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Safety and Efficacy of Fecal Microbiota, Live-jslm, in Preventing Recurrent *Clostridioides difficile* Infection in Participants Who Were Mildly to Moderately Immunocompromised in the Phase 3 PUNCH CD3-OLS Study

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Background. Fecal microbiota, live-jslm (RBL; Rebyota), is the first Food and Drug Administration–approved, single-dose, microbiota–based live biotherapeutic to prevent recurrent *Clostridioides difficile* infection (rCDI) in adults following standard-of-care antimicrobials. Patients who are immunocompromised are often considered at higher risk for *C difficile* infection, including recurrence, as compared with those who are immunocompetent. This subgroup analysis of PUNCH CD3-OLS (NCT03931941) evaluated RBL safety and efficacy in participants with rCDI who were considered mildly to moderately immunocompromised.

Methods. Participants with rCDI who had immunocompromising conditions and/or were taking immunosuppressive medications were included. Treatment-emergent adverse events (TEAEs) were collected for up to 6 months following RBL administration. Efficacy outcomes included treatment success at 8 weeks and sustained clinical response at 6 months.

Results. Overall, 793 participants were enrolled in PUNCH CD3-OLS and 697 received RBL; 141 were included in the immunocompromised subgroup. TEAEs within 8 weeks were reported by 44.7% and 48.0% of participants in the immunocompromised and nonimmunocompromised subgroups, respectively; most events were mild or moderate gastrointestinal disorders. Serious TEAEs within 8 weeks were reported by 4.3% and 3.8% of participants in the immunocompromised and nonimmunocompromised subgroups. No RBL-related systemic infections occurred. In the immunocompromised subgroup, the treatment success rate at 8 weeks was 75.7% and the sustained clinical response rate at 6 months was 88.7%; similar rates were observed in the nonimmunocompromised subgroup (73.3% and 91.6%).

Conclusions. Results of this subgroup analysis of PUNCH CD3-OLS suggest that RBL is safe and efficacious for the prevention of rCDI in participants with mildly to moderately immunocompromising conditions.

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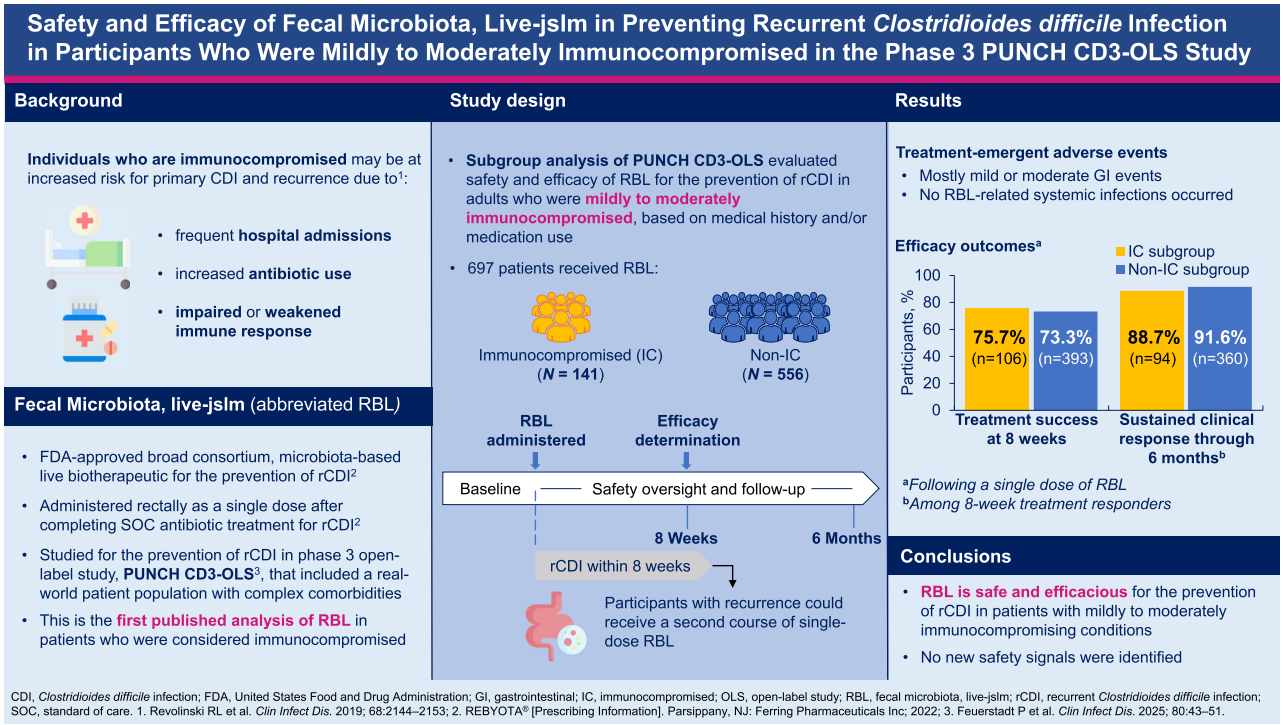
Graphical Abstract



Keywords. *Clostridioides difficile*; immunocompromised; live biotherapeutic; PUNCH CD3-OLS; RBX2660.

Clostridioides difficile infection (CDI) is the most common health care–associated infection in the United States [1], affecting approximately 500 000 Americans annually [2]. Recurrent CDI (rCDI) presents a major clinical burden. Approximately 15% to 30% of patients [3] treated for a primary CDI episode will experience recurrence, and this risk increases to 45% to 65% after 3 episodes [4–9]. In the United States, rCDI accounts for 75 000 to 175 000 cases of CDI annually [10]. Due to frequent interactions with health care systems, increased exposure to antibiotics, and additional risk conferred by compromised immune function, individuals who are immunocompromised (IC) may be at increased risk for primary CDI and recurrence [11, 12]. One retrospective cohort study found that having an immunocompromising condition doubled the risk of rCDI within 90 days after the primary episode [13].

Microbiota-based live biotherapeutics have shown efficacy in preventing rCDI and may help restore the composition and diversity of the gut microbiome [14]. Fecal microbiota, live-jslm (RBL; Rebyota, previously RBX2660), was approved by the US Food and Drug Administration (FDA) in November 2022 as the first single-dose, broad-consortia, microbiota-based live biotherapeutic for the prevention of rCDI in adults following standard-of-care antibiotic treatment [15]. No major safety signals were observed with RBL during clinical development [16]. However, a 2024 clinical practice guideline noted insufficient evidence to recommend RBL in adults with rCDI who are mildly or moderately IC and recommended against use of this therapeutic in patients who are severely IC, due to a potentially increased risk of infectious complications [17]. Outcomes in study participants who were IC and received RBL [14, 18–20] or other FDA-approved microbiota-based live biotherapeutics [21, 22] have not been reported and are of clinical interest.

PUNCH CD3-OLS (NCT03931941) is the largest study to date that investigated a microbiota-based live biotherapeutic for prevention of rCDI in a real-world patient population with complex comorbidities [23]. Here, safety and efficacy data are presented from an exploratory subgroup analysis of participants who were mildly to moderately IC in PUNCH CD3-OLS.

METHODS

Study Design

PUNCH CD3-OLS was a phase 3 single-arm study based on an open-label prospective design to evaluate the safety and tolerability of RBL in participants with rCDI [23]. Additional details on study design and RBL preparation are presented in the supplementary methods.

This study was conducted in the United States and Canada according to the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines, principles of

informed consent, and requirements of publicly registered clinical trials. All participants provided written informed consent, and the protocol received institutional review board/research ethics board approval before commencement.

Study Population

Participants enrolled in PUNCH CD3-OLS were aged ≥18 years with medical record documentation of rCDI, including first recurrence, or ≥2 episodes of severe CDI resulting in hospitalization. Participants had to have been undergoing a course of standard-of-care antibiotics for CDI diarrhea at the time of enrollment, and their diarrhea had to have been controlled, defined as <3 unformed/loose stools per day (Bristol Stool Scale type 6 or 7) for 2 consecutive days prior to the antibiotic washout period. Notable exclusion criteria were as follows: CD4 count <200 cells/mm^3 and/or absolute neutrophil count <1000 cells/μL at screening, receipt of fecal microbiota transplantation within the past 6 months, treatment with bezlotoxumab within the last year, and systemic antibiotic therapy for a condition other than CDI. Full inclusion/exclusion criteria have been published previously [23].

This subgroup analysis consists of participants who were considered IC based on medical history at the time of RBL administration and/or use of immunosuppressive medications for at least 2 weeks before RBL administration to 8 weeks after RBL administration. Medical conditions associated with impaired immune function were identified based on (1) the Centers for Disease Control and Prevention (CDC) guidelines for IC conditions (listed under CDC pneumococcal vaccination [24]: chronic renal failure, asplenia, HIV or HIV infection, congenital hemoglobinopathies, and immunodeficiency syndromes) and (2) medical conditions according to standardized MedDRA queries (Medical Dictionary for Regulatory Activities). End-stage renal disease was included as an immunocompromising condition [25] given its clinical similarity to "chronic renal failure." Authors of this subgroup analysis opted to also include participants with end-stage liver disease, as alterations in innate and adaptive immunity in this population can increase susceptibility to infections [26]. Immunocompromising medications were identified by Anatomical Therapeutic Chemical (ATC) classification. Corticosteroids ("glucocorticoids" or "corticosteroids for systemic use") were included and must have been taken for 5 consecutive days within the prespecified medication use window already described. A ≥20-mg/d dose of prednisone or prednisone equivalent was considered immunosuppressive. Prednisone-equivalent doses are listed in Supplementary Table 1. Noncorticosteroid immunocompromising medications were those with an ATC classification of "antineoplastic agents," "immunostimulants," or "immunosuppressants." Only systemic administration routes were included (oral, intramuscular, subcutaneous, or intravenous injection). Other medical conditions of interest that were not in CDC guideline criteria were identified per indication for qualifying immunosuppressive medication (eg, history of solid organ transplantation).

Study Outcomes

The primary end point for the overall PUNCH CD3-OLS study was the number of participants with RBL- or administration-related treatment-emergent adverse events (TEAEs) in the safety population. In this subgroup analysis, participants were classified as being IC or not (henceforth, non-IC). TEAEs were defined as any adverse event occurring on the same day of or after RBL administration. TEAEs were coded with MedDRA version 20.0. Safety data were censored at the time of on-study CDI recurrence (ie, treatment failure). The severity of TEAEs, relatedness of TEAEs, and number of select CDI-related TEAEs through 8 weeks after RBL administration were assessed: death, septic shock, toxic megacolon, colonic perforation, emergency colectomy, and intensive care unit admission. Infection monitoring was included as part of the comprehensive TEAE assessment.

Efficacy outcomes were treatment success and sustained clinical response. Treatment success was defined per the PUNCH CD3-OLS protocol as the absence of CDI diarrhea through 8 weeks after receiving RBL. Sustained clinical response was defined as treatment success of the presenting rCDI and no new episodes of CDI through 6 months after completing RBL treatment. Treatment failure (CDI recurrence) was defined as the presence of CDI diarrhea within 8 weeks of RBL administration. Recurrence was confirmed by a positive stool test result (C DIFF QUIK CHEK COMPLETE; Techlab, Inc) for *C difficile* glutamate dehydrogenase antigen and toxins A and B as determined by a central laboratory. If results were discordant (eg, glutamate dehydrogenase positive and toxin negative), polymerase chain reaction–based testing was performed. The site investigator determined the efficacy outcomes, which were then independently reviewed and confirmed by the Endpoint Adjudication Committee. In this subgroup analysis, efficacy outcomes were evaluated with consideration for select potentially clinically relevant variables (eg, number of immunocompromising medications).

Statistical Analysis

Descriptive statistics were reported for all outcomes. The safety analysis was performed on the safety population, defined as all participants who were exposed to RBL. The intent-to-treat population included all participants enrolled in the study. Efficacy analyses were performed on the modified intent-to-treat population, defined as all participants who received RBL but excluding those in whom administration was not completed and those who discontinued from the study prior to evaluation of treatment outcome.

RESULTS

Participants

Overall, 793 participants were enrolled. Of the 697 participants who received RBL, 141 (20.2%) were included in the IC subgroup and 556 (79.8%) in the non-IC subgroup. Within the IC subgroup, 82 participants (58.2%) were IC due to medications only, 41 (29.1%) had immunocompromising conditions only, and 18 (12.8%) were IC due to both immunocompromising medications and conditions. Most participants taking immunocompromising medications took 1 medication (n = 61, 61.0%), 23 (23.0%) took 2 medications, and 16 (16.0%) took ≥3 medications. According to the ATC classification, common systemic immunosuppressive medications included tumor necrosis factor α inhibitors, interleukin inhibitors, and purine analogs (Table 1). The most frequent indications for immunosuppressive medications were inflammatory bowel disease (n = 42), arthritis (n = 18), solid organ transplantation (n = 12), and cancer (n = 12). No participants had end-stage liver disease.

Most participants were female (58.9% vs 72.7%) and White (89.4% vs 95.0%), and approximately half (48.2% vs 48.6%) were aged ≥65 years (Table 2). Most participants had ≤3 previous CDI episodes (IC subgroup, 69.5%; non-IC subgroup, 64.7%), and most participants in the IC (90.8%, 128/141) and non-IC (83.5%, 464/556) subgroups had received vancomycin for the enrolling CDI episode. The median standard-of-care antibiotic course duration was 20.0 days for participants in the IC subgroup (IQR, 12.0–41.0) and the non-IC subgroup (IQR, 13.0–35.0).

Table 1. Immunocompromising Medications and Conditions: Safety Population (N = 141)

	Participants, No. (%)
Immunocompromising conditions[a]	59 (41.8)
Neoplasms: malignant and unspecified	42 (29.8)
Renal and urinary disorders	10 (7.1)
End-stage renal disease	8 (5.7)
Renal failure	2 (1.4)
Immune system disorders	6 (4.3)
HIV infection	2 (1.4)
Thalassemia	2 (1.4)
Concomitant immunosuppressive medications[a]	99 (70.2)
Selective immunosuppressants[b]	32 (22.7)
Other immunosuppressants[c]	29 (20.6)
Glucocorticoids	22 (15.6)
Tumor necrosis factor α inhibitors	17 (12.1)
Calcineurin inhibitors	12 (8.5)
Interleukin inhibitors	9 (6.4)
Purine analogs	4 (2.8)
Monoclonal antibodies	3 (2.1)
Protein kinase inhibitors	3 (2.1)
Anthracyclines and related substances	2 (1.4)
Interferons	2 (1.4)
Other antineoplastic agents	2 (1.4)
Platinum compounds	2 (1.4)
Pyrimidine analogs	2 (1.4)
Colony stimulating factors	1 (0.7)
Combination of antineoplastic agents	1 (0.7)
Nitrogen mustard analogs	1 (0.7)
Podophyllotoxin derivatives	1 (0.7)
Vinca alkaloids and analogs	1 (0.7)
Indication for immunosuppressive medications	
Inflammatory bowel disease[d]	42 (29.8)
Arthritis[e]	18 (12.8)
Solid organ transplantation[f]	12 (8.5)
Cancer[g]	12 (8.5)
Systemic lupus erythematosus[h]	4 (2.8)
Multiple sclerosis	2 (1.4)
Psoriasis	2 (1.4)
Scleroderma	2 (1.4)

Data are presented as No. (%) of participants unless noted otherwise.

Abbreviation: NOS, not otherwise specified.

[a]Participants could have ≥1 immunocompromising condition and be receiving multiple medications. Coded terms were used to identify and categorize participants with immunocompromising conditions.

[b]Vedolizumab (n = 14); leflunomide (n = 7); mycophenolate mofetil (n = 6); mycophenolate sodium (n = 2); sirolimus, tofacitinib, tofacitinib citrate, and upadacitinib (n = 1, each).

[c]Hydroxychloroquine (n = 9), methotrexate (n = 8), azathioprine (n = 6), hydroxychloroquine sulfate (n = 4), methotrexate sodium (n = 2), and lenalidomide (n = 1).

[d]Ulcerative colitis (n = 26), Crohn disease (n = 14), and NOS (n = 2).

[e]Rheumatoid arthritis (n = 15), psoriatic arthritis (n = 1), and NOS (n = 2).

[f]Kidney (n = 4), lung (n = 3), liver (n = 3), and NOS (n = 2).

[g]Adenocarcinoma (n = 2), leukemia (n = 2), lymphoma (n = 2), Ewing sarcoma (n = 2), and others (n = 1).

[h]Systemic lupus erythematosus, confirmed or suspected.

Safety

Within 8 weeks of RBL administration, TEAEs were reported by 44.7% (63/141) and 48.0% (267/556) of participants in the IC and non-IC subgroups, respectively (Table 3). TEAE rates were also similar in the IC (26.2%) and non-IC (22.1%) subgroups in the 8-week to 6-month period following RBL administration. The incidence of TEAEs in the IC subgroup taking 1, 2, or ≥3 immunocompromising medications was 56.9%, 70.4%, and 57.1%. In the IC and non-IC subgroups, most participants had TEAEs that were mild (18.4% and 20.9%) or moderate (19.9% and 19.8%). The most common TEAEs in both groups were gastrointestinal, including diarrhea, abdominal pain, and nausea. Most TEAEs in both groups were deemed related to a preexisting condition (IC, 18.4%; non-IC, 24.3%).

Within 8 weeks of RBL administration, serious TEAEs were reported by 4.3% (6/141) of participants in the IC subgroup and 3.8% (21/556) in the non-IC subgroup (Table 3). Between 8 weeks and 6 months of follow-up, serious TEAEs were reported by 7.1% (10/141) of participants in the IC subgroup and 2.7% (15/556) in the non-IC subgroup. No trends were observed in the types of serious TEAEs indicated. In the IC and non-IC subgroups, most serious TEAEs were related to a preexisting condition (8 weeks, 2.1% and 2.9%, respectively; 8 weeks to 6 months, 4.3% and 2.2%). One serious TEAE of ulcerative colitis flare was deemed definitely related to a preexisting

Table 2. Participant Demographics and Baseline Characteristics: Safety Population

	IC (n = 141)	Non-IC (n = 556)
Age, y		
Median (IQR)	64.0 (46.0–75.0)	64.0 (49.5–74.0)
<65	73 (51.8)	286 (51.4)
≥65	68 (48.2)	270 (48.6)
Sex: female	83 (58.9)	404 (72.7)
Race		
American Indian or Alaska Native	0	1 (0.2)
Asian	4 (2.8)	3 (0.5)
Black or African American	9 (6.4)	12 (2.2)
Multiple	0	4 (0.7)
Other	2 (1.4)	8 (1.4)
White	126 (89.4)	528 (95.0)
Ethnicity		
Hispanic or Latino	6 (4.3)	9 (1.6)
Not Hispanic or Latino	132 (93.6)	538 (96.8)
Not reported or unknown	3 (2.1)	9 (1.6)
No. of previous CDI episodes, mean (SD)		
≤3	98 (69.5)	360 (64.7)
>3	43 (30.5)	196 (35.3)
Diagnostic test results for most recent CDI episode[a]		
PCR positive	82 (58.2)	350 (62.9)
EIA positive	13 (9.2)	62 (11.2)
GDH positive	9 (6.4)	41 (7.4)
Toxins A and B positive	9 (6.4)	32 (5.8)
Other positive[b]	3 (2.1)	21 (3.8)
Charlson Comorbidity Index, median (IQR)	4.0 (1.0–7.0)	3.0 (1.0–4.0)
Enrolling CDI antibiotic		
Vancomycin	128 (90.8)	464 (83.5)
Fidaxomicin	12 (8.5)	86 (15.5)
Rifaximin	1 (0.7)	6 (1.1)
Enrolling CDI antibiotic duration		
Mean (SD)	33.2 (42.0)	31.2 (38.7)
Median (IQR)	20.0 (12.0–41.0)	20.0 (13.0–35.0)
≤14 d	51 (36.2)	182 (32.7)
>14 d	90 (63.8)	374 (67.3)

Data are presented as No. (%) of participants unless noted otherwise.

Abbreviations: CDI, *Clostridioides difficile* infection; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; IC, immunocompromised; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction.

[a]Enrolling tests could be used in combination and are not mutually exclusive. CDI diagnosis was determined by the treating physician; a positive test result was not protocol specified.

[b]Included a positive LAMP test result.

condition (underlying severe ulcerative colitis) and possibly related to RBL. The event was reviewed by the independent data safety monitoring board and determined not to be a product-related safety concern. No TEAEs leading to death occurred in the IC subgroup. No deaths (n = 4) or study withdrawals (n = 5) in the non-IC subgroup were related to RBL.

Infections were reported by 9.2% (13/141) of participants in the IC subgroup and 10.4% (58/556) in the non-IC subgroup within 8 weeks of RBL administration and by 11.3% (16/141) in the IC subgroup and 9.4% (52/556) in the non-IC subgroup between 8 weeks and 6 months of follow-up (Supplementary Table 2). None of the infections in the IC subgroup were assessed as being related to RBL or its administration. No cases of bacteremia or fungemia were noted in the IC subgroup. One case of bacteremia that was considered unrelated to RBL was identified in the non-IC subgroup.

Efficacy

In the modified intent-to-treat population (n = 676), treatment success was achieved in 75.7% (106/140) of participants in the IC subgroup and 73.3% (393/536) in the non-IC subgroup (Figure 1). In participants with treatment success, sustained clinical response through 6 months was maintained in 88.7% (94/106) and 91.6% (360/393) of those in the IC and non-IC subgroups, respectively. Efficacy outcomes were comparable across select subgroups: by number of previous CDI episodes, CDI antibiotic duration, number of immunocompromising medications, and type of immunocompromising medication (Supplementary Figure 1).

Second Course of RBL

Of the 31 and 120 participants who met criteria for treatment failure in the IC and non-IC subgroups, respectively, 23 in the IC subgroup and 98 in the non-IC subgroup opted to receive a second course of RBL within 21 days of treatment failure determination. In sum, 16.3% (23/141) of participants in the IC subgroup and 17.6% (98/556) in the non-IC subgroup received 2 courses of single-dose RBL.

The incidences of TEAEs following a second course of RBL were comparable between the IC and non-IC subgroups and were similar to those following a single course of RBL (Supplementary Table 3). No new safety signals were observed.

Table 3. TEAEs Within 8 Weeks and Between 8 Weeks and 6 Months of RBL Administration: Safety Population

	Within 8 wk (n = 697)		8 wk to 6 mo (n = 697)	
	IC (n = 141)	Non-IC (n = 556)	IC (n = 141)	Non-IC (n = 556)
Any TEAE	63 (44.7)	267 (48.0)	37 (26.2)	123 (22.1)
No. of events	137	657	74	257
TEAE by maximum severity				
Mild	26 (18.4)	116 (20.9)	7 (5.0)	44 (7.9)
No. of events	73	383	19	122

Table 3. Continued

	Within 8 wk (n = 697)		8 wk to 6 mo (n = 697)	
	IC (n = 141)	Non-IC (n = 556)	IC (n = 141)	Non-IC (n = 556)
Moderate	28 (19.9)	110 (19.8)	21 (14.9)	58 (10.4)
No. of events	51	210	39	104
Severe	9 (6.4)	37 (6.7)	8 (5.7)	18 (3.2)
No. of events	13	60	15	26
Potentially life-threatening	0	4 (0.7)	1 (0.7)	3 (0.5)
No. of events		4	1	5
Most frequent TEAE by system organ class and preferred term[a]				
Gastrointestinal disorder	36 (25.5)	169 (30.4)	8 (5.7)	43 (7.7)
No. of events	71	340	15	67
Diarrhea	15 (10.6)	70 (12.6)	4 (2.8)	15 (2.7)
No. of events	17	78	4	17
Abdominal pain	15 (10.6)	55 (9.9)	1 (0.7)	9 (1.6)
No. of events	16	62	1	9
Nausea	8 (5.7)	33 (5.9)	1 (0.7)	4 (0.7)
No. of events	8	36	1	4
Abdominal distension	7 (5.0)	31 (5.6)	1 (0.7)	1 (0.2)
No. of events	8	38	1	1
General disorders and administration site conditions	10 (7.1)	26 (4.7)	0	8 (1.4)
No. of events	13	31		10
Infections[b]	13 (9.2)	58 (10.4)	16 (11.3)	52 (9.4)
No. of events	15	63	18	68
Investigations[c]	4 (2.8)	31 (5.6)	0	5 (0.9)
No. of events	4	45		5
TEAEs by relatedness[d]				
Related to RBL	18 (12.8)	88 (15.8)	0	4 (0.7)
No. of events	36	162		4
Related to administration procedure	10 (7.1)	50 (9.0)	0	1 (0.2)
No. of events	13	83		2
Related to CDI	11 (7.8)	55 (9.9)	3 (2.1)	9 (1.6)
No. of events	16	85	4	13
Related to a preexisting condition	26 (18.4)	135 (24.3)	19 (13.5)	49 (8.8)
No. of events	41	252	32	77
Any serious TEAE	6 (4.3)	21 (3.8)	10 (7.1)	15 (2.7)
No. of events	7	28	13	24
Serious TEAEs by relatedness[d]				
Related to RBL	1 (0.7)[e]	0	0	0
No. of events	1			
Related to administration procedure	0	0	0	0
Related to CDI	0	1 (0.2)	0	1 (0.2)
No. of events		1		1
Related to a preexisting condition	3 (2.1)	16 (2.9)	6 (4.3)	12 (2.2)
No. of events	3	16	6	12
TEAEs leading to withdrawal from study	0	4 (0.7)	0	1 (0.2)
No. of events		4		1
TEAEs leading to death[f]	0	3 (0.5)	0	1 (0.2)
No. of events		3		1

Data are presented as No. (%) of participants unless noted otherwise. Coding was based on MedDRA version 20.0.

Abbreviations: CDI, *Clostridioides difficile* infection; IC, immunocompromised; MedDRA, Medical Dictionary for Regulatory Activities; RBL, fecal microbiota, live-jslm; TEAE, treatment-emergent adverse event.

[a]TEAEs were reported where participants with or without immunocompromising conditions had an incidence ≥5%.

[b]Preferred terms within the infections system organ class are listed in Supplementary Table 2.

[c]Investigations are diagnostics (eg, colon biopsy).

[d]Relatedness categories are not mutually exclusive.

[e]Flare of preexisting ulcerative colitis; this event was assessed as being definitely related to a preexisting condition and possibly related to RBL.

[f]Deaths were due to cardiac arrest (n = 1), cardiac failure (n = 1), spina bifida (n = 1), and pulmonary sepsis (n = 1). None of the deaths were related to RBL or its administration.

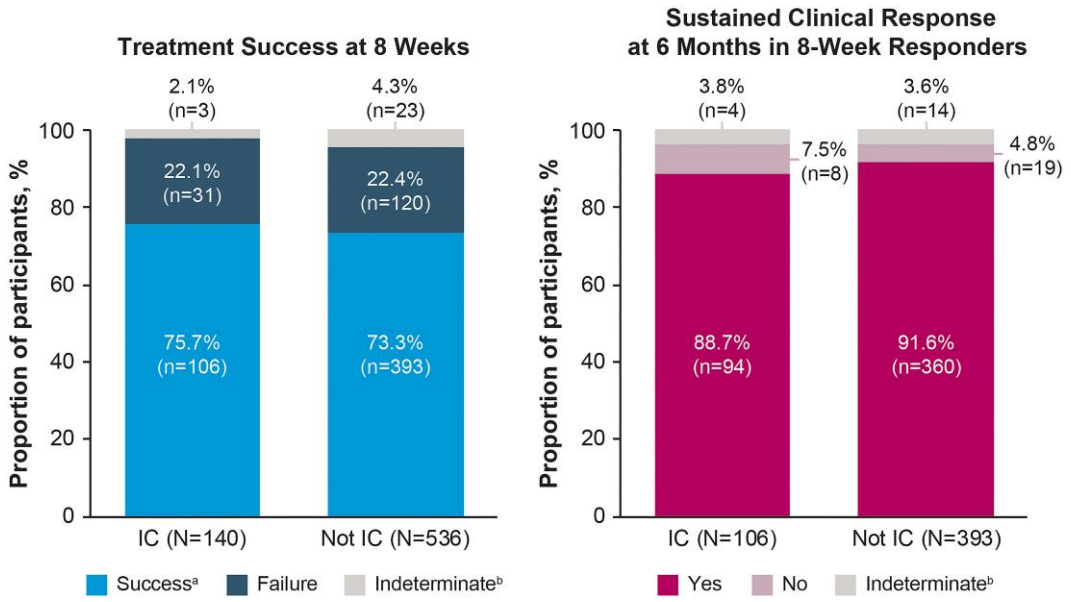


Figure 1. Treatment success within 8 weeks and sustained clinical response within 6 months of RBL administration (mITT population). Only participants with adjudicated treatment outcomes are included. [a]Treatment success is defined as the absence of CDI diarrhea through 8 weeks after RBL administration. [b]Efficacy outcome was indeterminate if CDI test was inconclusive or missed at time of visit. Abbreviations: CDI, *Clostridioides difficile* infection; IC, immunocompromised; RBL, fecal microbiota, live-jslm; mITT, modified intent to treat.

Following a second course of RBL, 39.1% of participants in the IC subgroup and 59.2% in the non-IC subgroup achieved treatment success (Supplementary Table 4). Of the participants who achieved treatment success following a second course of RBL, 88.9% and 87.9% of the IC and non-IC subgroups, respectively, had a sustained clinical response at 6 months. Overall, 82.1% (115/140) of participants in the IC subgroup and 84.1% (451/536) in the non-IC subgroup achieved treatment success, with either 1 or 2 RBL administrations.

DISCUSSION

To date, PUNCH CD3-OLS is the largest phase 3 study evaluating the safety and efficacy of a microbiota-based live biotherapeutic in a real-world population [23]. This study was the first in the RBL clinical development program to include participants with mildly to moderately immunocompromising conditions and/or those requiring immunosuppressive medication use, as well as those with more complex medical conditions. This subgroup analysis is the first published report of outcomes for the 141 participants who were mildly to moderately IC and received RBL in PUNCH CD3-OLS, including those with a history of solid organ transplantation, inflammatory bowel disease, arthritis, and cancer. This analysis provides important data regarding the safety and efficacy of RBL for rCDI in patients who are mildly to moderately IC, which can assist clinical decision making in real-world care settings.

This analysis found the safety and efficacy of RBL in patients who were mildly to moderately IC to be comparable to the overall study population [23]. Most participants in the IC and non-IC subgroups received 1 course of single-dose RBL, consistent with FDA-approved dosing. Most TEAEs were mild or moderate, gastrointestinal, and assessed as related to preexisting conditions or CDI. No trends were observed among serious TEAEs to suggest a new safety signal. In PUNCH CD3-OLS, only 1 serious TEAE was assessed as being possibly related to RBL among use in nearly 700 participants. No deaths in this study or across the development program have been assessed as being related to RBL [16]. Overall, safety results in this analysis are consistent with those from previous studies in the RBL clinical trial program [16]. Efficacy outcomes were comparable in the IC and non-IC subgroups and consistent with those reported for the overall PUNCH CD3-OLS population; outcomes were also comparable to those of PUNCH CD3, which excluded participants with immunocompromising conditions [14]. Efficacy outcomes were comparable across select subgroups, including those based on the number of previous CDI episodes, CDI antibiotic duration, and number and type of immunocompromising medications.

Increased risk of infection is often of clinical concern when a microbiota-based product is administered to a patient who is IC. A published report of bacteremia in 2019 associated with investigational fecal microbiota transplantation raised concern around the transmission of infections from donor stool to IC hosts [27], leading to an FDA-issued safety alert. American Gastroenterological Association clinical practice guidelines on fecal microbiota–based therapies published in early 2024

note that FDA-approved microbiota-based live biotherapeutics may confer a similar risk of infection transmission [17]. These guidelines do not make a recommendation for FDA-approved microbiota-based live biotherapeutics for CDI in patients who are IC, noting limited data availability by the review end date of March 2023 [17]. Topline results from PUNCH CD3-OLS were published in 2024 [23], with subgroup analyses reported subsequently. In this analysis, no infections in the IC subgroup were considered to be related to RBL or its administration per the treating clinician, study medical monitor, and study data safety monitoring board. There were no noted instances of bacteremia or fungemia in these participants at higher risk. RBL is manufactured following good manufacturing practice standards, and each healthy donor–derived dose undergoes standardized screening procedures that have been reviewed by the FDA as part of the drug approval process. No additional TEAEs were observed with 2 vs 1 course of single-dose RBL. Findings collectively suggest that RBL is safe in patients who are mildly to moderately IC. Studies in real-world settings outside a clinical trial will help validate these results.

This subgroup analysis from a single-arm, phase 3, open-label study has limitations—specifically, the absence of placebo and potential bias due to unblinding. Other design aspects should be considered to interpret the results. The definition of mildly to moderately immunocompromising conditions used to classify participants may not completely align with clinical definitions. For instance, the inclusion of participants with a history of neoplasms, regardless of prior treatment or current disease status, may have led to inclusion of individuals not typically considered IC. Patients who were severely IC were not enrolled in PUNCH CD3-OLS per exclusion criteria (eg, those with neutropenia); therefore, efficacy and safety findings in this subgroup analysis are reflective of a mildly to moderately IC population. The degree of immunosuppression was not investigated in this study. Additionally, outcomes for specific immunocompromising conditions or immunosuppressants were not evaluated, with the exception of steroids vs nonsteroids, due to small samples. Other studies suggest that CDI risk may vary by immunosuppressive medication class [28]. The number of prescribed immunosuppressants was characterized to investigate any potential outcome signals, though no trends were clearly observed.

Patients were also excluded from PUNCH CD3-OLS if they were receiving non-CDI antibiotics, which may be given as infection prophylaxis for patients with immunocompromising conditions or in the setting of immunocompromising medication use. Clinicians should therefore use clinical judgment when considering the appropriateness of administering RBL to a patient with frequent or long-term antibiotic use. A small subgroup analysis of PUNCH CD2 open-label study participants found that systemic antibiotic use after administration of RBL often did not result in CDI recurrence; however, that trial excluded participants who were IC [29]. With respect to CDI antibiotics, it is worth noting that many participants in this subgroup analysis are assumed to have received a vancomycin tapered/pulsed regimen, as inferred by the reported duration of treatment. Finally, CDI diagnostics used for enrollment into this study are somewhat challenging to interpret (eg, unclear use of multistep testing vs polymerase chain reaction alone). Additional details surrounding CDI testing were not evaluable in this population. Previous publications have highlighted diagnostic testing challenges in IC patients due to high rates of non–CDI-associated diarrhea and a potentially altered innate immune response to CDI [30]. While testing details would help provide additional context to our study findings, the relatively high efficacy rates suggest that RBL can confer clinical benefit to help prevent rCDI in patients with or without compromised immune systems.

CONCLUSIONS

The results of this subgroup analysis from a large phase 3 open-label study suggest that RBL can be used safely and is efficacious in preventing rCDI in participants who are mildly to moderately IC. There were no new safety signals in these patients with potentially higher risk.

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

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Data availability. Ferring will provide access to individual deidentified participant data, upon request via a secure portal, to researchers whose proposals meet the research criteria and other conditions. To gain access, data requestors must enter into a data access agreement with Ferring.

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