Clinical Infectious Diseases

MAJOR ARTICLE

Results From a Randomized, Placebo-Controlled Clinical Trial of a RBX2660—A Microbiota-Based Drug for the Prevention of Recurrent *Clostridium difficile* Infection

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Background. Despite advancements, recurrent *Clostridium difficile* infections (CDI) remain an urgent public health threat with insufficient response rates to currently approved antibiotic therapies. Microbiota-based treatments appear effective, but rigorous clinical trials are required to optimize dosing strategies and substantiate long-term safety.

Methods. This randomized, double-blind, placebo-controlled phase 2B trial enrolled adults with 2 or more CDI recurrences to receive: 2 doses of RBX2660, a standardized microbiota-based drug (group A); 2doses of placebo (group B); or 1 dose of RBX2660 followed by 1 dose of placebo (group C). Efficacy was defined as prevention of recurrent CDI for 8 weeks following treatment. Participants who had a recurrence within 8 weeks were eligible to receive up to 2 open-label RBX2660 doses. The primary endpoint was efficacy for group A compared to group B. Secondary endpoints included the efficacy of group C compared to group B, combined efficacy in the blinded and open-label phases, and safety for 24 months.

Results. The efficacy for groups A, B, and C were 61%, 45%, and 67%, respectively. The primary endpoint was not met ($P = .152$). One RBX2660 dose (group C) was superior to placebo (group B; $P = .048$), and the overall efficacy (including open-label response) for RBX2660-treated participants was 88.8%. Adverse events did not differ significantly among treatment groups.

Conclusions. One, but not 2, doses of RBX2660 was superior to placebo in this randomized, placebo-controlled trial. These data provide important insights for a larger phase 3 trial and continued clinical development of RBX2660.

Clinical Trials Registration. NCT02299570.

Keywords. *Clostridium difficile* infection; recurrence; microbiota-based therapy; placebo; clinical trial.

Clostridium difficile infection (CDI), which is the most common healthcare-associated infection in the United States, is an urgent public health threat [1–4] that causes significant morbidity, mortality, and healthcare costs [4, 5]. The incidence of recurrent CDI (rCDI) is increasing disproportionately to the incidence of primary CDI [6], with significantly worse outcomes in patients who develop rCDI [7–10]. In general, antibiotics (including CDI antibiotics) disrupt and decrease the diversity of the intestinal microbiota, creating a dysbiotic environment in which *C. difficile* can colonize, proliferate, and produce toxins responsible for symptomatic rCDI [11–13]. Accordingly, restoring the composition and diversity of the intestinal microbiota protects against recurrence [11, 14, 15].

Recognition of the role of the intestinal microbiota in health and disease has prompted interest in microbiota-based approaches for treating rCDI. Several studies have indicated that fecal microbiota transplantation (FMT) can help prevent rCDI, with relatively few adverse events (AEs) [16–19], but these treatments lack standardization of process and composition. Moreover, definitions and procedures for AE reporting, follow-up, and optimal dosing are not clearly established. Several standardized microbiota-based rCDI therapeutics are in clinical development [20, 21]. However, to date there are no published randomized, blinded, placebo-controlled US Food and Drug Administration–registration trials of standardized microbiota therapeutics for preventing rCDI. In the trial described here, we evaluated the efficacy and safety of the microbiota-based drug candidate RBX2660 compared to placebo for preventing future rCDI among patients with a history of rCDI.

METHODS

Study Design and Treatment

In this international, multicenter, randomized, doubled-blind, placebo-controlled phase 2B study, we aimed to demonstrate the efficacy and safety of 1 or 2 doses of RBX2660 to prevent rCDI

Received 12 January 2018; editorial decision 19 March 2018; accepted 28 March 2018; published online March 29, 2018.

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Clinical Infectious Diseases® 2018;67(8):1198–204

DOI: 10.1093/cid/ciy259

among patients with a history of multiple rCDIs. Participants were randomly assigned to receive 2 blinded treatments by enema as follows: group A, 2 doses of RBX2660; group B, 2 doses of placebo; and group C, 1 dose of RBX2660 followed by 1 dose of placebo. Administration of the first dose commenced 24–48 hours following completion of CDI treatment antibiotics, with the second dose administered 7 ± 2 days thereafter. Although the second enema treatment could be administered earlier than the 7-day interval if necessary to control suspected CDI recurrence. Participants who failed both blinded treatments were eligible to receive up to 2 open-label doses of RBX2660 7 ± 2 days apart, which could be administered after CDI antibiotics at the discretion of the investigator. Additional details are included in the online Supplementary Material.

Study Population

The study population included participants aged ≥18 years with a diagnosis of rCDI and either 2 or more documented recurrences of CDI after a primary episode or 2 or more documented episodes of severe CDI that resulted in hospitalization. CDI was defined as the presence of diarrhea (3 or more unformed stools in 24 hours for at least 2 consecutive days) and a positive stool test for *C. difficile* or its toxins, performed according to the enrolling physician's standard procedure. Recurrent CDI was defined as a diagnosis of CDI that began less than 8 weeks after completion of treatment for a previous episode. Participants were required to have a positive stool test within 60 days prior to enrollment for the qualifying rCDI episode, to have completed at least 2 courses of standard-of-care oral antibiotics, and to have rCDI symptoms controlled before treatment. Major exclusion criteria included ongoing or anticipated antibiotic therapy for a condition other than CDI, known or suspected causes of diarrhea other than CDI, a compromised immune system, a history of inflammatory bowel disease, or pregnancy. A detailed list of inclusion and exclusion criteria is provided in the Supplementary Material.

Study participants were enrolled at 21 centers in the United States and Canada from 10 December 2014 through 13 November 2015. The study protocol received institutional review board approval at each center. All participants provided written informed consent. A medical monitor along with a data safety monitoring board (DSMB) examined the trial on an ongoing case-by-case basis, as needed.

Randomization and Blinding

Participants were randomized using permuted blocks within 3 strata based on the antibiotic regimen for the enrolling episode (vancomycin, fidaxomicin, or metronidazole) and assigned 1 of 3 treatments (group A, B, or C) at a 1:1:1 ratio. There was no stratification by site; each drew from the same set of blocks. The randomization code, date, and time were captured in the clinical database. Study participants, investigators, and site personnel who performed follow-up procedures were blinded to the assignment and study drug administration, and the enema administrator was not involved in study follow-up. RBX2660 and placebo were shipped in a ready-to-use enema bag shrouded in an opaque brown sleeve, and the tubing set was shrouded by the enema administrator during the procedure and disposed in an opaque biohazard container after administration.

RBX2660 Preparation

RBX2660 is a microbiota suspension prepared from human stool. Donor selection and screening and RBX2660 preparation were as previously described [20, 22] and included 75 doses from 17 donors. Each dose consisted of a 150-mL suspension containing $\geq 10^7$ live organisms/mL in a single-dose ready-to-use enema bag. The placebo consisted of normal saline and formulation solution in the same proportions found in RBX2660. Each unit of RBX2660 and placebo were identified by a unique batch number and were traceable to a specific donor and recipient. Drug and placebo were stored frozen at −80°C in a secure location at the manufacturer and shipped frozen to the site in a temperature-controlled container. Products were thawed in a refrigerator for 24 hours and administered within 48 hours after thawing.

Study Endpoints

The primary endpoint was prevention of recurrence (treatment success) after 2 doses of RBX2660 compared to 2 doses of placebo. Success was defined as the absence of diarrhea and no retreatment for CDI any time after the first dose until 8 weeks after the second dose of assigned study treatment. Treatment failure was defined as meeting all 4 of the following criteria at <8 weeks after completion of both assigned blinded study treatments: diarrhea, a positive laboratory diagnosis for *C. difficile* or its toxins as conducted and reported by the study investigator, a need for retreatment for CDI, and no other cause for CDI symptoms. An independent DSMB reviewed each participant for final determination of treatment success or failure while blinded to the randomization. Some participants were declared treatment failures by the study investigator due to suspected CDI recurrence, even though all 4 criteria were not met. These were categorized by the DSMB as having an indeterminate response and considered treatment failures for efficacy analyses. In addition, some participants were declared failures and offered open-label treatment after only 1 blinded study treatment. These were recorded as protocol deviations but were classified as failures for efficacy analysis.

Secondary endpoints included safety and the following efficacy assessments: 1 dose of RBX2660 and 1 dose of placebo (group C) compared to either 2 doses of placebo (group B) or 2 doses of RBX2660 (group A) and the efficacy of up to 2 open-label RBX2660 doses administered to participants who failed blinded treatment. Participants recorded solicited AEs and their symptom severity daily through 7 days after the final

blinded dose. AEs were assessed at in-office visits at 1, 4, and 8 weeks after completing the assigned study treatment. Telephone assessments occurred weekly during weeks 2, 3, and 5–7 and will continue through 3, 6, 12, and 24 months.

Statistical Analyses

To achieve a power of 90% with a 2-sided level of significance of 0.05 for an estimated 80% success in group A vs 40% success in group B, 105 participants were required for a 1:1:1 randomization ratio. An additional 12 enrollments were included to account for 10% attrition, for a total of approximately 39 participants per group. After the first 5 participants were enrolled, a protocol modification was made to stratify participants according to the antibiotic they received for CDI treatment prior to study enrollment. Accordingly, the randomization schedule was recreated and the sample size was increased to 44 participants per group. Enrolled participants who withdrew prior to randomization were replaced without counting toward the size cap. All analyses were performed on participants in the intent-to-treat (ITT) population who received at least 1 assigned blinded treatment. Participants who were classified by the DSMB as having an indeterminate response to blinded treatment were analyzed as failures.

The primary efficacy endpoint was analyzed with the Pearson χ^2 test. Descriptive statistics were used for safety assessments. All analyses were performed using SAS version 9.3 or later (Cary, North Carolina).

RESULTS

Participants

A total of 150 participants at 21 centers in the United States and Canada were enrolled. Seventeen were screen failures and exited prior to randomization, and 133 were randomly assigned to receive 2 doses of RBX2660 (group A, n = 45), 2 doses of placebo (group B, n = 44), or 1 dose of RBX2660 followed by 1 dose of placebo (group C, n = 44). Three withdrew prior to treatment, 1 was withdrawn by the investigator prior to treatment, and 1 died before receiving treatment, leaving 128 participants in the safety population. One participant withdrew after experiencing anxiety during the first attempted enema, which was not completed, leaving 127 participants for the ITT efficacy analysis (Figure 1, Table 1). Positive *C. difficile* laboratory diagnosis prior to enrollment was established as follows: nucleic acid amplification tests (NAAT) including polymerase chain reaction assay or loop-mediated amplification (n = 103), enzyme immunoassay (EIA, n = 23), and not specified (n = 1). The 3 treatment groups included similar proportions of participants diagnosed using each method.

Primary Outcome

The efficacy for each blinded treatment group in the ITT analysis was as follows: group A = 61% (25/41), group B = 45% (20/44), and group C = 67% (28/42; Figure 2, Table 2). There was no significant difference between group A and group B ($P > .05$), thus the primary study endpoint was not met. There was no

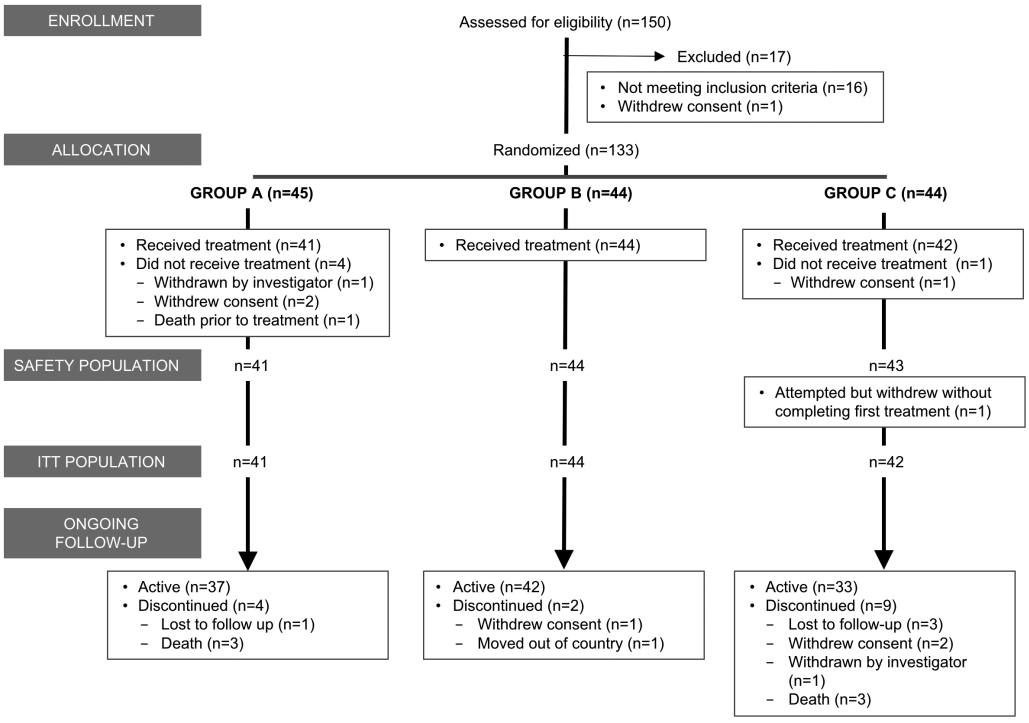

Figure 1. Flowchart of participants in multicenter, double-blind, placebo-controlled randomized trial of microbiota-based drug RBX2660 for prevention of recurrent *Clostridium difficile* infection. Abbreviation: ITT, intention to treat.

Table 1. Baseline Demographics and Clinical Characteristics of Participants in the Intent-to-Treat Population

Characteristic	Group A 2 Doses of RBX2660	Group B 2 Doses of Placebo	Group C 1 Dose of RBX2660 and 1 Dose of Placebo
n	41	44	42
Median age, years (range)	66 (24–89)	62 (19–92)	63 (18–88)
Female sex, no. (%)	25 (61)	30 (68)	24 (57)
Race–white, no. (%)	39 (95)	42 (96)	40 (95)
Antibiotic used at screening, no. (%)			
Vancomycin	38 (93)	40 (91)	37 (88)
Fidaxomicin	1 (2)	2 (5)	2 (5)
Metronidazole	2 (5)	2 (5)	2 (5)
None			1 (2)
Median number of CDI episodes (range)	4 (3–11)	3 (2–11)	4 (2–14)
Median duration of CDI episode, days (range)[a]	15 (1–74)	15 (1–98)	14 (1–71)

Abbreviation: CDI, *Clostridium difficile* infection.

[a]As reported by enrolling physician.

association between outcome and which antibiotic was being administered at screening (P = .15, Cochran-Mantel-Haenszel). Laboratory *C. difficile* diagnosis for failure determination was by NAAT (n = 33) or EIA (n = 7), with the remaining 14 participants classified as treatment failures by the site investigator despite not having a positive *C. difficile* laboratory diagnosis (n = 3, 6, and 5 in groups A, B, and C, respectively). Eleven of these were never tested and 3 tested negative (EIA). These 14 participants were adjudicated by the DSMB as having an indeterminate response but were included in the ITT analyses as failures.

Secondary Outcomes

Participants who received 1 RBX2660 dose followed by 1 placebo dose (group C) showed superior response relative to participants who received 2 placebo doses (group B, P = .049). Likewise, the response among participants who received at least 1 blinded dose of RBX2660 (groups A and C combined, 64%, 53/83) was significantly higher than group B participants who received placebo (P = .047).

All participants who were classified as treatment failures during the blinded phase of the study (n = 54) opted to receive 1 (n = 5) or 2 (n = 49) open-label RBX2660 treatments, with a median time of 8 days (range, 0–50 days) between failure determination and open-label RBX2660 treatment. Thirteen of these were offered open-label treatment after only a single blinded treatment (n = 5, 3, and 5 in groups A, B, and C, respectively).

Treatment success rates were 80% (4/5) and 78% (38/49) for participants who received 1 or 2 open-label RBX2660 treatments, respectively, with a combined success rate of 78% (42/54). Open-label treatment success rates according to blinded-phase study groups were as follows: 69% (11/16), 88% (21/24), and 71% (10/14) for groups A, B, and C, respectively. The overall success rate for participants who received at least 1 dose of RBX2660 in the blinded and/or open-label phases was 89% (95/107; median number of doses per participant = 2).

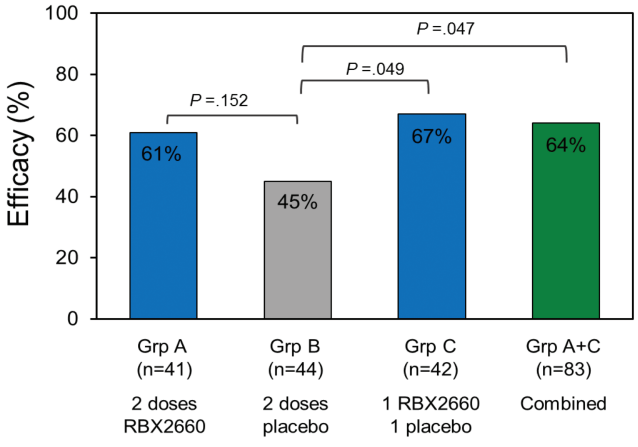

Figure 2. Efficacy of microbiota-based drug RBX2660 or placebo following blinded treatment. The proportions of participants in the blinded phase who responded to treatment with 2 doses of RBX2660 (group A); 2 doses of placebo (group B); and 1 dose of RBX2660 followed by 1 dose of placebo (group C).

Safety

In total, 379 AEs were reported in 82 (64.1%) participants during the blinded treatment phase (Table 3). There were no differences in the number or rate of AEs among blinded treatment groups. The most common AEs were gastrointestinal disorders, followed by general disorders, infections, and nervous system disorders. Safety follow-up beyond the blinded treatment phase is ongoing (mean of 8.3 months post-treatment; range, 0.1–15.9 months) and will continue to 24 months. As of this interim analysis, 580 AEs have been reported, with a similar distribution and type as observed in the blinded treatment phase (Supplementary Table 1).

Including the follow-up period, 45 serious AEs (SAEs) have been reported (Table 4). As determined by the site investigator and DSMB, none were related to the enema procedure, 31.1% were related to CDI, and 77.8% were related to a preexisting condition. Three of the SAEs were adjudged possibly related to the blinded study drug; 1 participant developed recurrent acute

Table 2. RBX2660 Efficacy in Blinded and Open-Label Arms of A Phase 2 Open-Label Clinical Trial Demonstrating the Safety of RBX2660 Microbiota Suspension for the Treatment of Recurrent *Clostridium difficile*-Associated Diarrhea Study

	Group A	Group B	Group C	Total
		Blinded arm		
Treatment	2 doses RBX2660	2 doses placebo	1 dose RBX2660, 1 dose placebo	
n	41	44	42	127
Success n (%)	25 (61)	20 (45)	28 (67)	
		Open-label arm		
n	16	24	14	54
Success n (%)	11 (69)	21 (88)	10 (71)	42 (78)
		Overall RBX2660 efficacy		
n	41	24	42	107
Success n (%)	36 (82)	21 (88)[a]	38 (90)	95 (89)[a]

[a]Overall RBX2660 efficacy analysis does not include participants who responded in group B of the blinded phase analysis.

myeloid leukemia, another reported abdominal cramping and pain, and a third experienced constipation that required hospitalization. Among the SAEs were 6 participant deaths, all of which were determined by the site investigator and medical monitor to be unrelated to RBX2660 or study procedures. The median time to death from the last enema administration was 67 days (range, 28–156 days). The reported causes of death included general decline of health (n = 2), renal failure (n = 2), respiratory failure, and methicillin-resistant *Staphylococcus aureus* bacteremia. Four of the 6 deaths were adjudged to be possibly, probably, or definitely related to a preexisting condition.

DISCUSSION

To address the need for rigorous safety and efficacy data for microbiota-based therapeutics, we report a randomized, double-blind, placebo-controlled evaluation of the safety and efficacy of RBX2660 for preventing rCDI. To our knowledge, this is the first trial of its type for a microbiota-based therapeutic.

We examined several possible reasons why the primary objective was not met ($P > .05$). The 61% response rate observed for group A is consistent with the previously reported activity of RBX2660 in a phase 2 trial; 51% of participants responded to a single RBX2660 dose [20]. The present results are also consistent with reported responses to single colonoscopic administration of FMT [10, 23–27]. Thus, the activity of RBX2660 was not unexpectedly low.

Next, we assessed the trial design. Based on our previous data for RBX2660, this trial was designed to test a regimen of 2 RBX2660 doses spaced 1 week apart (group A). Even though the protocol allowed for administration of the second enema after less than 7 days, 13 participants were declared failures by the site investigator and offered open-label RBX2660 after only a single blinded treatment (group A = 5, group B = 3, group C = 5). This may have affected the power of the trial to demonstrate the primary objective, because some might have responded had they received the full per-protocol treatment. This compliance finding and its potential impact on the outcome suggest a single-dose study design could provide a clearer analysis of efficacy. Moreover, the efficacy of group C was significantly better than placebo ($P = .049$), which suggests a single-dose RBX2669 regimen is sufficient.

Table 3. Overall Adverse Events and By-Organ-Class Adverse Events Reported in at Least 5% of the Safety Population During the Blinded Treatment Phase

	Events/Participants (% of Participants)			
Adverse Event	Group A (n = 41)	Group B (n = 44)	Group C[a] (n = 43)	Total (N = 128)
Overall	169/25 (61.0)	105/26 (59.1)	105/31 (72.1)	379/82 (64.1)
Gastrointestinal disorders	78/21 (51.2)	56/16 (36.4)	49/20 (46.5)	183/57 (44.5)
General disorders	12/8 (19.5)	15/7 (15.9)	14/9 (20.9)	41/24 (18.8)
Infections	8/6 (14.6)	8/6 (13.6)	5/5 (11.6)	21/17 (13.3)
Nervous system disorders	5/3 (7.3)	8/6 (13.6)	1/1 (2.3)	14/10 (7.8)
Renal and urinary disorders	6/5 (12.2)	2/2 (4.5)	2/2 (4.7)	10/9 (7.0)
Skin and subcutaneous tissue disorders	5/4 (9.8)	3/2 (4.5)	3/3 (7.0)	11/9 (7.0)
Musculoskeletal and connective tissue disorders	5/4 (9.8)	2/2 (4.5)	2/2 (4.7)	9/8 (6.3)
Injury, poisoning, procedural complications	3/2 (2.4)	0	10/5 (11.6)	13/7 (5.5)
Metabolism and nutrition disorders	7/4 (9.8)	1/1 (2.3)	2/2 (4.7)	10/7 (5.5)
Respiratory, thoracic, and mediastinal disorders	11/4 (9.8)	1/1 (2.3)	2/2 (4.7)	14/7 (5.5)

[a]The safety population included 1 participant in group C who was not in the intention-to-treat population due to withdrawal before completing a blinded study treatment.

Table 4. Serious Adverse Events and Relatedness Reported During Blinded and Open-Label Arms to a Mean Follow-up of 8.3 Months, Range 0.1 to 15.9 Months

Relatedness of Adverse Event	Events/Participants (% of Participants)			
	Group A (n = 41)	Group B (n = 44)	Group C[a] (n = 43)	Total (N = 128)
Overall severe adverse events	19/13 (31.7)	8/6 (13.6)	18/7 (16.3)	45/26 (20.3)
Related to RBX2660[b]	3/3 (7.3)	0	0	3/3 (2.3)
Related to procedure	0	0	0	0
Related to *Clostridium difficile* disease	8/5 (12.2)	1/1 (2.3)	5/3 (7.0)	14/9 (7.0)
Related to a preexisting condition	16/10 (24.4)	6/4 (9.1)	13/7 (16.3)	35/21 (16.4)
Deaths[c]	3 (7.3)[c]	0	3 (7.0)	6 (4.6)
Related to RBX2660	0	0	0	0
Related to procedure	0	0	0	0
Related to *C. difficile* disease	1 (2.4)	0	0	1 (0.8)
Related to a preexisting condition	1 (2.4)	0	3 (7.0)	4 (3.1)

[a]The safety population included 1 participant in group C who was not in the intention-to-treat population due to withdrawal before completing a blinded study treatment.

[b]Three severe adverse events were adjudged possibly related to the blinded study drug: 1 participant developed recurrent acute myeloid leukemia, another reported abdominal cramping and pain, and a third experienced constipation requiring hospitalization.

[c]One participant death was due to methicillin-resistant *Staphylococcus aureus* bacteremia and was adjudged to be unrelated to the treatment, procedure, or preexisting condition.

The higher-than-expected response rate among placebo-treated participants also contributed to lack of significance. This trial was powered with a conservative expectation of 40% placebo response based on a 30% placebo response observed in the only randomized FMT trial available at the time [23]. Consequently, the 46% response rate for group B did diminish the power of the trial. Since 2016, 3 additional trials have reported 43% to 58% response rates among placebo-treated cohorts [28–30], which is in line with our results and likely reflects a basal recurrence-free rate after standard-of-care antibiotics [31, 32]. Thus, our results, and now the literature, indicate that rCDI trials should be powered to account for higher response rates in placebo-treated cohorts.

The heterogeneity of CDI laboratory diagnostic practices, which is a known challenge for the field [33], also may have contributed to the statistical outcome. Despite the protocol definition of treatment failure, positive laboratory diagnosis was established in only 40 of 54 investigator-designated failures. These 14 participants were included in the ITT analysis as failures because an alternative diagnosis could not be established post hoc, that is, CDI recurrence could not be ruled out. This could have biased the study toward the null if some were not bona fide recurrences. Although the "optimal" method to diagnose CDI remains elusive, some analyses suggest that detection of *C. difficile* toxin in stool is more specific for CDI [34, 35], whereas NAAT testing alone leads to overdiagnosis [36]. As such, the fact that the majority of enrolled patients were diagnosed by NAAT may have also biased the study toward the null.

This study will add significant long-term safety data for microbiota-based rCDI therapies. The overall safety profile was favorable through a mean follow-up of 8.3 months, with 90.8% of reported AEs being mild to moderate, consistent with the open-label phase 2 trial of RBX2660 [20]. Safety follow-up is ongoing and will continue until 24 months post-treatment. This study also underscores the safety of enema administration, which is important given some reports that associated morbidity and mortality with duodenal or colonoscopic FMT [37, 38].

This study demonstrated that 1 dose of RBX2660 (group C) was more effective than placebo ($P < .05$). The observation that a second dose within 7 days did not provide additional benefit ($P > .59$, group C compared to group A) was somewhat surprising, based on prior open-label results for RBX2660 [20]. However, the second dose in that trial was only administered to participants who recurred, which is a distinctly different population. The present data suggest that a single RBX2660 dose is sufficient to elicit maximal benefit after each episode of rCDI. Consistent among both trials is the conclusion that patients who have a recurrence after a single dose can benefit from a subsequent dose if needed. Indeed, the overall response rate of 88.8% among participants who received at least 1 RBX2660 dose highlights the potential clinical benefit of repeated doses of RBX2660.

CONCLUSIONS

In this double-blinded, randomized, placebo-controlled trial, 2 RBX2660 doses spaced 1 week apart were not superior to placebo, but a single dose of RBX2660 was significantly better than placebo. Participants who had a recurrence after blinded study treatment benefitted from open-label RBX2660. RBX2660 was safe and well tolerated. Future clinical evaluation is warranted to confirm long-term RBX2660 benefit and safety.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The data safety monitoring board for this study included Teena Chopra (chairperson, Wayne State University), Monika Fischer (Indiana University), and Adam Hamm (Chiltern International Limited). The first draft of the manuscript was written with the assistance of Harriet Guthertz, an independent medical writer funded by Rebiotix Inc.

Financial support. The work was supported by Rebiotix Inc., Roseville, Minnesota.

Potential conflicts of interest. E. R. D., D. N. G., G. H., C. H. L., and R. O. serve on the Rebiotix Physician Advisory Board. S. K. has received research support and consulting fees from Rebiotix. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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