Clinical Infectious Diseases

MAJOR ARTICLE

RBX7455, a Non-frozen, Orally Administered Investigational Live Biotherapeutic, Is Safe, Effective, and Shifts Patients' Microbiomes in a Phase 1 Study for Recurrent *Clostridioides difficile* Infections

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(See the Editorial Commentary by Youngster on pages e1621–3.)

Background. Recurrent *Clostridioides difficile* infections (rCDI) are a global public health threat. To reduce rCDI, microbiota-restoring therapies are needed, particularly standardized, easy-to-administer formulations.

Methods. This phase I open-label trial assessed the safety, efficacy in preventing rCDI recurrence, and intestinal microbiome effects of RBX7455, a room temperature-stable, orally administered investigational live biotherapeutic. Adult participants with 1 or more prior episodes of rCDI received: 4 RBX7455 capsules twice daily for 4 days (group 1); 4 RBX7455 capsules twice daily for 2 days (group 2); or 2 RBX7455 capsules twice daily for 2 days (group 3). For all groups, the first dose was administered in clinic, with remaining doses self-administered at home. Adverse events were monitored during and for 6 months after treatment. Treatment success was defined as rCDI prevention through 8 weeks after treatment. Participants' microbiome composition was assessed prior to and for 6 months after treatment.

Results. Nine of 10 group 1 patients (90%), 8 of 10 group 2 patients (80%), and 10 of 10 group 3 patients (100%) were recurrence-free at the 8-week endpoint with durability to 6 months. Seventy-five treatment-emergent adverse events were observed in 27 participants with no serious investigational product-related events. Prior to treatment, participants' microbiomes were dissimilar from the RBX7455 composition with decreased Bacteroidia- and Clostridia-class bacteria, whereas after treatment, responders' microbiomes showed increased Bacteroidia and Clostridia.

Conclusions. Three dosing regimens of RBX7455 were safe and effective at preventing rCDI. Responders' microbiomes converged toward the composition of RBX7455. These results support its continued clinical evaluation.

Clinical Trials Registration. NCT02981316.

Keywords. *Clostridioides difficile* infection; recurrence; microbiota-based therapeutic; oral administration; clinical trial.

Clostridioides difficile infection (CDI) is the leading hospital-acquired infection in the United States, impacting 500 000 people annually [1] and accounting for 15% of all diagnosed infections [2]. In a recent meta-analysis, the incidence of CDI in the United States was calculated to be 8.3 cases per 10 000 patient days [3]. As such, CDI is associated with significant patient morbidity and mortality as well as a large financial burden, with annual attributable costs of 6.3 billion dollars in the United States [4].

A major risk factor for CDI is systemic antibiotic use, which disrupts normal gut flora, lowering resistance to *C. difficile* colonization [5]. The standard guideline-based treatment for CDI is antibiotic therapy with either vancomycin or fidaxomicin [6]. However, recurrent CDI (rCDI), defined as a new episode of CDI occurring within 8 weeks of successful treatment, is common [7] and occurs in approximately 15–30% of primary CDI episodes [8, 9] and 45–65% of recurrent CDI episodes [10, 11]. Indeed, given the historic nature of many of the studies, these figures likely underestimate this risk of recurrence, because the incidence of rCDI is on the increase, particularly for multiply recurrent episodes [12].

The recommended treatment of an initial episode of rCDI is antibiotic therapy. However, with further episodes, antibiotic treatment followed by microbiota-based therapies, most commonly fecal microbiota transplantation (FMT), to restore the intestinal microbiome is warranted [6]. A recent systematic review of randomized controlled trials identified 8 heterogeneously designed studies that included FMT, with an aggregate cure rate of 81% and recurrence rates varying from 0 to 56.3%

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[13]. However, to date there are no standardized Food and Drug Administration (FDA)-approved microbiota treatments for rCDI, and studies of FMT have widely varied in stool quantity, preparation, storage, and administration procedures [14]. To address this, several standardized investigational therapeutics are in formal clinical development [15, 16]. In one example, a randomized, double-blinded, placebo-controlled phase 2B trial indicated that trial participants with rCDI who received at least 1 dose of RBX2660, a liquid suspension investigational microbiota-based therapeutic administered by enema, had a reduced recurrence rate compared to participants who received only placebo [15].

FMT or investigational microbiota-based treatments for rCDI are commonly delivered via colonoscopy or enema by a clinician within a clinical environment, but there are also accumulating data to suggest the effectiveness of oral administration, including frozen [17–19] and lyophilized [20, 21] FMT preparations. Although these trials reported high efficacy, subjects were required to take large numbers of capsules (average 27–40) in 1 "dose," which may be difficult to adhere for a substantial portion of the eligible rCDI population. Moreover, most oral administrations reported to date have required freezer storage, which necessitates administration in a healthcare setting.

The aim of the present study was to evaluate the safety and efficacy of RBX7455—a standardized, lyophilized, non-frozen, orally administered live biotherapeutic drug candidate—in participants with a history of CDI recurrence. The unique stability of RBX7455 to room temperature storage facilitates the option for patient self-administration in a nonhealthcare setting. In addition, we examined the fecal microbiome profiles of the patients prior to and up to 6 months following therapy with RBX7455.

METHODS

Study Population

Study participants were enrolled between 27 December 2016 and 15 May 2018 at a single tertiary referral center, the Mayo Clinic, Rochester, Minnesota, which served as the regulatory sponsor. The study protocol received Institutional Review Board approval prior to its commencement and was conducted under an FDA Investigational New Drug (IND) application. All patients provided written informed consent.

The study population included patients aged 18 years old or older with documentation of rCDI, defined as at least 1 recurrence after a primary episode, who had completed at least 2 rounds of standard-of-care oral antibiotic therapy. The inclusion of first-recurrent CDI (1 recurrence after a primary episode) differs from recent studies that only included multirecurrent CDI (at least 2 recurrent episodes) [15]. This difference was aimed at assessing the potential for reducing recurrence rates in the first-recurrent CDI population, which has not been as extensively evaluated. For eligibility purposes, patients must have had a positive stool test (either a nucleic acid amplification test or enzyme immunoassay for the *C. difficile* toxin) for the presence of *C. difficile* within 30 days prior to enrollment and had to have demonstrated resolution of diarrhea (fewer than 3 watery bowel movements per day) prior to study treatment.

Major exclusion criteria included: continued diarrhea despite antibiotic therapy; requirement of continuous antibiotic therapy for a condition other than CDI; previous fecal transplant; previous treatment with RBX2660; history of inflammatory bowel disease, irritable bowel syndrome, chronic diarrhea, or celiac disease; history of colostomy; evidence of active colitis; intended exposure to antibiotics within 6 months following study enrollment; compromised immune system (white blood cell count < 1000 cells/μL); current or expected use of systemic steroids; and pregnancy.

Study Design and Treatment

This open-label, single arm, dose-ranging study was designed to determine the safety and efficacy of 3 dosing regimens of the investigational RBX7455 drug product. For all participants, antibiotics were discontinued at least 24 and no more than 48 hours prior to the first dose of RBX7455. In group 1, participants received 4 RBX7455 capsules twice daily for 4 days; in group 2, 4 RBX7455 capsules twice daily for 2 days; and in group 3, 2 RBX7455 capsules twice daily for 2 days. Participants were requested but not required to provide stool samples at baseline, defined as after enrollment and prior to assigned treatment, and at day 1, and at weeks 1, 4, 8, 12, and 24 after treatment. These stool samples were provided via a home collection kit for whole stool, shipped overnight under cold conditions to Rebiotix, where upon receipt samples were immediately aliquoted and frozen without stabilizers at −80°C until analysis.

RBX7455 Manufacturing and Administration

RBX7455 was provided for the study by Rebiotix, a Ferring Company (Roseville, MN, USA). RBX7455 is manufactured starting with a microbiota-based suspension (RBX2660) that is prepared from human stool. Donor selection, pathogen screening, and preparation of RBX2660 are previously described [22] and were conducted according to an IND application in compliance with FDA guidelines at the time of manufacture. Multiple aliquots of RBX2660 sourced from a single donor and containing $\geq 10^7$ live, human-derived bacteria / mL were combined, reduced in volume, and nonviable particulate material was minimized via centrifugation. A proprietary formulation of lyoprotectant and cryoprotectant excipients was added, and the mixture was lyophilized, with the resulting product milled and doubly encapsulated (V-caps® enteric, Capsugel,

a Lonza Company, Morristown, NJ, USA) via a proprietary process. These capsules are resistant to opening in gastric pH conditions and release contents in postgastric pH conditions. Finished capsules were packaged into foil bags to limit moisture uptake and were stored at 2 to 8°C at Rebiotix (Roseville, MN, USA) until delivery to the study site pharmacy, where they were stored at 2 to 8°C until dispensation to study participants, at which point it was permitted to store RBX7455 at room temperature. Participants were administered the first assigned dose under supervision in the clinic, with self-administration thereafter permitted at home. Dosing compliance was monitored via study diary and by requesting participants to return empty packaging after dosing was completed.

Using a proprietary assay, the viable bacterial content of RBX7455 (colony forming units per capsule [CFU / capsule]) was measured at the time of manufacture and at specified time points up to 12 months during controlled storage at 2–8°C or 23–27°C. Four different batches of RBX7455 were utilized in the course of this study. All were confirmed to have viable bacterial content that exceeded proprietary minimal specifications at the time of dosing. All doses for each participant were from a single manufacturing batch and the same donor.

Study Endpoints

Primary Endpoints

Success was defined as the absence of rCDI at 8 weeks following completion of the final treatment. Treatment failure was defined as meeting all of the following criteria: recurrence of diarrhea <8 weeks after completion of RBX7455 therapy, a positive stool test for *C. difficile* (either a nucleic acid amplification test or enzyme immunoassay for the *C. difficile* toxin), a need for retreatment for CDI, and no other cause for diarrhea.

Safety was assessed via in-office study visits at 1 and 8 weeks after the start of study treatment, telephone assessments at 2 and 4 days and 3 and 6 months, and a subject diary utilized from enrollment to 7 days after study treatment. The diary solicited for the occurrence of anticipated possible treatment-emergent adverse events (TEAE), including flatulence, abdominal distension or bloating, rectal irritation or pain, chills or severe shivering, abdominal pain or cramping, increased diarrhea, constipation, rectal bleeding, nausea, vomiting, or a fever ≥38.0°C. Assessments beyond 8 weeks included the possibility of CDI recurrence. The in-office and telephone assessments and the study diary were used to assign the frequencies and severity grades of TEAEs in each treatment group from the first day of assigned study treatment through 6 months following the last dose of assigned study treatment. The investigator made a causality assessment for all TEAEs as definitely, probably, possibly, or not related to the investigational product.

Exploratory Endpoints

Comparison of fecal microbial composition and diversity changes was an exploratory endpoint. Participation in the sample collection phase of the trial was optional per consent requirements. To preclude selection bias, all received samples were included in the analysis, with the exception that samples received after treatment failure were not included, because participants received standard-of-care antibiotic and/or FMT at failure determination. Participant fecal samples and representative RBX7455 samples were stored frozen at −80°C with no added stabilizers until extraction and sequencing using a whole genome sequencing method (CoreBiome, Minneapolis, MN, USA). Relative taxonomic abundances and alpha diversity for each sample were determined from operational taxonomic units (OTU) data, and multidimensional scaling analysis (MDS) was used to map all individual samples onto 2-dimensional space with a Bray-Curtis dissimilarity metric with nonparametric scaling [23]. To assess longitudinal changes among patient-matches samples, DMRepeat [24] was used to conduct a repeated measures analysis on the subset of samples from treatment-responsive participants from whom baseline, 1-, 4-, and 8-week samples were received (3, 4, and 6 participants in groups 1, 2, and 3, respectively).

Data Monitoring and Statistical Analysis

Data were monitored by nonstudy personnel for accuracy and completeness using source document verification. Univariate descriptive statistics and frequency distributions were calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) were tabulated for the treatment groups to identify potential confounding variables.

RESULTS

Participants

A total of 31 patients were enrolled. One enrollee withdrew prior to treatment, leaving 30 that received RBX7455, with 10 participants assigned to the 3 dosing groups sequentially without randomization (Figure 1). One participant withdrew from follow-up observation after being adjudicated as a treatment failure, and the remaining 29 completed the 6-month follow-up. The demographic and baseline characteristics of the 30 treated participants are summarized in Table 1. There were no statistically significant differences among groups with respect to sex (Fisher exact test), age, baseline antibiotic used, or number or duration of prior CDI episodes (1-way ANOVA).

Clinical Outcomes

All assigned doses were recorded in subject diaries as successfully taken as prescribed. Nine of 10 participants in group 1, 8 of 10 in group 2, and 10 of 10 group 3 were recurrence-free at the 8-week endpoint, with an overall success rate of

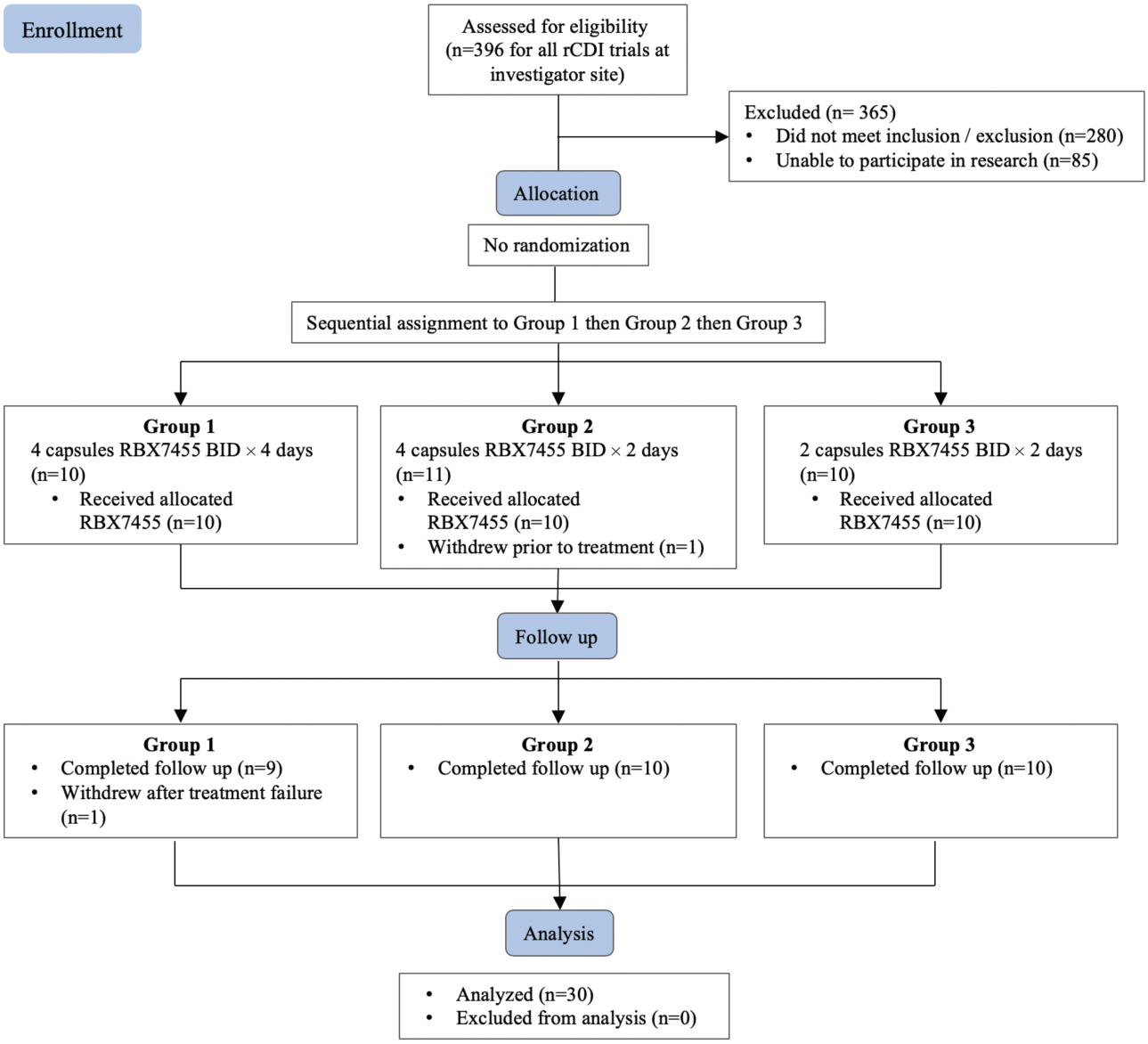

Figure 1. Consort diagram showing participant enrollment, allocation, follow-up, and analysis. Abbreviation: rCDI, recurrent *Clostridioides difficile* infection.

Table 1. Demographic Characteristics of Participants Receiving RBX7455

	Group		
	1	**2**	**3**
Median age (range) years	67.5 (27–83)	55 (22–81)	63.2 (22–85)
Female sex (n)	9	5	8
Ethnicity, White (%)	100	100	100
Antibiotic administered at screening			
Vancomycin	9	9	9
Fidaxomicin	0	1	1
Metronidazole	1	0	0
Mean number of CDI episodes (range)	2.3 (2–3)	2.8 (2–4)	2.5 (2–4)
3 or more CDI episodes (n)	3	6	4
Median duration of qualifying CDI episodes (range)	15.5 (10–26)	18 (12–29)	15 (10–29)
With history of CDI hospitalization (n)	4	1	1

Abbreviation: CDI, *Clostridioides difficile* infection.

Table 2. Efficacy and Safety Following Administration of RBX7455

	Group 1	Group 2	Group 3
Success at 8 weeks, n (%)	9 (90)	8 (80)	10 (100)
Recurrences at 8 weeks, n (%)	1 (10)	2 (20)	0 (0)
Recurrence-free at 6 months, n (%)	9 (90)	8 (80)	10 (100)
		Safety events, n/participants (%)	
Total TEAEs	34/9 (90)	15/10 (100)	26/8 (80)
Possibly/probably related to treatment[a]	7/5 (50)	5/5 (50)	14/6 (60)
Related to CDI	5/5 (50)	5/5 (50)	12/5 (50)
Related to preexisting condition	4/4 (40)	5/4 (40)	7/3 (30)
Serious	5/1 (10)[b]	1/1 (10)[c]	0/0 (0)
		Most prevalent organ classes	
Gastrointestinal disorders	13/7 (70)	7/7 (70)	13/6 (60)
Infections	4/2 (20)	2/2 (20)	7/3 (30)

Abbreviations: CDI, *Clostridioides difficile* infection; TEAE, treatment-emergent adverse event.

[a]Possibly or probably related to treatment. None reported as definitely related to RBX7455 treatment.

[b]Not related to treatment. Participant hospitalized 96 days after treatment for conditions unrelated to treatment.

[c]Not related to treatment. Participant hospitalized 111 days after treatment for condition unrelated to treatment.

90% (Table 2). There was not a significant difference in clinical effectiveness among the 3 dose levels ($P > .05$; 1-way ANOVA) or between first recurrent (17 of 17 responded) and multirecurrent participants (10 of 13 responded; $P > .05$, Fisher exact test). Among the 3 nonresponders (1 in group 1 and 2 in group 2), the time between completing treatment and recurrence was 6, 2, and 44 days. Among 27 treatment responders, there were no additional rCDI episodes between 8 weeks and 6 months.

A total of 75 TEAEs were observed in 27 participants (Table 2). The most common TEAEs were gastrointestinal including transient diarrhea (n = 10), abdominal discomfort (n = 3), and constipation (n = 5). There was no significant difference in TEAE frequency among treatment groups (χ^2 test). Serious TEAE were reported for 2 participants. One was hospitalized 96 days after treatment for chest and abdominal pain and shortness of breath, possibly related to prior diagnosis of urinary tract infection (UTI) and not related to RBX7455 treatment. A second was hospitalized 111 days after treatment for a diverticulitis-associated fistula not related to RBX7455, which resolved without sequelae. No deaths occurred in the duration of the study.

Microbiome Outcomes

All 30 treated participants provided fecal samples that were included in this analysis (Table S1), for a total of 148 samples including 11 RBX7455 product samples representing all 4 batches dosed. Nonparametric multidimensional scaling analysis and relative abundance analyses indicated that treatment responsive participants' bacterial microbiome compositions were divergent from the RBX7455 composition prior to treatment, with Gammaproteobacteria- and Bacilli-class bacteria predominating (Figures 2 and 3). Posttreatment microbiomes converged toward the RBX7455 composition after treatment within days and remained so through the 24-week period, with Bacteroidia and Clostridia predominating and a decrease in Gammaproteobacteria, Bacilli, and Verrucomicrobiae. At the genus level, restoration of Bacteroidia appeared to be driven by *Bacteroides* and *Alistipes* genera; restoration of Clostridia included multiple genera (Figure S1). Decreases in Bacilli were predominantly *Lactobacillus* and decreases in Gammaproteobacteria were exclusively genera in the Enterobacteriaceae family. There was not a clear trend of alpha diversity changes from before to after treatment (Figure 3), possibly due to small sample numbers. In the 3 nonresponders, baseline compositions were similar to those of responders, and although compositional changes were observed after treatment, little Bacteroidia restoration was observed (Figure S2).

To assess whether longitudinal microbiome changes were different among treatment groups, a repeated measures analysis was conducted with patient-matched samples from baseline, 1, 4, and 8 weeks after treatment, including 3, 4, and 6 participants from groups 1, 2, and 3, respectively, using a DMRepeat algorithm designed for Dirichlet multinomial relative abundance data [25]. Repeated measures examine an aggregate time course of posttreatment microbiome changes within individual patients and, as such, may be considered a pharmacodynamic measure of RBX7455 treatment. In this analysis, there was a significant difference between groups 2 and 3 at 4 and 8 weeks ($P < .05$), with more Clostridia restoration and Gammaproteobacteria decrease in group 3, but the difference was not significant at 1 week after treatment (Figure S3). Likewise, there was no significant difference at any time point between groups 1 and 2 or groups 1 and 3. Thus, there was no consistent trend among dosing groups that would confirm a dose-responsiveness with

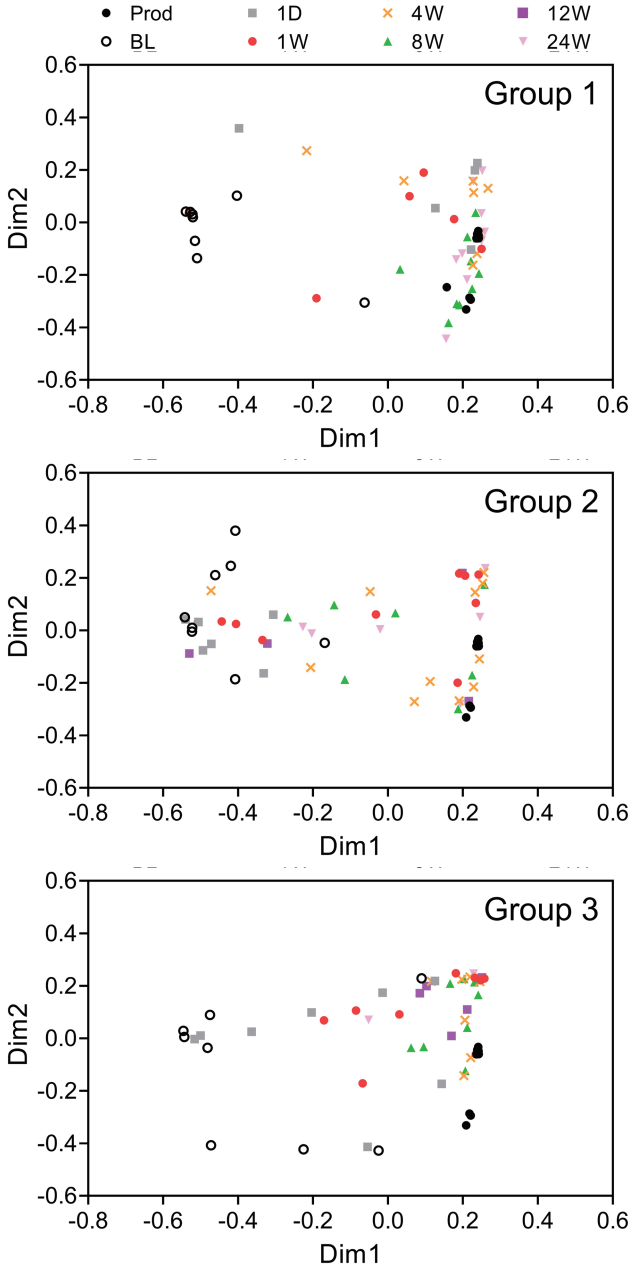

Figure 2. Nonparametric multidimensional similarity analysis for microbiome compositions of RBX7455 (Prod) and participant microbiome compositions before treatment (BL) and 1 day (1D) or 1, 4, 8, 12, and 24 weeks (1W, 4W, 8W, 12W, and 24W) after treatment.

respect to microbiome changes, which is accordant with clinical outcomes.

DISCUSSION

The primary finding in this study was that 3 different dosing regimens of RBX7455 were effective at preventing rCDI, with success rates at 8 weeks of between 80% and 100% and no dose dependence. The efficacy demonstrated with RBX7455 is highly consistent with published studies of FMT reported by Madoff et al. at 81% [13], within which the 2 RCTs that examined oral preparations reported a mean efficacy of 87% [17–21]. Moreover, there were no additional recurrences between the 8-week and 6-month assessment points, indicating durable efficacy of RBX7455, which has not been examined in controlled studies of oral FMT. Finally, the safety profile of RBX7455 for rCDI was acceptable in this study, comparable to other reports for FMT, and possibly advantageous compared to administration by colonoscopy, for which there are known procedure-related risks [25].

This study has also importantly demonstrated that the microbiome compositions of RBX7455 treatment responders shifted after receiving therapy. Participants' microbiomes normalized from Gammaproteobacteria and Bacilli predominance (before treatment) to Bacteroidia and Clostridia predominance (after treatment), demonstrating profiles similar to the RBX7455 composition and to recognized healthy compositions [26–28]. This is consistent with microbiome shifts measured after treatment with RBX2660, an enema-administered investigational live biotherapeutic [29]. The observed changes after RBX7455 may underpin its mechanism for reducing rCDI, because Bacteroidia and commensal Clostridia are associated with increased resistance to *C. difficile* colonization [30, 31], and because Gammaproteobacteria, which are reduced after treatment, are associated with increased CDI susceptibility and gastrointestinal inflammation [32, 33].

A key potential benefit of RBX7455 over existing FMT formulations is that it is produced using standardized good manufacturing practices (GMP) processes under strict quality-controlled conditions. This provides greater batch-to-batch consistency and ensures a documented, high quantity of viable bacteria per dose. Furthermore, the process for RBX7455 yields a formulation in which bacteria remain viable even during storage at room temperature throughout dosing. This feature allowed for participants in this trial to self-administer RBX7455 outside of a healthcare setting and still achieve a positive clinical outcome. This patient feasibility improvement has the potential to broaden the population that could benefit from RBX7455.

The main limitations of the study are the size of the population under investigation, lack of a control arm, and the possibility that the encouraging results may be related to a type-2 error. However, given the fact that many previous studies have reported comparable results for microbiome restorative therapies, this is unlikely.

CONCLUSIONS

In this study, 3 dosing regimens of RBX7455 were shown to be safe and effective at preventing rCDI for 8 weeks after treatment, with no additional recurrences for up to 6 months posttreatment. In addition, treatment with RBX7455 appeared to modify the microbiome in patients responding to therapy such that it converged toward the composition of RBX7455.

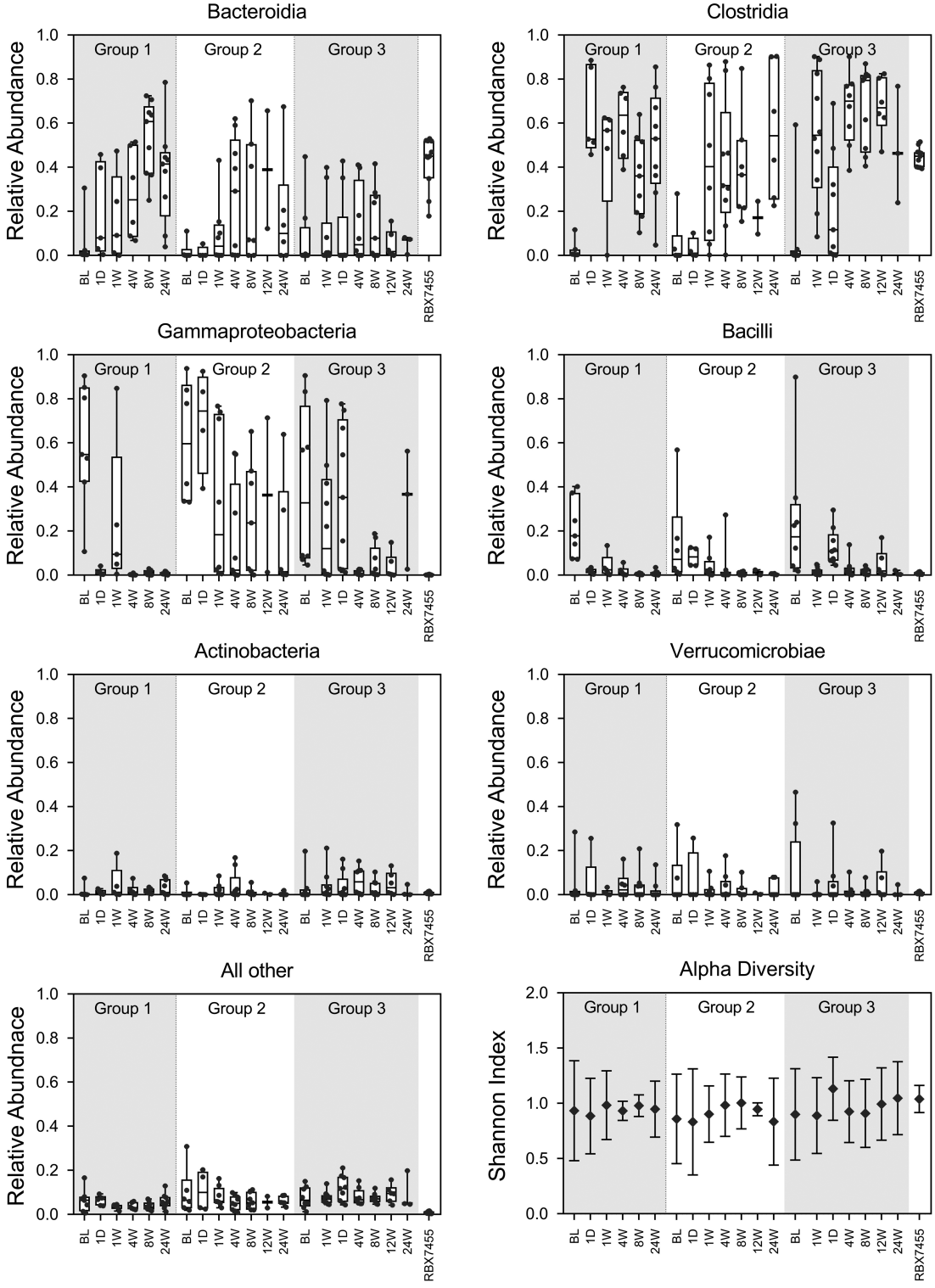

Figure 3. Taxonomic compositions at the class level and alpha diversity of responder microbiomes and RBX7455. Relative abundance data are shown in box-and-whiskers format, and alpha diversity is shown as a mean with standard deviations. Classes with <5% total relative abundances are groups together as "Other".

Although this study is small, these results are encouraging and support the continued clinical evaluation of RBX7455 in randomized controlled trials.

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

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Potential conflicts of interest. S. K. has received research support from Rebiotix. K. B. and C. J. are employees of Rebiotix. K. B. has patents WO2019213595 and WO2019075426A1 (licensed to Rebiotix, A Ferring Company) pending. C. J. has the following patents issued: 9675648, 9433651, 10603341, 9642880, 10610547, 10624932, 10471107, 10493111, 2014275025, 2017201330, 2018220114, 3003330, 242934, 6330032, 6363259, KR102093537, KR102099503, 9511099, 10434124, 9511100, 10434125, 10434126, 9782445, 10688137, 10226431, 10391064, 20180243349, JP6616431, KR102066242, RU2714841C1, 9694039, 10383901, and 20180289750; and the following patents issued: CN111247254A, 2952500, 2019-0365831, 3455373, 109415760, 3022847, 2017/0327862, 2020037573, 3307289, 107921072, 2986915, 20180078586, 2928652, 3375447, 105473152, and 2914636. S. K. reports stock options from Jetson; and fees paid to Mayo Clinic from Vedanta, outside the submitted work. D. P. reports consulting fees from Seres Therapeutics, Assembly Biosciences, and Vedanta, outside the submitted work. All other authors have no potential conflicts. 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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