-01, the exclusion criteria for ITT subjects who received the treatment to which they were randomized and were evaluable for treatment success/failure at 56 days after assigned treatment were related to withdrawal of consent, lost to follow-up, retention of enema, and major protocol deviations. In comparison, the PP population of study 2017-01 included all subjects who successfully received blinded treatment except for protocol deviations and subjects who exited prior to the 8-week efficacy evaluation, if the reason for exit was not related to CDI symptoms.

Table 9 provides the aligned Phase 2 study 2014-01 data for borrowing in the refined Bayesian analysis. There were no qualitative changes in the results with alignment of the treatment success definition and primary endpoint assessment period. Applying study 2017-01 analysis population definitions to study 2014-01 decreased the number of 2014-01 subjects in the ITT population by five subjects, increased the number of subjects in the mITT population by two subjects, and increased the number of subjects in the PP population by 38 subjects.

Table 9. Aligned Study 2014-01 Data for Borrowing in the Refined Bayesian Analysis

Endpoint	mITT Group C 1-Dose RBX2660 1-Dose Placebo	mITT Group B 2-Dose Placebo	ITT Group C 1-Dose RBX2660 1-Dose Placebo	ITT Group B 2-Dose Placebo	PP Group C 1-Dose RBX2660 1-Dose Placebo	PP Group B 2-Dose Placebo
Number of subjects (n)	39	43	43	44	37	43
Treatment success (n)	25	19	25	19	25	19
Treatment failure (n)	14	24	18	25	12	24
Success rate	0.641	0.442	0.581	0.432	0.676	0.442

Source: Adapted from Table 5, 8, 9 and 10 in Rebiotix response to CBER information request #15 (IR#15) dated July 1, 2022 (STN 125739/0.25).
mITT= Modified intent to treat, ITT= intent to treat, PP= Per protocol

Refined Analysis Results

Analysis results of the primary efficacy endpoint for the different analysis populations (mITT, ITT, and PP) in Study 2017-01 that borrowed final data from the corresponding study populations (mITT, ITT, and PP) from study 2014-01 are presented in Table 10.

The primary efficacy analysis using the mITT population resulted in an estimated treatment success rate of 0.71 in the RBX2660 group and 0.57 in the placebo group; the difference in treatment success rates was 0.13 (95% credible interval: 0.02 to 0.24). The posterior probability that RBX2660 was superior to placebo was 0.991, which met the second success threshold of 0.9750338 but did not meet the first success threshold of 0.9993275. The primary efficacy endpoint analysis using the ITT and the PP populations led to the same conclusion.

Table 10. Posterior Probability for Superiority and Posterior Estimates From the Bayesian Hierarchical Model With Study 2017-01 Analysis Population Definitions Applied to Study 2014-01

Population	Placebo Success Rate	RBX2660 (blinded) Success Rate	Treatment Effect
mITT	--	--	--
Mean	0.57	0.71	0.13
95% Credible Interval	0.48, 0.67	0.64, 0.77	0.02, 0.24
Posterior Probability	--	--	0.991
ITT	--	--	--
Mean	0.57	0.69	0.12
95% Credible Interval	0.47, 0.67	0.62, 0.76	0.01, 0.23
Posterior Probability			0.986
PP	--	--	--
Mean	0.56	0.72	0.15
95% Credible Interval	0.47, 0.66	0.65, 0.78	0.04, 0.26
Posterior Probability	--	--	0.997

Source: Adapted from STN 125739/0, Amendment 25, Final efficacy result Table 7
mITT= Modified intent to treat, ITT= intent to treat, PP= Per protocol
Note: This statistical analysis includes data from Phase 2 study (Protocol 2014-01) and Phase 3 (2017-01) studies

Applicant's Initial Analysis

The Applicant used non-final ITT data from Study 2014-01 as historical data during evaluation of trial operating characteristics at the design stage and in the Statistical Analysis Plan.

The Applicant later discovered that six subjects who were randomized but not dosed were erroneously excluded from the non-final Study 2014-01 ITT population. The Applicant corrected the issue in the final Study 2014-01 Clinical Study Report. Nevertheless, following the Statistical Analysis Plan, the Applicant presented this analysis using non-final ITT data from Study 2014-01 as the primary efficacy analysis (Table 11) and included a sensitivity analysis using the final ITT data of Study 2014-01 for borrowing in the Study 2017-01 Clinical Study Report (not shown). Both analyses led to the same conclusion as the refined primary efficacy analysis requested by the FDA, i.e., the results met the second success criterion but missed the more stringent first success criterion.

Table 11. Posterior Probability for Superiority and Posterior Estimates from the Bayesian Hierarchical Model, mITT, ITT, and PP Populations (Integrated Bayesian Analysis Borrowing Non-Final 2014-01 ITT Data Which was Used at the Design Stage)

Population	Placebo Success Rate	RBX2660 (blinded) Success Rate	Treatment Effect
mITT	--	--	--
Mean	0.58	0.70	0.12
95% Credible Interval	0.48, 0.68	0.64, 0.77	0.01, 0.23
Posterior Probability	--	--	0.986
ITT	--	--	--
Mean	0.57	0.69	0.13
95% Credible Interval	0.47, 0.67	0.63, 0.76	0.02, 0.23
Posterior Probability	--	--	0.987
PP	--	--	--
Mean	0.57	0.71	0.14
95% Credible Interval	0.47, 0.68	0.64, 0.77	0.02, 0.25
Posterior Probability	--	--	0.991

Source: Adapted from Table 11 in Study 2017-01 CSR and Protocol 2017-01 for RBX2660 Final Analysis Report, November 20, 2020 (STN 125739/0.4).
mITT= Modified intent to treat, ITT= intent to treat, PP= Per protocol

Subgroup analysis on primary efficacy endpoint

Subgroup analyses of efficacy for Study 2017-01 study only are presented in Table 12 by age group, sex, race, ethnicity, number of previous episodes of CDI recurrence at baseline, and vancomycin use duration for qualifying CDI episode for the mITT population. Numerical differences in the point estimate of the difference in success rate between RBX2660 and placebo were observed. Similar trends were also observed in the ITT and PP populations. However, due to the small sample sizes and because randomization might not be preserved in the subgroups, differences between subgroups should be interpreted with caution.

Table 12. Subgroup Analyses of Treatment Success Within 8 Weeks, mITT population

Subgroup / Analysis Population	Placebo n/N (%)	RBX2660 n/N (%)	Difference between RBX2660 and Placebo % (95% CI)
Age group	--	--	--
<65 years	35/53 (66.0)	66/90 (73.3)	7.3 (-8.4, 23.0)
≥65 years	18/32 (56.3)	60/87 (69.0)	12.7 (-7.0, 32.5)
Sex	--	--	--
Male	15/26 (57.7)	42/55 (76.4)	18.7 (-3.4, 40.7)
Female	38/59 (64.4)	84/122 (68.9)	4.4 (-10.3, 19.2)
Race	--	--	--
White	47/76 (61.8)	117/165 (70.9)	9.1 (-3.9, 22.0)
Non-White	6/9 (66.7)	9/12 (75.0)	8.3 (-31.0, 47.7)
Ethnicity	--	--	--
Hispanic or Latino	2/4 (50.0)	2/2 (100.0)	50.0 (1.0, 99.0)
Not Hispanic or Latino	51/81 (63.0)	124/175 (70.9)	7.9 (-4.6, 20.4)
Number of previous episodes of CDI recurrence at baseline*	--	--	--
≤3	38/57 (66.7)	80/111 (72.1)	5.4 (-9.4, 20.2)
>3	15/28 (53.6)	46/66 (69.7)	16.1 (-5.4, 37.7)
Number of previous episodes of CDI recurrence at baseline **	--	--	--
<3	20/33 (60.6)	42/53 (79.2)	18.6 (-1.3, 28.6)
≥3	33/52 (63.5)	84/124 (67.7)	4.3 (-11.1, 19.7)
Vancomycin use duration for qualifying CDI episode	--	--	--
≤14 days	18/26 (69.2)	32/45 (71.1)	1.9 (-20.3, 24.0)
>14 days	28/50 (56.0)	75/109 (68.8)	12.8 (-3.5, 29.1)

Source: Adapted from Table 21 in Study 2017-01 CSR
mITT= modified intent to treat
*Applicant's results submitted with the original BLA
**Adapted from Table 11 and 12 in Rebiotix response to CBER information request #15 (IR#15) dated July 1, 2022 (STN 125739/0.25).

4.2.5.2. Secondary efficacy analyses

The secondary efficacy endpoint of sustained clinical response was defined as treatment success for the presenting CDI recurrence at 8 weeks and no new CDI episodes for greater than 8 weeks after treatment during the 6 months of follow-up. In the mITT population, the sustained clinical response rate difference between the RBX2660 (65.5%) and placebo (56.5%) arms was 9.1% and not statistically significant (Table 13). The analyses using the ITT and PP populations showed similar results. Additionally, the Applicant evaluated the proportion of subjects with no new CDI episodes after Week 8 and through 6 months follow-up among the

subjects who achieved treatment success at 8 weeks after treatment. The proportions were 92.1% in the RBX2660 group and 90.6% in the placebo group in the mITT population.

Table 13. Comparison of Sustained Clinical Response, Study 2017-01

Endpoint	ITT RBX2660 N=180	ITT Placebo N=87	mITT RBX2660 N=177	mITT Placebo N=85	PP RBX2660 N=167	PP Placebo N=78
Treatment success, n (%)	116 (64.4)	48 (55.2)	116 (65.5)	48 (56.5)	110 (65.9)	43 (55.1)
Treatment failure, n (%)	64 (35.6)	39 (44.8)	61 (34.5)	37 (43.5)	57 (34.1)	35 (44.9)
Difference	9.3	--	9.1	--	10.7	--
95% CI	-3.3, 21.9	--	-3.6, 21.7	--	-2.4, 23.9	--
p-value	0.145	--	0.156	--	0.106	--

Source: Table 1 in response to CBER information request IR#21 (STN 125739/0.32)
mITT= Modified intent to treat, ITT= intent to treat, PP= Per protocol

4.3. Other Studies

RBX2660 was evaluated in several open label studies (2013-001, 2015-01, 2019-01) and in a retrospective study, 2019-02. In these studies, the Applicant collected the 8-week CDI recurrence data and analyzed them in a descriptive manner. However, interpretation of these open-label and retrospective data is limited by the lack of concurrent placebo control, inclusion of a different dosing regimen (2 doses) than intended for licensure, and differences between study populations in the open-label and placebo-controlled studies. Therefore, discussion of treatment success outcomes from these open-label and retrospective studies is not included in this briefing document.

5. Integrated Summary of Safety

5.1. Methodology

The integrated summary of safety (ISS) included safety data from a pooled analyses of all subjects who were exposed to at least one study treatment in five prospective studies (three Phase 2 studies: 2013-001, 2014-01, 2015-01, and two Phase 3 studies: 2017-01 and 2019-01). Subjects who were enrolled but not treated were not included in the ISS. In addition, 110 subjects who enrolled into the historical control arm of study 2015-01 and 78 subjects from the retrospective study 2019-02 were not included in the safety population. Based on the differences in study design, the ISS was organized by study groupings as follows:

- The Full ISS included any subject who was exposed to at least one dose of RBX2660 (blinded or open-label) or placebo from the five prospective studies (2013-001, 2014-01, 2015-01, 2017-01 and 2019-01. See Table 1 and Table 2 for summaries of studies included in the ISS). The Full ISS population was comprised of 749 subjects exposed to at least one dose of RBX2660 and 83 subjects exposed only to placebo. The safety review specifically focused on the 429 subjects who received one dose of RBX2660 (dosing regimen proposed for licensure). Of the 429 subjects who received one dose of RBX2660, most were enrolled in the open-label Phase 3 study 2019-01 (n=211; 49.2%) and the placebo-controlled Phase 3 study 2017-01 (n=163; 38.0%). The majority of the placebo recipients (n=63; 75.9%) were enrolled in 2017-01.

  Considerations in the interpretation of comparisons between the placebo and treatment groups in the Full ISS include 1) the open-label nature of many of the RBX2660 doses in the

ISS population; 2) subjects crossed over to receive RBX2660 in an open-label fashion due to recurrence of CDI, which may reflect increased risk for adverse events due to underlying risk factors that predispose to rCDI or morbidities attributable to the CDI; and 3) subjects were followed for 6 months after the last dose of study treatment, resulting in a longer duration of follow up for subjects who received multiple doses.

- The Blinded ISS included any subject who was exposed to RBX2660 or placebo in the randomized, double-blind, placebo-controlled studies 2014-01 and 2017-01. Subjects in the placebo group who experienced a CDI recurrence and received open-label RBX2660 were removed from the placebo group and counted as being exposed to RBX2660 in the safety analyses. This population was comprised of 83 subjects exposed to placebo and 312 subjects exposed to RBX2660, including 193 who received blinded RBX2660, 48 who received blinded placebo followed by RBX2660, and 71 who received blinded and then open-label RBX2660. The safety review specifically focused on the 193 subjects who received one or two doses of blinded RBX2660. Randomization is no longer preserved between the blinded placebo and RBX2660 groups as a result of exclusion of the subjects who experienced a CDI recurrence and received open-label RBX2660. The observed safety profiles may not be representative of those expected in the underlying groups.

For each ISS group, the safety data are analyzed by treatment group (treatment, blinding, and sequence) and by number of exposures (1-4 doses of RBX2660).

All studies included at least 6 months of safety follow-up from the last dose. Two studies (2014-01 and 2015-01) included follow up through 24 months; data from 6 months through 24 months of follow up were analyzed separately.

The Applicant provided a safety update to the BLA six months after BLA submission, with safety data from an additional 229 subjects that were enrolled in study 2019-01, who were exposed to at least one dose of RBX2660 and increased the overall RBX2660 exposure from 749 subjects to 978 subjects. There were no new deaths reported in the safety update. There were two serious TEAEs (CDI and ulcerative colitis and CDI) reported by the investigator to be possibly related to RBX2660; however, the FDA considered the events to have plausible alternative etiologies, including rCDI and pre-existing conditions. In general, the safety update did not reveal any new safety trends to change the safety profile compared to what was provided in the ISS at the time of BLA submission. Thus, the safety analysis for the BLA was conducted using the initial ISS dataset provided by the Applicant.

5.2. Exposure and Demographics

In the Full ISS, a complete treatment course consisted of one dose (studies 2017-01, 2019-01, 2015-01, and 2013-001) or two doses administered one week apart (studies 2014-01 and 2015-01). All studies except Study 2015-01 offered a second dose of RBX2660 to subjects who experienced a recurrence within 8 weeks of completing treatment.

The number of subjects contributing to the safety population by treatment exposure and study are described in Table 14 below.

Table 14. Treatment Exposures, Full ISS, ISS Population

Study	Placebo Only 1-2 Doses (N=83) n (%)	RBX2660 1 Dose (N=429) n (%)	RBX2660 2 Doses (N=294) n (%)	RBX2660 3 Doses (N=14) n (%)	RBX2660 4 Doses (N=12) n (%)	Total (N=832) n (%)
2013-001	0	19 (4.4)	15 (5.1)	0	0	31 (4.1)
2014-01	20 (24.1)	30 (7.0)	52 (17.7)	14 (100.0)	12 (100.0)	128 (15.4)
2015-01	0	6 (1.4)	143 (48.6)	0	0	149 (17.9)
2017-01	63 (75.9)	163 (38.0)	41 (13.9)	0	0	267 (32.1)
2019-01	0	211 (49.2)	43 (14.6)	0	0	254 (30.5)

Source: STN 125739/0, Clinical Study Report for ISS, Table 15
ISS= Integrated summary of safety

The demographics and baseline characteristics of subjects included in the ISS by number of exposures are described in Table 15 below.

Table 15. Demographic and Baseline Characteristics by Treatment Exposure, Full ISS, ISS population

Characteristic	Placebo Only 1-2 Doses (N=83)	RBX2660 1 Dose (N=429)	RBX2660 2 Doses (N=294)	RBX2660 3 Doses (N=14)	RBX2660 4 Doses (N=12)
Age (years)	--	--	--	--	--
Mean	58.1	59.5	63.4	67.5	66.3
Min-Max	19.0 – 90.0	18.0 – 94.0	20.0 – 103.0	25.0 – 89.0	44.0 – 87.0
Age group (years), n (%)	--	--	--	--	--
<65	52 (62.7)	245 (57.1)	135 (45.9)	5 (35.7)	5 (41.7)
≥65	31 (37.3)	184 (42.9)	159 (54.1)	9 (64.3)	7 (58.3)
≥75	12 (14.5)	86 (20.0)	96 (32.7)	7 (50.0)	4 (33.3)
Sex, n (%)	--	--	--	--	--
Male	23 (27.7)	143 (33.3)	105 (35.7)	7 (50.0)	4 (33.3)
Female	60 (72.3)	286 (66.7)	189 (64.3)	7 (50.0)	8 (66.7)
Race, n (%)	--	--	--	--	--
American Indian or Alaska Native	0	3 (0.7)	0	0	0
Asian	0	3 (0.7)	3 (1.0)	0	0
Black or African American	6 (7.2)	13 (3.0)	13 (4.4)	1 (7.1)	0
White	75 (90.4)	401 (93.5)	275 (93.5)	13 (92.9)	12 (100.0)
Other	2 (2.4)	5 (1.2)	3 (1.0)	0	0
Multiple	0	4 (0.9)	0	0	0
Number of CDI episodes prior to enrollment, n (%)	--	--	--	--	--
≥1	83 (100.0)	424 (98.8)	293 (99.7)	14 (100.0)	12 (100.0)
1-3	60 (72.3)	281 (65.5)	134 (45.6)	1 (7.1)	4 (33.3)
≥3	57 (68.7)	304 (70.9)	268 (91.2)	14 (100.0)	12 (100.0)

Characteristic	Placebo Only 1-2 Doses (N=83)	RBX2660 1 Dose (N=429)	RBX2660 2 Doses (N=294)	RBX2660 3 Doses (N=14)	RBX2660 4 Doses (N=12)
Number of CDAD/CDI episodes before first enema treatment, n(%)	--	--	--	--	--
1	0	7 (1.6)	0	0	0
2	26 (31.3)	113 (26.3)	25 (8.5)	0	0
3	34 (41.0)	161 (37.5)	109 (37.1)	1 (7.1)	4 (33.3)
4	16 (9.3)	87 (20.3)	68 (23.1)	8 (57.1)	4 (33.3)
5	5 (6.0)	30 (7.0)	42 (14.3)	4 (28.6)	1 (8.3)
6	2 (2.4)	15 (3.5)	26 (8.8)	1 (7.1)	0
7	0	5 (1.2)	11 (3.7)	0	0
8	0	2 (0.5)	6 (2.0)	0	2 (16.7)
9	0	2 (0.5)	3 (1.0)	0	1 (8.3)
10	0	1 (0.2)	1 (0.3)	0	0
12	0	0	1 (0.3)	0	0
14	0	1 (0.2)	0	0	0
25	0	0	1 (0.3)	0	0

Source: STN 125739/0, Adapted from Clinical Study Report for the ISS, Tables 19 and 23
CDAD= *C. difficile* associated diarrhea; CDI= *C. difficile* infection

The mean age of subjects was comparable between the one-dose RBX2660 group (59.5 years) the placebo only group (58.1 years) and comparable between the blinded RBX2660 group (61.1 years) and any RBX2660 group (61.3 years). The proportion of subjects >65 and >75 years of age increased as the number of treatment exposures increased, which likely reflects age as a risk factor for rCDI. The remaining demographic characteristics were generally comparable between the placebo only group and each of the RBX2660-exposed groups. The proportion of subjects with a history of ≥ 3 previous events of CDI increased as the number of treatment exposures increased, which likely reflects prior rCDI as a risk factor for a subsequent recurrence.

Overall, the demographic and baseline characteristics were comparable between the one-dose RBX2660, Blinded RBX2660, and Any RBX2660 groups. However, the mean age of subjects who received RBX2660 was higher compared to the placebo only group.

5.3. Safety Analysis

Table 16 below summarizes safety outcomes in the Full ISS population for RBX2660.

Table 16. Safety Outcomes, Full ISS, ISS Population

TEAE Category	Placebo Only (1-2 Doses) N=83 n (%)	RBX2660 1 Dose N=429 n (%)	RBX2660 2 Doses N=294 n (%)	RBX2660 3 Doses N=14 n (%)	RBX2660 4 Doses N=12 n (%)	Any[a] RBX2660 N=749 n (%)	Total N=832 n (%)
Subjects with AEs	54 (65.1)	285 (66.4)	236 (80.3)	11 (78.6)	11 (91.7)	543 (72.5)	597 (71.8)
TEAEs	--	--	--	--	--	--	--
Subjects with TEAEs	50 (60.2)	265 (61.8)	234 (79.6)	11 (78.6)	11 (91.7)	521 (69.6)	571 (68.6)
Subjects with severe TEAEs	7 (8.4)	40 (9.3)	48 (16.3)	1 (7.1)	6 (50.0)	95 (12.7)	102 (12.3)
Subjects with potentially life-threatening (maximum severity) TEAEs	1 (1.2)	9 (2.1)	10 (3.4)	1 (7.1)	2 (16.7)	22 (2.9)	23 (2.8)
TEAEs leading to withdrawal from study	0	4 (0.9)	3 (1.0)	0	0	7 (0.9)	7 (0.8)
TEAEs leading to death	0	5 (1.2)	10 (3.4)	1 (7.1)	2 (16.7)	18 (2.4)	18 (2.2)
TEAE relatedness	--	--	--	--	--	--	--
Related to RBX2660	16 (19.3)	97 (22.6)	72 (24.5)	4 (28.6)	5 (41.7)	178 (23.8)	194 (23.3)
Related to Enema procedure	17 (20.5)	65 (15.2)	54 (18.4)	3 (21.4)	3 (25.0)	125 (16.7)	142 (17.1)
Related to *C. difficile* infection	17 (20.5)	90 (21.0)	109 (37.1)	7 (50.0)	7 (58.3)	213 (28.4)	230 (27.6)
Related to a pre-existing condition	29 (34.9)	155 (36.1)	145 (49.3)	5 (35.7)	10 (83.3)	315 (42.1)	344 (41.3)
Serious TEAEs	6 (7.2)	36 (8.4)	56 (19.0)	4 (28.6)	10 (83.3)	106 (14.2)	112 (13.5)
Serious TEAE relatedness	--	--	--	--	--	--	--
Related to RBX2660	0	1 (0.2)	2 (0.7)	0	2 (16.7)	5 (0.7)	5 (0.6)
Related to Enema procedure	0	0	1 (0.3)	0	0	1 (0.1)	1 (0.1)
Related to *C. difficile* infection	1 (1.2)	15 (3.5)	17 (5.8)	3 (21.4)	4 (33.3)	39 (5.2)	40 (4.8)
Related to a pre-existing condition	3 (3.6)	29 (6.8)	40 (13.6)	3 (21.4)	8 (66.7)	80 (10.7)	83 (10.0)
Serious TEAEs leading to withdrawal from study	0	3 (0.7)	2 (0.7)	0	0	5 (0.7)	5 (0.6)
Serious TEAEs leading to death	0	5 (1.5)	10 (3.4)	1 (7.1)	2 (16.7)	18 (2.4)	18 (2.2)

Source: STN 125739/0, Adapted from Integrated Summary of Safety, Table 33 and 34
a. Any RBX2660 group includes subjects who received any number of RBX2660 doses

Table 17 below summarizes safety outcomes in the Blinded ISS population for RBX2660, including only the blinded data.

Table 17. Safety Outcomes, Blinded ISS, ISS Population

TEAE Category	Blinded Placebo Only N=83 n (%)	Blinded RBX2660 Only N=193 n (%)
Subjects with AEs	54 (65.1)	145 (75.1)
TEAEs	--	--
Subjects with TEAEs	50 (60.2)	135 (69.9)
Subjects with severe TEAEs	7 (8.4)	19 (9.8)
Subjects with potentially life-threatening (maximum severity) TEAEs	1 (1.2)	6 (3.1)
TEAEs leading to withdrawal from study	0	1 (0.5)
TEAEs leading to death	0	5 (2.6)
TEAE relatedness	--	--
Related to RBX2660	16 (19.3)	51 (26.4)
Related to Enema procedure	17 (20.5)	37 (19.2)
Related to *C. difficile* infection	17 (20.5)	45 (23.3)
Related to a pre-existing condition	29 (34.9)	83 (43.0)
Serious TEAEs	6 (7.2)	20 (10.4)
Serious TEAE relatedness	--	--
Related to RBX2660	0	1 (0.5)
Related to Enema procedure	0	0
Related to *C. difficile* infection	1 (1.2)	3 (1.6)
Related to a pre-existing condition	3 (3.6)	19 (9.8)
Serious TEAEs leading to withdrawal from study	0	1 (0.5)
Serious TEAEs leading to death	0	5 (2.6)

Source: STN 125739/0, Adapted from Integrated Summary of Safety, Table 31

5.3.1. Solicited Adverse Events

As the largest double blind, randomized, placebo-controlled study, 2017-01 provides the most representative and appropriate population for assessment of solicited adverse events. Therefore, comparison of solicited adverse events between placebo and blinded treatment in study 2017-01 is presented here. The most frequently reported solicited adverse events from days 1 through 7 during the blinded period in study 2017-01 were flatulence, abdominal distension or bloating and abdominal pain or cramping. Most of the solicited events were mild or moderate in severity. The most common severe solicited events were abdominal pain/cramping, increased diarrhea, and abdominal distension/bloating. In general, there were more solicited events in subjects exposed RBX2660 compared to placebo only, but more severe and potentially life-threatening solicited events were reported in placebo compared to RBX2660 as follows abdominal distension/bloating, increased diarrhea, constipation, rectal irritation or pain and nausea.

Table 18 below summarizes the solicited adverse events across the exposure groups.

Table 18. Summary of Solicited Adverse Events by Maximum Post-Treatment Severity During Days 1-7 in Study 2017-01 (SP)

Solicited Event	Blinded Placebo Only N=87 n (%)	Blinded RBX2660 Only N=180 n (%)
Subjects with at least one solicited AE	84 (96.6)	170 (94.4)
Gas (flatulence)	--	--
None	8 (9.2)	24 (13.3)
Mild	33 (37.9)	84 (46.7)
Moderate	44 (50.6)	69 (38.3)
Abdominal distension or bloating	--	--
None	16 (18.4)	63 (35.0)
Mild	26 (29.9)	65 (36.1)
Moderate	33 (37.9)	37 (20.6)
Severe	10 (11.5)	12 (6.7)
Increased diarrhea	--	--
None	27 (31.0)	74 (41.1)
Mild	25 (28.7)	41 (22.8)
Moderate	22 (25.3)	40 (22.2)
Severe	9 (10.3)	21 (11.7)
Potentially life-threatening	2 (2.3)	1 (0.6)
Abdominal pain or cramping	--	--
None	15 (17.2)	58 (32.2)
Mild	28 (32.2)	60 (33.3)
Moderate	22 (25.3)	42 (23.3)
Severe	17 (19.5)	16 (8.9)
Potentially life-threatening	3 (3.4)	1 (0.6)
Constipation	--	--
None	61 (70.1)	147 (81.7)
Mild	12 (13.8)	21 (11.7)
Moderate	7 (8.0)	8 (4.4)
Severe	3 (3.4)	1 (0.6)
Potentially life-threatening	2 (2.3)	0 (0.0)
Fever	--	--
None	73 (83.9)	150 (83.3)
Mild	9 (10.3)	17 (9.4)
Moderate	3 (3.4)	7 (3.9)
Severe	0 (0.0)	2 (1.1)
Potentially life-threatening	0 (0.0)	1 (0.6)
Chills/severe shivering	--	--
None	60 (69.0)	123 (68.3)
Mild	19 (21.8)	36 (20.0)
Moderate	5 (5.7)	14 (7.8)
Severe	1 (1.1)	4 (2.2)
Potentially life-threatening	0 (0.0)	0 (0.0)
Rectal irritation or pain	--	--
None	39 (44.8)	97 (53.9)
Mild	22 (25.3)	57 (31.7)
Moderate	19 (21.8)	17 (9.4)
Severe	5 (5.7)	5 (2.8)
Potentially life-threatening	0 (0.0)	1 (0.6)

Solicited Event	Blinded Placebo Only N=87 n (%)	Blinded RBX2660 Only N=180 n (%)
Rectal bleeding	--	--
None	68 (78.2)	151 (83.9)
Mild	12 (13.8)	23 (12.8)
Moderate	3 (3.4)	3 (1.7)
Severe	1 (1.1)	0 (0.0)
Potentially life-threatening	1 (1.1)	0 (0.0)
Nausea	--	--
None	46 (52.9)	113 (62.8)
Mild	22 (25.3)	36 (20.0)
Moderate	11 (12.6)	23 (12.8)
Severe	5 (5.7)	4 (2.2)
Potentially life-threatening	1 (1.1)	1 (0.6)
Vomiting	--	--
None	79 (90.8)	161 (89.4)
Mild	4 (4.6)	12 (6.7)
Moderate	0 (0.0)	3 (1.7)
Severe	1 (1.1)	1 (0.6)
Potentially life-threatening	1 (1.1)	0 (0.0)

Source: STN 125739/0, Study 2017-01 Clinical Study Report, Table 29

5.3.2. Unsolicited Treatment Emergent Adverse Events

Blinded ISS

In the Blinded ISS, the proportion of subjects reporting unsolicited TEAEs was higher in the blinded RBX2660 group (69.9%) compared to the blinded placebo group (60.2%). The most commonly reported TEAEs were gastrointestinal, including diarrhea, abdominal pain and distention, nausea, flatulence, constipation and vomiting. Numerical imbalances (higher proportion of participants in the RBX2660 group) were noted for the following events: abdominal pain (19.7% blinded RBX2660 vs. 8.4% blinded placebo), nausea (10.9% vs. 3.6%), flatulence (7.3% vs. 1.2%), abdominal distention (5.7% vs. 3.6%), anxiety (3.6% vs. 1.2%), depression (3.1% vs. 0%), chronic obstructive pulmonary disease (3.1% vs 0%), decreased diastolic blood pressure (2.6% vs 0%), cough (2.6% vs 0%), and asthenia (2.1% vs 0%).

Related events were reported by a higher proportion of subjects after blinded RBX2660 (26.4%) compared to blinded placebo (19.3%). The most commonly reported related TEAEs included gastrointestinal events (diarrhea, abdominal pain and distention, nausea, flatulence, constipation and anorectal discomfort), all of which were reported by a higher proportion of subjects in the blinded RBX2660 group.

The proportions of subjects reporting severe and life-threatening unsolicited TEAEs were higher in the blinded RBX2660 group (9.8% and 3.1%, respectively) compared to the blinded placebo group (8.4% and 1.2%, respectively). No life-threatening events were considered related by the investigator.

Full ISS

<u>One-dose RBX2660 group</u>

In the Full ISS, the proportion of subjects reporting unsolicited TEAEs was comparable between the placebo only (60.2%) and one-dose RBX2660 (61.8%) groups. The most commonly reported TEAEs were gastrointestinal, including diarrhea, abdominal pain and distention,

nausea, flatulence, constipation and vomiting. Numerical imbalances (higher proportion of participants in the one-dose RBX2660 group) were noted for the following events: abdominal pain (14.9% one-dose RBX2660 vs. 8.4% placebo), nausea (10.0% vs. 3.6%), flatulence (8.4% vs. 1.2%), abdominal distention (5.6% vs. 3.6%), CDI (2.1% vs. 0%), decreased diastolic blood pressure (1.9% vs 0%), and depression (2.3% vs. 0%).

Related events were reported by a similar proportion of subjects in the one-dose RBX2660 group (22.6%) compared to the placebo group (19.3%). The most commonly reported related TEAEs included gastrointestinal events (diarrhea, abdominal pain and distention, nausea, flatulence, constipation and anorectal discomfort), all of which (other than constipation) were reported by a higher proportion of subjects in the blinded RBX2660 group.

The proportions of subjects reporting severe and life threatening unsolicited TEAEs were higher in the one-dose RBX2660 group (9.3% and 2.1%, respectively) compared to the placebo group (8.4% and 1.2%, respectively).

Any RBX2660 group
The proportions of subjects reporting TEAEs, severe TEAEs, and potentially life threatening TEAEs was higher in the Any RBX2660 group (n=749; 69.6%, 12.7%, and 2.9%, respectively) compared to the placebo group (60.2%, 8.4%, and 1.2%, respectively). In general, the pattern of the most commonly reported TEAEs was consistent with the other analyzed treatment groups. Related events were reported by a similar proportion of subjects in the Any RBX2660 group (23.8%) compared to the placebo group (19.3%).

5.3.3. Serious TEAEs

Blinded ISS

In the Blinded ISS, the proportion of subjects reporting serious TEAEs was higher in the blinded RBX2660 group (10.4%) compared to the blinded placebo group (7.2%). Serious TEAEs were most commonly reported in the SOCs of *Gastrointestinal disorders* and *Infections and infestations*. Numerical imbalances (higher proportion of participants in the blinded RBX2660 group and reported by more than one subject) were noted for the following events: abdominal pain, general physical health deterioration, CDI, and chronic obstructive pulmonary disease (all 1.0% blinded RBX2660 vs 0% placebo). The remaining serious events were reported by one subject each (0.5%).

The following events were reported in the blinded placebo group: Arnold-Chiari malformation, colitis, sepsis, gastroenteritis, dehydration, acute kidney injury, acute respiratory failure (n=1 each, 1.2%), and cellulitis (n=2, 2.4%).

Full ISS

One-dose RBX2660 group
In the Full ISS, the proportion of subjects reporting serious TEAEs was comparable between the one-dose RBX2660 (8.4%) group and the placebo only (7.2%) group. Serious events were most commonly reported in the SOCs of *Infections and infestations* (CDI, *C. difficile* colitis, urinary tract infection, pneumonia and sepsis), *Gastrointestinal disorders* (abdominal pain, diarrhea, ileus, colitis) and *Respiratory, thoracic and mediastinal disorders* (chronic obstructive pulmonary disease, acute respiratory failure and dyspnea). Numerical imbalances (higher proportion of participants in the one-dose RBX2660 group and reported by more than one subject) were noted for the following events: CDI (2.1% one-dose RBX2660 vs. 0% placebo); *C. difficile* colitis

and chronic obstructive pulmonary disease (each 0.7% one-dose RBX2660 vs. 0% placebo); bacteremia, pneumonia, urinary tract infection, general physical health deterioration, vomiting, ileus, diarrhea, abdominal pain and cardiac failure congestive each (0.5% one-dose RBX2660 vs. 0% placebo). The remaining serious events were reported by one subject each (0.2%).

Any RBX2660 group
Including all RBX2660 recipients, the imbalance in serious TEAEs was larger, with 14.2% of the subjects in the Any RBX2660 group reporting events compared to 7.2% of subjects in the placebo only group. This larger imbalance is attributable to the high rate of serious TEAEs in the multiple dose populations (19%, 28.6%, and 83.3% of subjects in the two, three, and four-dose RBX2660 groups, respectively). Serious events were most commonly reported in the SOCs of *Gastrointestinal disorders* and *Infections and infestations*. Numerical imbalances (higher proportion of participants in the Any RBX2660 group and reported by more than one subject) were noted for the following events: CDI (2.1% Any RBX2660 vs 0% placebo); urinary tract infection (1.1% Any RBX2660 vs 0% placebo); chronic obstructive pulmonary disease, cardiac failure congestive (each 0.9% Any RBX2660 vs 0% placebo); *C. difficile* colitis, pneumonia (each 0.8% Any RBX2660 vs 0% placebo); abdominal pain, diarrhea, (each 0.7% Any RBX2660 vs 0% placebo); ileus, bacteremia (each 0.5% Any RBX2660 vs 0% placebo); gastrointestinal hemorrhage, respiratory failure (each 0.4% Any RBX2660 vs 0% placebo); nephrolithiasis, upper abdominal pain, colitis, constipation, intestinal obstruction, nausea, esophagitis, vomiting, pyrexia, acute myocardial infarction, transient ischemic attack, alcohol withdrawal syndrome, atrial fibrillation, leukocytosis, hyperkalemia, dyspnea, and alcohol poisoning (each 0.3% Any RBX2660 vs 0% placebo).

Events reported by a higher proportion of the placebo only group compared to the Any RBX660 group included: sepsis (0.7% Any RBX2660 vs 1.2% placebo); colitis and acute respiratory failure (each 0.3% Any RBX2660 vs 1.2% placebo), and acute kidney injury (0.4% Any RBX2660 vs 1.2% placebo).

All remaining serious TEAEs in the Any RBX2660 group were reported by one subject each (0.1%).

Through 8 weeks
For the time period from baseline to 8 weeks after administration of the first RBX2660 exposure, serious TEAEs were reported in 3.6% (3/83) of subjects in the placebo only compared to 6.1% (26/429) subjects in the one RBX2660 dose group, and 8.9% (67/749) subjects in the Any RBX2660 group.

Although the overall imbalances in serious TEAEs between the blinded and Any RBX2660 groups when compared to the placebo group are notable, a review of the events did not identify apparent trends in serious TEAEs by MedDRA SOC or Preferred Term that would suggest a causal association. Following review of individual case narratives, the FDA did not identify any serious TEAEs that were considered causally related to RBX2660.

Related Serious TEAEs

Five subjects had serious TEAEs considered related to RBX2660 by the investigator, all of which were plausibly related to alter pre-existing condition or CDI in the opinion of the FDA.

Three serious TEAEs were reported in the context of rCDI following treatment. In these cases, the FDA considers the serious events to be plausibly related to the rCDI and not to RBX2660.

- A 44-year-old white female with history of Marfan syndrome, interstitial cystitis, hypertension, angina, fibromyalgia, depression, morbid obesity and rCDI (4 episodes) received 2 doses of RBX2660 and was diagnosed with a serious adverse event of abdominal pain on day 10 post RBX2660 exposure. The subject reported nausea, vomiting and eight bowel movements with abdominal pain worse after eating and was diagnosed with rCDI. The investigator reported the serious event of abdominal pain as related to RBX2660 and CDI. The investigator reported the rCDI as unrelated to RBX2660 and related to CDI.
- A 58-year-old white male with history of diabetes and atrial fibrillation received one of two doses of RBX2660 and reported two serious adverse events of rCDI on day 4 and recurrent CDI and diarrhea on day 22 post RBX2660. The subject did not receive the second dose because of a serious adverse event of rCDI that was diagnosed on day 4 and considered resolved on day 17. Diarrhea requiring hospitalization was reported as a serious adverse event on day 24 and considered resolved on day 27 post RBX2660. Recurrent CDI was reported as a serious adverse event on day 31 and 64 post RBX2660 and considered resolved on day 41 and 69 post RBX2660 respectively. The investigator reported the three rCDI episodes as possibly related to RBX2660 and related to CDI, and the diarrhea was reported as being possibly related to the RBX2660 and CDI.
- A 94-year-old white female with history of chronic kidney disease stage IV, hypertension, hyperglycemia, gastroesophageal reflux disease, depression with anxiety, anemia in chronic disease and recurrent CDI (5 recurrences) received 2 doses of RBX2660 and reported serious adverse events of ileus, leukocytosis, CDI (reported as relapsed severe CDI) and pyrexia (fever of 102.5°F) on day 21 post RBX2660 exposure. Her clinical course was complicated by serious unrelated adverse events of atrial fibrillation on day 25, acute myocardial infarction on day 26 and malnutrition on day 27. She underwent additional fecal transplant on day 29 to manage the CDI non-invasively given that her decline in clinical status was not amenable to surgical intervention; however, the fecal transplant was not successful. The subject died on day 31 post RBX2660 exposure due to the serious event of CDI and other co-morbidities. The investigator considered the events of ileus, leukocytosis, CDI and pyrexia to be possibly related to RBX2660 and the enema procedure and definitely related to CDI disease and pre-existing condition.

Details of the remaining two events are as follows:

- A 53-year-old white male with history of acute myeloid leukemia (AML) in remission following stem cell transplant received 2 doses of RBX2660 and was diagnosed with a serious adverse event of recurrent acute myeloid leukemia on day 69 post RBX2660 exposure. The investigator reported the recurrent AML as related to RBX2660 and pre-existing condition. The subject received multiple chemotherapy regimens and the event was noted to be resolved on day 253 post RBX2660. However, subject was diagnosed with relapsed AML on day 357 post RBX2660 and subsequently died. The death was considered unrelated to RBX2660 by the investigator. FDA does not consider the event of AML relapse to be related to RBX2660 in this subject, given the pre-existing diagnosis and lack of temporal relationship of RBX2660 and onset of AML symptoms.
- A 59-year-old white female with history of Parkinson’s disease and chronic constipation received two doses of RBX2660 and reported a serious adverse event of worsening chronic constipation on day 45 post RBX2660. The investigator reported worsening chronic constipation as related to RBX2660; FDA does not consider this event as related to RBX2660 due to a lack of temporal relationship (onset 45 days post RBX2660 exposure).

5.3.4. Deaths

Across studies, exposure groups and treatment groups through 6 months post study treatment, a total of 2.4% of subjects (18/749) in the Full ISS Any RBX2660 group experienced fatal TEAEs compared to zero in the placebo only group. This imbalance was also observed when comparing the proportion of subjects with fatal TEAEs in the Blinded ISS blinded RBX2660 group (2.6%) and the Full ISS one-dose RBX2660 group (1.2%) to the placebo only group (0%). The proportion of subjects reporting any TEAEs leading to death increased as the number of treatment exposures increased, ranging from 5/429 (1.2%) subjects in the one-dose RBX2660 group to 2/12 (16.7%) subjects in the four-dose RBX2660 group. Of the 18 fatal TEAEs observed in the RBX2660 clinical program, 17 were adjudicated as being unrelated to treatment. FDA agrees with the assessment of causality for these cases. One death due to relapsed CDI on Day 21 (study 2015-01) was considered possibly related to RBX2660 and the enema procedure and definitely related to CDI by the investigator. Following review of the narrative and case report form by FDA, the event was considered not to be causally related to RBX2660 but was considered definitely related to CDI.

Of the subjects who died, two had onset of the fatal TEAE and death within 30 days of the last treatment. One of the subjects had rCDI 14 days after the last (2nd) RBX2660 dose and died 10 days later (described in Section 5.3.3 above). The other subject was a 63 year old white male with history of end stage renal disease, diabetes mellitus and rCDI (4 episodes) who received three doses of RBX2660 and one dose of placebo and was reported to have serious adverse events of sepsis, bacteremia, respiratory failure and staphylococcal infection on Day 24 post last RBX2660 exposure. The subject died on day 28 post RBX2660 due to sepsis that was noted to be secondary to methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia and possibly related to healthcare acquired associated pneumonia. The investigator reported the serious event of sepsis and bacteremia as unrelated to RBX2660 and FDA concurs with this assessment.

Two additional subjects had onset of the fatal TEAE within 30 days of the last treatment, including acute respiratory failure 24 days after the last (2nd) RBX2660 dose and renal failure 8 days after the last (4th) RBX2660 dose. Details of these two subjects are presented below.

- A 76-year-old white male with history of prostate and lung cancer and left thoracotomy with lobectomy and rCDI (6 episodes) who received two doses of RBX2660 was reported to have a serious adverse event of acute respiratory failure on day 31 post RBX2660, when he had respiratory distress, tachycardia and shortness of breath following a cystoscopy procedure to replace a ureteral stent for right-sided hydronephrosis secondary to history of hormone refractory metastatic prostate cancer. He was diagnosed with acute hypoxic respiratory failure secondary to acute exacerbation of chronic obstructive pulmonary disease and left lower lobe pneumonia. On day 56 post RBX2660, the subject was reported to have worsening of acute respiratory failure and died on day 75 post RBX2660 due to acute respiratory failure, with contributing factors listed as pneumonia and lung cancer. The investigator reported the serious event of acute respiratory failure as unrelated to RBX2660 and FDA concurs with this assessment.
- A 84-year-old white female with history of renal insufficiency and rCDI (3 episodes) who received RBX2660 while admitted to the hospital for treatment of rCDI. The subject remained well without recurrent diarrhea with planned discharge; however, she was reported to have a serious event of renal impairment on day 19 post RBX2660 and was treated with intravenous fluids and interruption of antihypertensive medications. She was

reported to have recurrent *C. difficile* diarrhea on day 23 post RBX2660, and *Escherichia coli* urinary tract infection with worsening renal function on day 26 post RBX2660. The *C. difficile* diarrhea reportedly ended on day 34 post RBX2660, and two additional doses of RBX2660 were administered on day 35 and day 41. On day 49, subject was diagnosed with anuria and renal failure. Hemodialysis was initiated on day 51 post RBX2660. Additional diagnosis of rCDI was reported on day 50 post RBX2660; however, the subject was unable to tolerate oral or rectal antibiotic treatment and continued to have watery bowel movements. Dialysis was discontinued on day 69 and the subject died on day 74 post RBX2660 due to renal failure. The investigator reported the serious of renal impairment, anuria and sepsis as unrelated to RBX2660, the enema or CDI. FDA considered the death unrelated to RBX2660 and likely related to the rCDI and history of renal impairment.

Details of all fatal cases are presented in Table 19.

Table 19. Summary of Fatal Adverse Events

Age/Sex Study	Number of RBX2660 Doses	Adverse Events	Time to TEAE From Last RBX2660 Dose (Days)	Time to Death from Last RBX2660 Dose (Days)	Relatedness to RBX2660 or CDI¥
88/M 2014-01	1	General physical health deterioration	56	57	Unrelated: poor pre-treatment health status, multiple co-morbidities including anemia and BKA secondary to gangrene
83/M 2014-01	1	General physical health deterioration	88	100	Unrelated: poor pre-treatment health status, multiple co-morbidities including osteomyelitis and decubitus ulcers
75/M 2017-01	1	Cardio-respiratory arrest	37	37	Unrelated: history of coronary artery bypass x 4
94/M 2019-01	1	Pulmonary sepsis	153	153	Unrelated: co-morbidities including end stage CHF
44/F 2019-01	1	Spina bifida with osteomyelitis of coccyx, *C. difficile* infection contributing	35	36	Probably related to CDI
83/F 2013-01	2	Pelvic fracture, respiratory failure	19 33	36 36	Unrelated: chronic respiratory failure exacerbation following pelvic fracture
73/F 2014-01	2	Intestinal ischemia	Onset unknown	564	Unrelated
76/M 2014-01	2	Acute respiratory failure	24	68	Unrelated: history of upper lobectomy, respiratory failure worsened post-ureteral stent placement on Study Day 31
77/M 2015-01	2	Death (due to unknown reasons)	175	175	Unrelated: death certificate noted lung cancer, COPD and colitis
94/F 2015-01	2	*C. difficile* infection	14	24	Definitely related to CDI
67/M 2015-01	2	Cardiac failure COPD	111	253	Unrelated: Significant prior respiratory disease x 6 years prior to study entry
68/F 2015-01	2	Sepsis	147	178	Unrelated: multiple co-morbidities and multi-organism infections
91/F 2015-01	2	Nephropathy	85	613	Unrelated: cardiovascular and renal co-morbidities

Age/Sex Study	Number of RBX2660 Doses	Adverse Events	Time to TEAE From Last RBX2660 Dose (Days)	Time to Death from Last RBX2660 Dose (Days)	Relatedness to RBX2660 or CDI¥
79/F 2017-01	2	Multimorbidity (COPD, decubitus ulcer, cardiac failure and *C. difficile* infection)	151	151	Unrelated: multiple system co-morbidities
62/M 2019-01	2	Cardiac arrest	168	168	Unrelated: multiple co-morbidities including quadriparesis, CHF
63/M 2014-01	3	Bacteremia/sepsis Staphylococcal infection Respiratory failure	25 25 25	29 29 29	Unrelated: subject with multiple co-morbidities including decubitus ulcers, PICC line, +MRSA blood culture
87/F 2014-01	4	Respiratory failure	157	157	Unrelated, subject reported respiratory failure following colectomy for adenocarcinoma of colon.
84/F 2014-01	4	Renal failure	9	34	Possibly related to CDI: history of renal insufficiency that worsened after CDI recurrence

Source: STN 125739/4, Module 5, Adapted from study narratives and case report forms of deaths.
Glossary: BKA = below the knee amputation, COPD = chronic obstructive pulmonary disease, CHF = congestive heart failure, MRSA = methicillin-resistant staphylococcus aureus, PICC = peripherally inserted central catheter, M= male, F= female

Although none of the fatal events, considered individually, were consistent with causal relationship to RBX2660 and no pattern of events was identified, the imbalance in fatal events is notable and is observed in the Any RBX2660 group, the one-dose RBX2660 group, and the blinded RBX2660 group when compared to placebo. The increased death rates with increasing number of RBX2660 doses may reflect both the small sample size of the four-dose RBX2660 group and the severity of the underlying CDI in those subjects requiring multiple enemas. Most subjects with TEAEs leading to death had underlying medical conditions and most died at least 30 days after the last enema was received.

5.3.5. Study Discontinuations Due to TEAE

Across all the studies included in the ISS, seven subjects experienced TEAEs leading to study discontinuation, five of whom received one dose of RBX2660 and two of whom received two doses of RBX260. Of the seven events, four resulted in discontinuation because the events were fatal (complications of spina bifida, cardiorespiratory arrest, pulmonary sepsis and events of multimorbidity that occurred on days 35, 37, 153, and 151, respectively). Please see Section 5.3.4 for additional information on these fatal events. The remaining three TEAEs that led to discontinuation were due to diarrhea, two of which were mild or moderate and reported as probably related to a pre-existing condition (reported days 3 and 47 posttreatment, respectively) and one of which was severe (reported on day 1), and considered definitely related to CDI.

In the long-term follow up period (6 months to 24 months follow-up), two additional subjects discontinued from the study due to fatal TEAEs of cardiac arrest (day 182 posttreatment) and cerebrovascular accident (day 725 posttreatment), both of whom received two doses of RBX2660.

None of the TEAEs leading to study discontinuation were considered related to RBX2660 or the enema procedure.

5.3.6. Adverse Events of Special Interest and Standardised MedDRA Queries

Specific PTs were not pre-specified as AESIs in the protocols. The MedDRA SMQs Hyperglycemia/new onset diabetes mellitus and Immune-mediated/autoimmune disorders were used to identify potential AESIs, in order to enhance detection of any potential safety signals. Across all the studies included in the ISS, PTs in the SMQ Hyperglycemia/new onset diabetes mellitus were reported in 10/749 (1.3%) of subjects in the Any RBX2660 group compared to 2/83 (2.4%) in the placebo only group, and PTs in the SMQ Immune-mediated/autoimmune disorders were reported in 10/749 (1.3%) in the Any RBX2660 group compared to 1/83 (1.2%) in the placebo only group. The overall rates of AESIs were similarly low in subjects exposed to RBX2660 as in subjects exposed to placebo; no patterns or clusters were observed to support causality and no safety signals were identified.

After collection of data in studies 2014-01 and 2017-01, the following SMQs were used to detect safety signals: Gastrointestinal and nonspecific inflammation and dysfunctional conditions; Gastrointestinal perforation, ulceration, hemorrhage or obstruction; Hyperglycemia/new onset diabetes mellitus; Noninfectious diarrhea; Medication errors; Sepsis; Shock; Systemic lupus erythematosus; Vasculitis; and Immune mediated/autoimmune disorders. Analyses of the results of these SMQs did not identify any safety concerns.

5.3.7. Long-term Follow-up

Studies 2014-01 and 2015-01 included long-term safety data for the 6 to 24 months follow-up period. A total of 222 subjects in the Any RBX2660 group and 19 subjects in the placebo only group were included in this analysis. TEAEs in this time period were reported by a higher percentage of subjects in the Any RBX2660 group (125/222; 56.3%) compared to subjects in the placebo only group (9/19; 47.4%) in this time period. Of these events, two were considered related, including an event of vertigo 363 days after treatment (relatedness imputed as it was not reported) and an event of diarrhea 183 days after treatment, which resulted in study discontinuation. Most of the AEs were mild or moderate in severity. TEAEs leading to death were reported by 10.5% of subjects in the placebo only group and 7.2% of subjects in the Any RBX2660 group.

6. Special Populations

6.1. Pediatric

Safety and efficacy of RBX2660 in patients less than 18 years of age have not been assessed. The Applicant has provided a letter of designation stating that the product received orphan designation (Designation No. DRU-2013-4210) for use of fecal microbiota (RBX2660) for the "prevention of recurrent CDI in individuals with prior recurrent CDI resolved following antibiotic treatment." Section 505B(k) of the FD&C Act contains a statutory exemption from the requirement to conduct pediatric studies under the Pediatric Research Equity Act (PREA) for certain drugs with orphan designation. Therefore, no pediatric study plan was provided with the BLA.

6.2. Elderly

Of the 749 subjects treated with RBX2660, 52.1% (390/749) were <65 years of age, 47.9% (359/749) of the subjects were ≥65 years of age, and 25.8% (193/749) of the subjects were ≥75 years of age.

There was an increase in the severity of TEAEs with increasing age, including the frequency of TEAEs leading to death. Of the subjects exposed to RBX2660, severe and potentially life-threatening TEAEs were more frequent in subjects ≥65 years old (13.9% and 4.5%, respectively) compared to subjects <65 years old (11.5% and 1.5%, respectively). Potentially life-threatening TEAEs occurred more frequently in subjects in the ≥75 years of age group (6.7%), all of which occurred in the RBX2550 group. In general, the observed TEAEs in the older age group were related to pre-existing conditions and recurrent CDI and unrelated to RBX2660.

In the Any RBX2660 group, serious TEAEs were reported by a higher proportion of subjects ≥75 years of age (47/193; 24.4%), compared to subjects ≥65 years of age (65/359; 18.1%) and subjects <65 years of age (41/390; 10.5%).

In the Any RBX2660 group, serious TEAEs leading to death were reported more frequently in subjects ≥75 years of age (12/18 deaths), with a lower number (3/18 deaths) reported in subjects <65 years of age. The frequencies of TEAEs and serious TEAEs assessed by the investigator as related to the RBX2660 were similar across age groups, and FDA agrees with the Applicant's assessments of causality. The majority of the severe, potentially life-threatening and serious TEAEs were in the older age groups which reflects that age >65 years of age is an

independent risk factor for rCDI. None of the TEAEs leading to death were related to RBX2660 exposure.

6.3. Pregnancy

No subject in the ISS population had a positive pregnancy test through 6 months after the last enema. In the long term follow up period (between 6 months and 24 months after the last enema) there were three pregnancies reported (one from study 2014-01 and two from study 2015-01):

- One subject who received two doses of placebo reported a ruptured ectopic pregnancy at 222 days post-enema.
- Two subjects who received two doses of RBX2660 delivered healthy infants at 570 days and 720 days post-enema, respectively.

6.4. Immunocompromised Subjects

A limited number of subjects with inflammatory bowel disease and other immunocompromising conditions were enrolled in the study population. The data were insufficient to make conclusions regarding safety and efficacy of RBX2660 in immunocompromised subjects.

7. Pharmacovigilance Plan

The Applicant's pharmacovigilance plan lists an important potential risk for transmission of infection; the plan is under ongoing review. Per the Applicant, "The safety of RBX2660 will be continuously monitored including preparation and submission of expedited reports and aggregate Periodic Adverse Drug Experience reports." The Applicant has not proposed any safety-related postmarketing studies at this time. Postmarketing safety monitoring will include routine pharmacovigilance with submission of expedited reports for serious and unlabeled adverse events; non-expedited reports for all other adverse events, and periodic safety reports at quarterly intervals for the first three years after licensure and annually thereafter.

8. Summary

The BLA includes data from six clinical studies: three Phase 2 trials (2013-001, 2014-01 and 2015-01), two Phase 3 trials (2017-01 and 2019-01), and one retrospective study (2019-02). Assessment of efficacy was based on Bayesian analysis of efficacy data from a single pivotal trial, 2017-01, and one supportive trial, 2014-01. Studies 2013-001, 2014-01, 2015-01, 2015-01 and 2019-01 provided safety data for a period of at least 6 months after the last dose of RBX2660 or placebo enema.

The primary efficacy endpoint analysis for the Phase 3 study 2017-01 (mITT population), performed with a Bayesian analysis borrowing information from Phase 2 study 2014-01, resulted in an estimated difference in treatment success rates of 0.13 (95% credible interval: 0.02 to 0.24). The posterior probability that RBX2660 was superior to placebo was 0.991. The efficacy results met the second success threshold (posterior probability of superiority 0.9750338) that is considered equivalent to positive statistical evidence from a single adequate and well-controlled trial. However, the efficacy results did not meet the first success threshold (posterior probability of superiority 0.9993275) that would have been considered a statistically very persuasive finding in a single trial that could substitute for positive statistical evidence from two independent adequate and well-controlled trials.

The safety evaluation was conducted in a population comprised of subjects enrolled in five prospective studies in the clinical development program for RBX2660 enema. Solicited adverse events (gas or flatulence, abdominal distension or bloating, rectal bleeding, irritation or pain, chills/severe shivering, abdominal pain or cramping, increased diarrhea, constipation, nausea, vomiting and fever) were collected from subjects via subject diary from the date of enrollment through the seventh day after receiving the assigned treatment (studies 2013-001, 2017-01 and 2019-01) or through the seventh day after receiving the second assigned study treatment (studies 2014-01 and 2015-01). The most frequently reported solicited AEs from day 1 through day 7 were gas (flatulence), abdominal distension or bloating, and abdominal pain or cramping. Most solicited AEs were mild or moderate in severity.

The proportion of participants reporting TEAEs was 61.8% in the one-dose RBX2660 group, 69.9% in the blinded RBX2660 group, and 69.6% in the Any RBX2660 group compared to 60.2% in the placebo group. In all groups, the most commonly reported events were gastrointestinal. For both the one-dose and blinded RBX2660 groups compared to placebo, numerical imbalances in events of abdominal pain, nausea, flatulence, and abdominal distention were observed. The proportion of participants reporting severe and life-threatening TEAEs was higher in the RBX2660 groups compared to the placebo group.

The proportion of participants reporting serious TEAEs was 8.4% in the one-dose RBX2660 group, 10.4% in the blinded RBX2660 group, and 14.2% in the Any RBX2660 group, compared to 7.2% in the placebo group. A high rate of serious TEAEs was observed in the multiple dose populations (19%, 28.6%, and 83.3% of subjects in the two, three, and four-dose RBX2660 groups, respectively). The most frequently reported serious TEAEs were in the SOCs of *Infections and infestations*, *Gastrointestinal disorders*, and *Respiratory, thoracic and mediastinal disorders*. None of the serious TEAEs were considered plausibly related to RBX2660 by FDA.

The proportion of participants reporting fatal TEAEs was 1.2% in the one-dose RBX2660 group, 2.6% in the blinded RBX2660 group, and 2.4% in the Any RBX2660 group, compared to 0% in the placebo group. The proportion of subjects reporting any TEAEs leading to death increased as the number of treatment exposures increased, ranging from 3.4% in subjects who received two doses of RBX2660 to 16.7% of subjects who received 4 doses of RBX2660. One death due to relapsed CDI on Day 21 (study 2015-01) was considered possibly related to RBX2660 by the investigator. Following review of the narratives and case report form by FDA, the event was considered not to be causally related to RBX2660 but was considered definitely related to CDI.

Overall, the safety review demonstrated imbalances in gastrointestinal TEAEs and SAEs, including fatal events, between the RBX2660 groups and the placebo group. No specific pattern or trend was identified in review of TEAEs, serious TEAEs, TEAEs leading to discontinuation or AESIs that would suggest a specific risk among recipients of RBX2660 compared to placebo.

9. Topics for VRBPAC Discussion

The VRBPAC will convene on September 22, 2022, to discuss and vote on whether the available data are adequate to support the safety and effectiveness of Rebyota.

10. References

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