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MAJOR ARTICLE

Efficacy of Fecal Microbiota, Live-jslm (REBYOTA®), Among Patients Exposed to Non–*Clostridioides difficile* Infection Antibiotics: Post Hoc Subgroup Analysis of a Phase 2 Open-Label Study

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Background. Antibiotic use is a major risk factor for recurrent *Clostridioides difficile* infection (CDI) due to the associated disruption in gut microbiota. Fecal microbiota, live-jslm (REBYOTA®; RBL, previously RBX2660), is the first microbiota-based live biotherapeutic approved by the US Food and Drug Administration to prevent recurrent CDI in adults following standard-of-care antibiotic treatment. To investigate the impact of non-CDI antibiotics on the durability of RBL, a subgroup analysis was conducted on PUNCH™ Open-Label study participants who received non-CDI antibiotics during the period between RBL administration and up to 2 years after.

Methods. Participants in PUNCH™ Open-Label who received non-CDI antibiotics after RBL administration were included in this subgroup analysis. Treatment response was defined as the absence of CDI diarrhea needing retreatment at the last evaluable time point (8 weeks, 6 months, 1 year, or 2 years) after RBL administration.

Results. Among participants from PUNCH™ Open-Label, 43 received non-CDI antibiotics after RBL administration but before CDI recurrence as evaluated over a 2-year period. Across all evaluable time points, 86% (37/43) of participants had a treatment response regardless of when non-CDI antibiotic exposure occurred. Treatment response was sustained for a median 470 days (IQR, 212–648) from the first day of non-CDI antibiotic use. Most participants (5/6) with CDI recurrences received a high-risk antibiotic.

Conclusions. RBL remained efficacious in participants with a history of recurrent CDI after subsequent non-CDI antibiotic exposure.

Clinical Trials Registration. NCT02589847 (https://clinicaltrials.gov/study/NCT02589847).

Keywords. antibiotics; clinical trials; *Clostridioides difficile* infection; microbiota-based therapy; recurrence.

Clostridioides difficile infections (CDIs) are associated with significant morbidity, mortality, and costs of care [1, 2]. The recurrent nature of CDI contributes to this burden, with 10% to 35% of patients with an initial episode experiencing a subsequent episode. Risk increases thereafter, with additional recurrence occurring in up to 65% of patients with a history of ≥2 CDIs [3–7].

While numerous risk factors exist for CDI (eg, advanced age, use of proton pump inhibitors, underlying chronic comorbidities), antibiotic exposure is the most widely recognized factor [8]. Cumulative antibiotic exposure may also affect risk, with consideration for dose, number of agents, spectrum of activity, and duration of therapy. In a large retrospective cohort study among hospitalized adults receiving ≥2 days of antibiotics, the adjusted hazard ratios for CDI for those who received 2, 3–4, or ≥5 antibiotic agents were 2.5, 3.3, and 9.6, respectively, as compared with patients who received only 1 antibiotic [9].

Antibiotics decrease bacterial abundance and diversity within the gut microbiota, leading to dysbiosis and loss of colonization resistance against potential pathogens, such as *C difficile* [10, 11]. Loss of nutrient competition, direct antagonist interactions among organisms, and decreased metabolic function,

Received 12 January 2024; editorial decision 11 June 2024; accepted 13 June 2024; published online 17 June 2024

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https://doi.org/10.1093/ofid/ofae341

including impaired secondary bile acid synthesis, are implicated in ongoing dysbiosis [12, 13]. Reduction in secondary bile acids can be detrimental, as they have inhibitory effects on *C difficile* spore germination and outgrowth [14]. Gut microbiota restoration can mitigate these risks by restoring colonization resistance [1].

Live biotherapeutic products (LBPs) are a class of US Food and Drug Administration (FDA)–approved microbiota-based therapies that have been evaluated in phase 2 and 3 studies for prevention of recurrent CDI (rCDI) in those with a history of CDI and following standard-of-care (SOC) antibiotic treatment [15, 16]. LBPs are generated by using standardized proprietary processes that adhere to good manufacturing practices. They also undergo rigorous screening procedures and pathogen testing to maximize patient safety [1, 17]. Fecal microbiota, live-jslm (RBL, previously RBX2660), is the first FDA-approved single-dose, rectally administered microbiota-based LBP. It is indicated for prevention of rCDI in individuals ≥18 years old following SOC antibiotic treatment for rCDI [15].

Given that LBPs are believed to restore gut microbiota diversity and colonization resistance, exposure to antibiotics post administration, especially broad-spectrum or high–CDI risk antibiotics, may diminish these beneficial effects. Therefore, it is clinically important to evaluate durability of effect in the setting of subsequent antibiotic exposure. A post hoc analysis of the clinical response to RBL was conducted in participants from the phase 2 PUNCH™ Open-Label study (ClinicalTrials.gov NCT02589847) [1] who subsequently received non-CDI antibiotics for up to 2 years. Overall, PUNCH™ Open-Label found RBL to be safe and efficacious in preventing rCDI as compared with a historical control group, with post hoc analysis showing that 91% (88/97) of RBL responders remained CDI recurrence-free 2 years after administration [1]. This is the first known analysis reporting outcomes associated with antibiotic exposure after LBP administration.

METHODS

PUNCH™ Open-Label Study

PUNCH™ Open-Label—an international multicenter phase 2 clinical study based on a prospective, open-label, single-arm design—evaluated the safety, efficacy, and durability of RBL for the prevention of rCDI. The primary end point was treatment success, defined as the absence of CDI diarrhea needing CDI antibiotic retreatment within 8 weeks after RBL receipt. After a 24- to 48-hour CDI SOC antibiotic washout period, up to 2 doses of RBL were rectally administered 7 ± 2 days apart without preceding bowel preparation.

Participants were enrolled if they were aged ≥18 years with a diagnosis of rCDI and had either ≥2 documented recurrences of CDI after a primary episode and had completed ≥2 rounds of SOC oral antibiotic therapy, or if they had ≥2 documented episodes of severe CDI resulting in hospitalization. A positive stool polymerase chain reaction test result for *C difficile* or positive toxin A/B enzyme immunoassay result ≤60 days prior to enrollment and the use of antibiotics for rCDI symptom control at enrollment were required. Additional study design details, including full inclusion and exclusion criteria and the definition of CDI recurrence, have been described [1].

The study protocol received institutional review board and research ethics board approval at each participating center prior to commencement and was conducted under an FDA investigational new drug application. The study was performed in accordance with the Declaration of Helsinki. An independent medical monitor provided safety oversight. All participants provided written informed consent.

Post Hoc Analysis

Post hoc analysis included PUNCH™ Open-Label participants who received non-CDI antibiotics within 2 years after RBL administration. Non-CDI antibiotic exposure was defined as ≥1 dose of an antibiotic given via an oral, intravenous, subcutaneous, or intramuscular route to treat infections other than CDI. Participants who received only topical antibiotics were excluded. Metronidazole (oral or intravenous) to treat CDI was considered a CDI antibiotic and was excluded. Metronidazole was considered a non-CDI antibiotic if used for indications other than CDI.

Treatment response was defined as the absence of CDI diarrhea needing retreatment as of the last evaluable time point (8 weeks, 6 months, 1 year, or 2 years) after RBL administration. Patients without evaluable outcomes were excluded from this post hoc analysis, such as those who experienced CDI recurrence prior to non-CDI antibiotic exposure or did not require non-CDI antibiotics in the period between RBL administration and the evaluation time point. CDI therapy was classified by the SOC agents that the patient was receiving on the last day of CDI antibiotic treatment, to capture the regimen most reflective of clinical response.

Other variables of interest included non-CDI treatment antibiotic agent (characterized by class), number of antibiotic classes used at a time (to distinguish monotherapy from combination therapy), duration of therapy, number of antibiotic courses, antibiotic indication (per Centers for Disease Control and Prevention definitions [18]), route of administration, and use of concomitant CDI prophylaxis. As patients received non-CDI treatment antibiotics at various times after RBL administration, the following were also assessed: time to non-CDI treatment antibiotic use after the second dose of RBL, time to CDI recurrence from end of non-CDI antibiotic therapy, and duration of recurrence-free period.

Antibiotics were further characterized according to their associated risk of causing CDI, with fluoroquinolones, third- and

Table 1. Baseline Characteristics and Treatment Response Rates of Participants Administered RBL Who Were Subsequently Treated With Non-CDI Antibiotics (N = 43)

	Participants, No. (%)	
	Overall	Treatment Response[a]
Age, y		
<65	17 (39.5)	15 (88.2)
≥65	26 (60.5)	22 (84.6)
Sex		
Female	29 (67.4)	25 (86.2)
Male	14 (32.6)	12 (85.7)
Race		
White	41 (95.3)	35 (85.4)
Black	2 (4.7)	2 (100)
No. of previous CDI episodes[b]		
2	1 (2.3)	1 (100)
3	18 (41.9)	16 (88.9)
4	14 (32.6)	10 (71.4)
≥5	10 (23.3)	10 (100)
CDI antibiotics prescribed as SOC therapy		
Vancomycin: alone	33 (76.7)	28 (84.8)
Vancomycin: combination	4 (9.3)	3 (75.0)
Metronidazole	5 (11.6)	5 (100)
Fidaxomicin	1 (2.3)	1 (100)
CDI antibiotic prophylaxis		
Yes	5 (11.6)	4 (80)
No	38 (88.4)	33 (86.8)
No. of non-CDI antibiotics used at 1 time		
1[c]	34 (79.1)	28 (82.4)
2	8 (18.6)	8 (100)
3	1 (2.3)	1 (100)
No. of non-CDI antibiotic courses		
1	28 (65.1)	24 (85.7)
2	6 (14.0)	6 (100.0)
3	5 (11.6)	4 (80.0)
4	4 (9.3)	3 (75.0)
Non-CDI antibiotic DOT, d[d]		
≥1 to ≤5	8 (18.6)	7 (87.5)
>5 to ≤10	13 (30.2)	10 (76.9)
>10 to ≤21	11 (25.6)	10 (90.9)
>21	9 (20.9)	8 (88.9)
Route of non-CDI antibiotic administration[e]		
IV/SC	13 (30.2)	9 (69.2)
Oral only	30 (69.8)	28 (93.3)
Non-CDI antibiotic exposure by risk category[f]		
High risk	23 (53.5)	18 (78.3)
Not high risk	20 (46.5)	19 (95.0)
High-risk non-CDI antibiotic exposure[f]		
Fluoroquinolones	13 (30.2)	…
Third- or fourth-generation cephalosporins	11 (25.6)	…
Broad-spectrum penicillins[g]	5 (11.6)	…
Carbapenems	1 (2.3)	…
Top 4 indications for non-CDI antibiotics[h]		
Urinary system infection	17 (39.5)	…
Lower respiratory system infection	9 (20.9)	…
Perioperative prophylaxis	6 (14.0)	…
Gastrointestinal system infection	6 (14.0)	

Abbreviations: CDI, *Clostridioides difficile* infection; DOT, duration of therapy; IV, intravenous; RBL, fecal microbiota, live-jslm; SC, subcutaneous; SOC, standard of care.

[a]In this post hoc analysis, treatment response was defined as the absence of CDI diarrhea needing treatment at the last evaluable time point (8 weeks, 6 months, 1 year, or 2 years) after non-CDI antibiotic exposure. Percentage is by row.

[b]Representative of all previously documented CDI events per reported medical history.

[c]Participants could have received multiple consecutive agents.

[d]For 2 participants, the end date for the duration of non-CDI antibiotics was missing.

[e]Participants who received IV/SC antibiotics could have also received oral antibiotics.

[f]Antibiotics are defined as high-risk agents if they are associated with a higher risk of CDI; no participants received clindamycin. Participants may have received more than 1 type of high-risk non-CDI antibiotic.

[g]Piperacillin/tazobactam or amoxicillin/clavulanate.

[h]Participants could have been treated for more than 1 indication.

fourth-generation cephalosporins, broad-spectrum penicillins, carbapenems, and clindamycin deemed "high risk" for CDI [19–21].

Statistical Analyses

As this post hoc analysis was not adequately powered for inferential statistical analyses, all analyses were descriptive only.

RESULTS

Demographics, Baseline Characteristics, and Non-CDI Antibiotic Exposure

Of the 149 participants in the PUNCH™ Open-Label study, 43 received non-CDI antibiotics after RBL administration but before CDI recurrence, if any occurred, as evaluated over a 2-year period. All participants exposed to non-CDI antibiotics received 2 doses of RBL in the open-label study (range, 2–8 days apart). Most participants were ≥65 years old (60.5%), White (95.3%), and female (67.4%); had 3 prior CDI episodes (41.9%) or 4 (32.6%); and were treated with oral vancomycin monotherapy for the enrolling CDI episode (76.7%; Table 1).

Most participants were treated with a non-CDI antibiotic from a single drug class at a time (ie, monotherapy; 79.1%) as a single non-CDI antibiotic treatment course (65.1%). Among the 86 reported antibiotics exposures, the most used agents were fluoroquinolones (n = 13), sulfamethoxazole/trimethoprim (n = 10), third-generation cephalosporins (n = 9), and tetracyclines (n = 9). A complete list is shown in Supplementary Table 1.

Approximately half of participants (53.5%, 23/43) received a high-risk agent, such as a fluoroquinolone (30.2%). Antibiotics were most frequently prescribed for urinary system infections (39.5%) or lower respiratory system infections (20.9%). The median antibiotic course duration was 8 days (IQR, 4.5–12.5),

and most participants received non-CDI antibiotics in oral form only (69.8%; Table 1). Few participants (11.6%, 5/43) received concomitant CDI prophylaxis during their non-CDI antibiotic course.

A summary of each participant's non-CDI antibiotic exposure history is shown in Figure 1. The median time to non-CDI antibiotic exposure after the second dose of RBL was 155 days (IQR, 55–349).

Efficacy

Treatment response rates at each evaluable time point of 8 weeks, 6 months, 1 year, and 2 years after the second dose of RBL were 91.7% (11/12), 95.7% (22/23), 90.6% (29/32), and 83.3% (30/36), respectively (Figure 2). Across all evaluable time points, 86.0% (37/43) of participants had a treatment response regardless of when the non-CDI antibiotic exposure occurred. Treatment response rates in participants who received non-CDI antibiotics within the first 8 weeks (91.7%, 11/12) were comparable to participants who received non-CDI antibiotics between 2 months and 6 months (100.0% [12/12]) and between 6 months and 1 year (90.0% [9/10]; Table 2). Numerically lower 2-year response rates were observed in participants who received non-CDI antibiotics between 6 months and 2 years vs the first 6 months.

Treatment response rates were similar when participants were stratified by age, number of previous CDI episodes, number of antibiotic agents, duration of therapy, number of courses, or use of concomitant CDI antibiotic prophylaxis (Table 1). Treatment response rates were numerically lower for participants who received a high-risk antibiotic relative to one not considered high risk (78.3% vs 95.0%) and for those who received intravenous or subcutaneous therapy relative to oral therapy only (69.2% vs 93.3%).

Among participants with treatment response (n = 37), success was sustained for a median 470 days (IQR, 212–648) from the first day of the initial non-CDI antibiotic use and 367 days (IQR, 107–554) from the last day of the non-CDI antibiotic course.

CDI Recurrences After Non-CDI Antibiotic Exposure

Descriptive characteristics of the 6 participants who experienced rCDI after antibiotic exposure are shown in Table 3. Five received a high-risk agent, and 4 received a single antibiotic course. One participant experienced rCDI during antibiotic therapy; this person did not receive concomitant CDI prophylaxis.

The median number of days to CDI recurrence was 39 (IQR, 20.5–134) from the first day of the initial non-CDI antibiotic

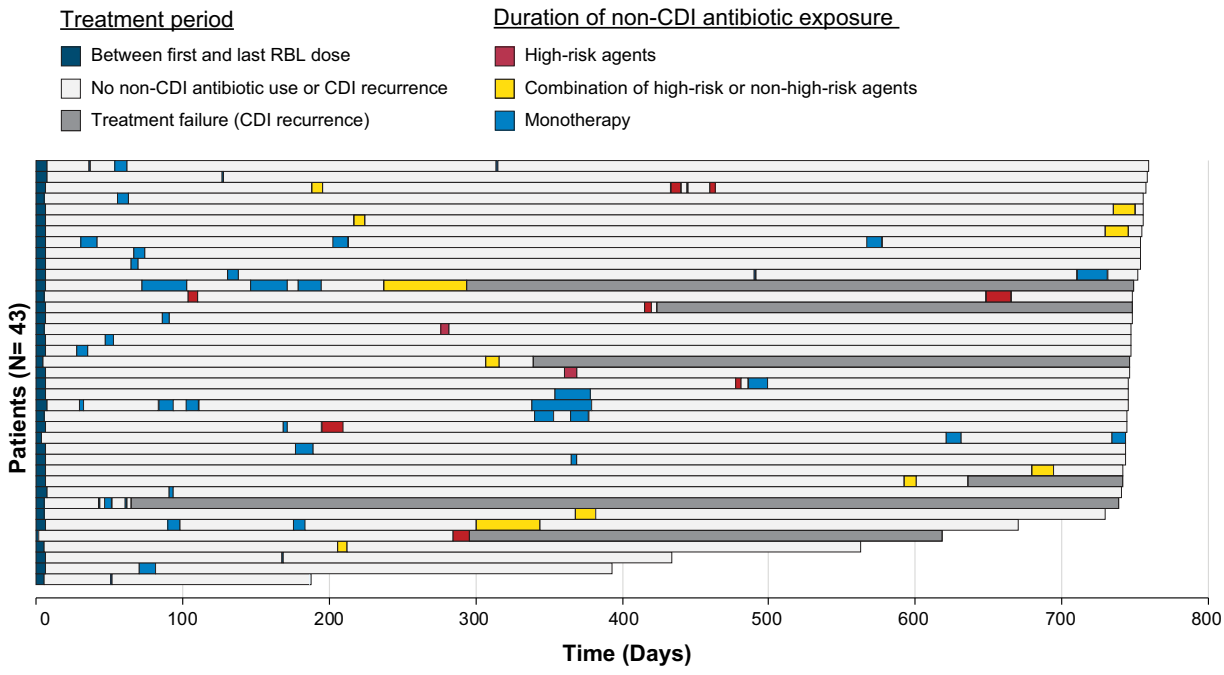

Figure 1. Time to non-CDI antibiotic use after RBL administration for each participant. Figure 1 provides a comprehensive overview of non-CDI antibiotic use for 39 of the 43 participants who received non-CDI antibiotics in the PUNCH™ Open-Label study after RBL administration. Four participants were omitted due to missing end dates for their first or second course of non-CDI antibiotics, all of which were non–high risk. From day 0 (the day of the first RBL dose), all participants received the second RBL dose between 2 and 8 days later (dark blue bars). Periods where participants were not receiving any non-CDI antibiotics and did not experience CDI recurrence are depicted by the white bars. For periods where participants received non-CDI antibiotic courses, the type of agent is denoted by color: high risk, red bars; mixed courses of high risk and non–high risk, yellow bars; and antibiotic monotherapy, light blue bars. CDI recurrences are denoted by the gray bars. CDI, *Clostridioides difficile* infection; RBL, fecal microbiota, live-jslm.

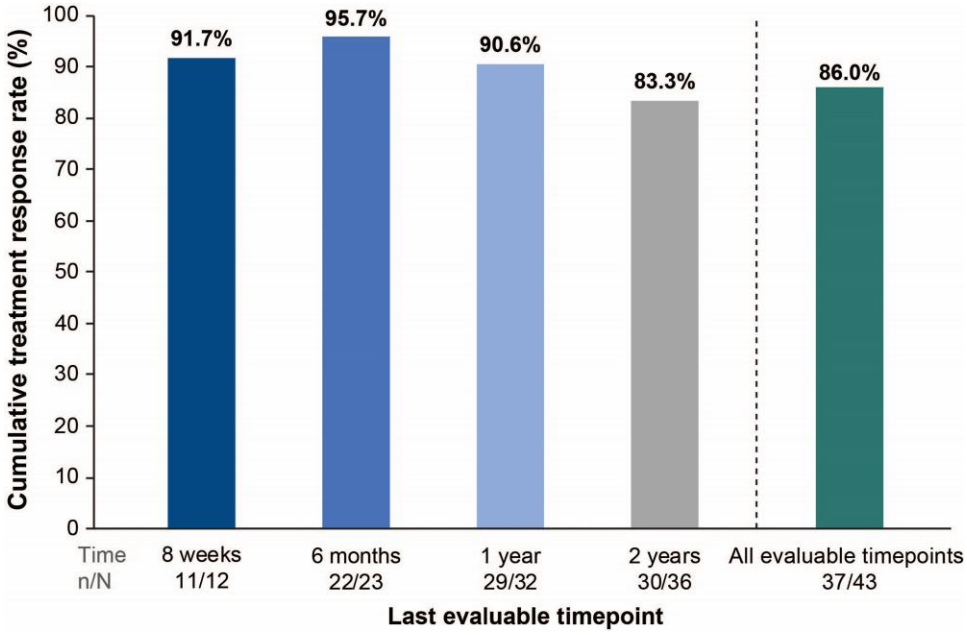

Figure 2. Treatment response rates among participants exposed to non-CDI antibiotics up to 2 years after RBL administration. Participants received non-CDI antibiotics after RBL administration before CDI recurrence or assessment period. CDI, *Clostridioides difficile* infection; RBL, fecal microbiota, live-jslm.

use and 12 (IQR, 0.25–35) from the last day of the non-CDI antibiotic course.

DISCUSSION

This post hoc analysis of PUNCH™ Open-Label reports treatment response rates between 83% and 96% at various time points (8 weeks–2 years) after RBL administration and subsequent non-CDI antibiotic exposure. Participants who received non-CDI antibiotics were most often aged ≥65 years, had 3 or 4 CDI episodes, and had previously received oral vancomycin for the enrolling CDI episode. Most participants received a single antibiotic course for about 1 week, several months after their qualifying rCDI episode. The results of this analysis were likely affected by non-CDI antibiotic exposure timing relative to the CDI episode, antibiotic agent choice, and duration of non-CDI antibiotic therapy. With consideration of these factors, an overall treatment response rate of 86% in participants with a history of rCDI remaining CDI recurrence-free is a positive signal for the durable effectiveness of RBL. These findings represent the first reported analysis of non-CDI antibiotic exposure after LBP administration from the longest LBP study duration of 2 years.

Topline results of this post hoc analysis are largely consistent with the full analysis set of PUNCH™ Open-Label. In that study, up to 2 doses of RBL resulted in a treatment response rate of 78.9% (112/142) at 8 weeks. Of those patients who experienced clinical response at 8 weeks, 91% (88/97) reported sustained clinical response at 2 years [1]. Findings from this post hoc analysis suggest that microbiome restoration with RBL is durable out to 2 years despite antibiotic exposure, with an 83.3% (30/36) treatment response rate. Notably, all patients in this post hoc analysis received 2 doses of RBL, in contrast to the FDA-approved dosing strategy of a single rectal dose after SOC rCDI treatment and an antibiotic washout period of 24 to 72 hours. Two doses administered approximately 7 days apart did not confer any additional benefit relative to 1 dose in the phase 2 randomized controlled trial, leading to incorporation of a single-dose strategy in the pivotal phase 3 trial. While administration of 2 doses may have affected findings of this analysis, microbiome composition analyses in patients across the clinical program have shown similar trends in restoration, particularly during the window of vulnerability across dosing strategies (1–8 weeks following CDI antibiotic treatment) [22]. Therefore, microbiome composition data paired with CDI outcomes data suggest that restoration may be sufficient with a single RBL dose to confer protective benefit against subsequent antibiotic exposure. A similar analysis conducted in the phase 3 open-label study population, which involved receipt of a single dose of RBL, would support the validation of this hypothesis. The phase 3 open-label study evaluating RBL in several hundred patients was completed at the end of 2023, which may offer more real-world evidence in clinically relevant populations, including those exposed to non-CDI antibiotics.

A notable characteristic among participants who experienced rCDI in this post hoc analysis was receipt of a high-risk agent, with fluoroquinolone and broad-spectrum cephalosporin use

Table 2. Treatment Response Rates in Participants Administered RBL After Non-CDI Antibiotic Exposure

Time When Non-CDI Antibiotics Were Received	Treatment Response,[a] No. (%)			
	8 wk	6 mo	1 y	2 y
Within the first 8 wk	11/12 (91.7)	10/11 (90.9)	9/10 (90.0)	8/9 (88.9)
Between 2 and 6 mo	…	12/12 (100.0)	11/12 (91.7)	9/10 (90.0)
Between 6 mo and 1 y	…	…	9/10 (90.0)	6/8 (75.0)
Between 1 and 2 y	…	…	…	7/9 (77.8)

Abbreviations: CDI, *Clostridioides difficile* infection; RBL, fecal microbiota, live-jslm.

[a]In this post hoc analysis, treatment response was defined as the absence of CDI diarrhea needing retreatment at the last evaluable timepoint (8 weeks, 6 months, 1 year, or 2 years) after RBL administration. For each time point, participants were excluded from the analysis if they had experienced CDI recurrence prior to the administration of a non-CDI antibiotic or had not received a non-CDI antibiotic during the period between RBL administration and the evaluation time point.

being common. These findings are not surprising in that high-risk non-CDI antibiotics, such as fluoroquinolones and β-lactams, are associated with dysbiosis and reduced microbial diversity [23]. Factors that can affect gut microbiota dysbiosis after antibiotic exposure include the spectrum of antibiotic activity (eg, anaerobic), pharmacokinetics (eg, biliary elimination rate), total dose, and duration of treatment [24]. In a retrospective analysis of adult admissions across 21 US hospitals, the odds of subsequent CDI increased by 12.8% for every antibiotic day of therapy prior to the index admission [25]. This risk was greatest with second- and later-generation cephalosporins, carbapenems, fluoroquinolones, and clindamycin.

Generally, patients appear to be at highest risk for CDI during antibiotic exposure and in the first month after use [26]. One participant in this post hoc analysis experienced rCDI during the non-CDI antibiotic course. Many clinical trials and retrospective observational studies commonly report rCDI within approximately 4 weeks after antibiotic exposure. This period, known as the window of vulnerability, coincides with reconstitution of the gut microbiota [27]. Clinical risk may extend out to 3 months, as evidenced by 1 study finding that use of non-CDI antibiotics within 3 months of CDI antibiotic treatment was associated with a 3-fold increased risk for rCDI [28]. Early non-CDI antibiotic exposure (eg, within 8 weeks) has been shown as a risk factor for rCDI in patients who had previously received microbiome restoration therapy [29–31]. Given the median time of 155 days (IQR, 55–349) to non-CDI antibiotic administration and the high overall treatment response rate, most participants in this post hoc analysis likely transitioned out of the window of vulnerability and experienced gut microbiota restoration that was sufficient to protect against rCDI.

Beyond traditional antibiotic exposure factors, severity of illness and/or medical complexity may have affected outcomes in this study. Nearly one-third (30.2%) of participants received an antibiotic agent intravenously or subcutaneously during their treatment course, suggesting that they were either medically

Table 3. Participants With CDI Recurrences

Age, y; Sex	No. of CDI Episodes	Antibiotic Class	Antibiotic Indication, CDC Class (Infection)	Antibiotic Details	Time to rCDI From End of Antibiotic Therapy	Time Point of Recurrence
79; male	3	Aminoglycoside, combinations of sulfonamides and trimethoprim, lipopeptide	UTI	Start date: D31; 3 courses over 17 d (duration, 1–5 d)	Next day after end of third course (monotherapy)	8 wk
69; male	3	Tetracycline, first-generation cephalosporin, broad-spectrum penicillins (3 class agents), glycopeptide, third-generation cephalosporin, carbapenem, fluoroquinolone	Skin and soft tissue infection (folliculitis), skin and soft tissue infection (chest wound), bacteremia	Start date: D58; 4 courses over 218 d (duration, 16–57 d)	During fourth course (high-risk and non–high-risk agents)	1 y
77; female	4	Third-generation cephalosporin, broad-spectrum penicillins	UTI (pyelonephritis)	Start date: D297; one 9-d course	23 d after end of first course (high-risk agent)	1 y
78; female	4	Fluoroquinolone	Lower respiratory system infection (respiratory failure)	Start date: D281; one 11-d course	Treatment failure unrelated to antibiotic use[a]	2 y
35; female	4	Third-generation cephalosporin	Eye, ear, nose, throat, or mouth infection (rhinitis)	Start date: D401; one 5-d course	4 d after start of first course (monotherapy)	2 y
50; female	4	Glycopeptide,[b] nitrofuran derivative, fluoroquinolone	UTI	Start date: D576; one 9-d course	39 d after end of first course (high-risk and non–high-risk agents)	2 y

Abbreviations: CDC, Centers for Disease Control and Prevention; CDI, *Clostridioides difficile* infection; D, days since last RBL dose; RBL, fecal microbiota, live-jslm; rCDI, recurrent CDI; UTI, urinary tract infection.

[a]CDI recurrence occurred 298 days after the end of the non-CDI antibiotic course.

[b]Participant received concomitant CDI prophylaxis for 3 days.

complex and/or had severe infections requiring hospitalization. Hospitalization is a risk factor for rCDI, and it is unsurprising that treatment response outcomes were numerically lower for those who received an intravenously or subcutaneously administered agent during their antibiotic course as compared with those who received an oral agent only. It is important to consider that the risk of rCDI was likely affected by the antibiotic agent (eg, spectrum of activity, concentrations achieved in the gut based on pharmacokinetic profile) rather than solely the route of administration [24].

CDI prophylaxis may be considered for high-risk patients in clinical settings. This strategy did not appear to prevent rCDI in this cohort; however, too few participants (n = 5) received prophylactic CDI antibiotics to reliably assess this association. CDI prophylaxis, which is most often oral vancomycin, is controversial. Prophylaxis may help prevent CDI, particularly in the short term, but may increase the risk of rCDI in the longer term since oral vancomycin itself furthers dysbiosis [32, 33]. Concurrent use of oral vancomycin with or soon after administration of RBL is also likely to be counterproductive owing to its potential depletion of RBL-provided beneficial microbes.

There are several factors to consider when interpreting the results of this post hoc analysis. PUNCH™ Open-Label did not have a placebo comparator group; therefore, our non-CDI antibiotic exposure analysis was limited to RBL recipients only. However, open-label studies often include more "real-world" patient cohorts relative to randomized controlled trials and therefore represent clinically relevant populations. Additionally, this analysis was descriptive and focused on characterizing outcomes associated with antibiotic exposure. Variables such as receipt of other dysbiosis-associated medications (eg, proton pump inhibitors) were not controlled for and may have affected the results. However, studies that have controlled for other agents, including proton pump inhibitor use, have consistently observed antibiotic exposure as a strong independent risk factor for subsequent rCDI [34].

Hospitalization or severity of illness data related to the index CDI episode or the systemic infection episode were not available for inclusion in this analysis. Route of administration of non-CDI antibiotic was identified to generate clinical assumptions about patient complexity and/or severity of infection at the time of non-CDI antibiotic administration. *C difficile*–level data were also unavailable, limiting any ability to determine whether rCDI was due to relapse (same strain as the qualifying CDI event) or reinfection (different strain). Despite these limitations, our findings provide evidence that RBL offers durable protection after antibiotic exposure in a real-world population and in a setting likely including other rCDI risk factors.

CONCLUSION

The findings of this post hoc analysis suggest that RBL effectively prevents rCDI in patients with multiple rCDIs despite systemic non-CDI antibiotic exposure. As expected, recurrences were more common after high-risk non-CDI antibiotic exposure. This trend reinforces the value of antibiotic stewardship for patients at high risk of CDI, including rCDI. Irrespective of the inherent limitations of this analysis, these results suggest that RBL may restore the gut microbiota to a sufficient threshold protective against rCDI for many patients despite subsequent non-CDI antibiotic use.

Supplementary Data

Supplementary materials are available at *Open Forum 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. We thank the participants and their families and caregivers and all the investigators and site staff. Medical writing support, under the guidance of the authors, was provided by Farah Dalwai, PhD (ApotheCom, London, UK; funded by Ferring Pharmaceuticals, Parsippany, NJ, USA.).

Author contributions. All authors conceptualized and designed the study. All authors were involved in the acquisition, analysis, or interpretation of data. All authors provided critical revision of the manuscript, and all agreed to the submission of the manuscript.

Patient consent statement. The study protocol received institutional review board and research ethics board approval at each participating center prior to commencement and was conducted under an FDA investigational new drug application. All participants provided written informed consent. Any information, including illustrations, is anonymized.

Financial support. This work was supported by Ferring Pharmaceuticals, Parsippany, NJ, USA.

Potential conflicts of interest. K. R. R. served as a consultant and speaker for Ferring Pharmaceuticals and received research funding (paid to her institution) from Merck & Co, Inc, and AstraZeneca. A. J. G.-L. received prior research funding paid to her institution from Cidara Therapeutics, Paratek Pharmaceuticals, Merck, and Seres Therapeutics and served as a consultant for Ferring Pharmaceuticals and Innoviva Specialty Therapeutics. Y. G. has served as a consultant for Ferring Pharmaceuticals, Seres Therapeutics, and Vedanta and as a principal investigator for Vedanta and has received honoraria for speaking for Ferring and Seres. C. D. A. received prior research funding from Merck paid to her institution; served in an advisory role to Cidara Therapeutics, AiCuris, Merck, DSM-Firmenich, and Pfizer; and received honoraria for speaking from the Academy for Continued Healthcare Learning, American Society of Health System Pharmacists, and Clinical Care Options. B. G. is an employee of Rebiotix Inc, a Ferring Company. M. R. B. is an employee of Ferring Pharmaceuticals. X. T. and V. P. were employees of Ferring Pharmaceuticals at the time of the analysis. C. V. C. has served as a consultant for Ferring Pharmaceuticals and Seres Therapeutics. A. M. S. received prior research funding paid to his institution from Paratek Pharmaceuticals; serves on the advisory board of Bio-K+; has served as a consultant for Ferring Pharmaceuticals; and has received honoraria for speaking from the American Society of Health System Pharmacist, MJH Life Sciences, and Academy for Continued Healthcare Learning.

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