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MAJOR ARTICLE

Microbiota-Based Live Biotherapeutic RBX2660 for the Reduction of Recurrent *Clostridioides difficile* Infection in Older Adults With Underlying Comorbidities

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Background. Advanced age and underlying comorbidities are associated with greater rates of recurrence in patients with *Clostridioides difficile* infection (CDI). Reducing the likelihood of recurrence through treatment with an antimicrobial followed by a microbiota replacement therapy can decrease the burden of this infection and improve patient outcomes. We report the efficacy and safety of RBX2660, a microbiota-based live biotherapeutic, in older adults with recurrent CDI, grouped by comorbidities.

Methods. In this post hoc subgroup analysis of the PUNCH CD3 trial, we assessed outcomes in older adults (age ≥65 years) grouped by Charlson Comorbidity Index severity scores at screening (moderate [3–4] and severe [≥5]) and by the presence of underlying cardiac, renal, or gastrointestinal disorders.

Results. RBX2660 treatment success rates in older adults with comorbidities were consistent across subgroups and similar to those in the total RBX2660-treated population. A greater percentage of RBX2660-treated older adults remained free of CDI recurrence through 8 weeks following treatment compared with placebo-treated participants in all but 2 subgroups assessed. Across all subgroups, most treatment-emergent adverse events (TEAEs) were mild or moderate in severity and related to a preexisting condition. None of the serious or life-threatening TEAEs that occurred were related to RBX2660 or its administration. Occurrence of TEAEs did not cluster in any subgroup.

Conclusions. RBX2660 is efficacious and safe in older adults with recurrent CDI and underlying comorbidities.

Keywords. *Clostridioides difficile*; *Clostridium difficile*; live biotherapeutic product; microbiome restoration; microbiome-based therapeutic.

Disruptions to gut microbiota composition and diversity can result in microbiome disruption, termed dysbiosis, and subsequent intestinal proliferation of opportunistic pathogens such as *Clostridioides difficile* [1, 2]. *Clostridioides difficile*, a gram-positive, spore-forming anaerobic bacterium, is the most common cause of healthcare-associated infections in the United States [3–5]. Standard-of-care antibiotics used to treat *C difficile* infection (CDI) may further exacerbate gut dysbiosis, leading to future recurrent CDI episodes. Up to 35% of patients with an initial CDI episode and as much as 65% of patients with 2 or more CDI episodes are estimated to experience subsequent recurrence [6–10].

Recurrent CDI (rCDI) is a serious and potentially fatal illness that places a profound burden on patients and the healthcare system, with an estimated 30-day mortality rate of 9% in people ≥65 years of age diagnosed with healthcare-associated CDI, and an estimated annual cost of $2.8 billion in the United States [11, 12]. Older age and comorbidities, including cardiac, renal, and gastrointestinal (GI) disorders, may increase the risk of recurrence, morbidity, and mortality in patients with rCDI [13, 14].

Current guideline-recommended standard-of-care antibiotic treatment for CDI and rCDI, particularly vancomycin, is able to control the vegetative phase of infection but does not affect spores and can worsen a patient's underlying dysbiosis, leaving them vulnerable to rCDI [13, 15, 16]. This vicious cycle makes rCDI treatment especially challenging and can be further complicated in patients with underlying comorbidities. CDI-related complications and mortality rates increase with each subsequent infection [17, 18]. Innovative treatments to

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address dysbiosis and reduce rCDI are needed to improve the impact of this infection—to decrease morbidity and mortality rates, reduce economic burden on patients and the healthcare system, and, most importantly, improve patients' quality of life.

Microbiome-based therapeutics are being actively developed to restore the gut microbiome and reduce rCDI. RBX2660 is a microbiota-based live biotherapeutic product developed to reduce CDI recurrence following antibiotic treatment for rCDI as early as first recurrence. RBX2660 is supplied as a single-dose, rectally administered microbiota suspension that contains a broad consortium of spore-forming and non-spore-forming bacteria, including Bacteroidetes and Firmicutes. RBX2660 is developed through a proprietary standardized manufacturing process that adheres to Good Manufacturing Practice and undergoes standardized rigorous screening procedures including pathogen testing to ensure patient safety as previously described [19–21].

Medically complex patients are often encountered in medical practice and are very commonly affected by both initial and rCDI. Here, we report a post hoc subgroup analysis of the PUNCH CD3 [21] phase 3 trial to demonstrate the efficacy and safety of RBX2660 in older adults with rCDI and comorbidities that may increase their risk for recurrence and mortality.

METHODS

Study Design

This is a post hoc subgroup analysis of the PUNCH CD3 trial (NCT03244644), a prospective, multicenter, randomized, double-blind, placebo-controlled phase 3 study to evaluate the efficacy and safety of RBX2660 for the prevention of rCDI in participants ≥18 years old with documentation of rCDI per the study definition, including either (*i*) at least 1 recurrence after a primary episode and had completed at least 1 round of standard-of-care oral antibiotic therapy or (*ii*) had at least 2 episodes of severe CDI resulting in hospitalization within the last year [21]. A positive stool test for the presence of toxigenic *C difficile* within 30 days before enrollment was required. *Clostridioides difficile* laboratory testing according to the site's standard of care was acceptable for enrollment, and toxin testing was not required. Polymerase chain reaction (PCR) was the predominant standard-of-care diagnostic test at the time the study was conducted, and >70% of PUNCH CD3 participants were confirmed by PCR. Patients with a history of inflammatory bowel disease (IBD; eg, ulcerative colitis, Crohn's disease, or microscopic colitis) or diagnosis of irritable bowel syndrome (IBS) as determined by Rome III criteria were excluded from PUNCH CD3. Following completion of standard-of-care antibiotic therapy, participants were randomized to receive 1 blinded dose of RBX2660 or placebo in a 2:1 ratio.

The study design included an optional retreatment for patients who experienced a CDI recurrence after blinded therapy. The open-label RBX2660 (OL RBX2660) treatment was administered within 21 calendar days of failure determination. If a participant received OL RBX2660 treatment, the follow-up requirements started over from the day of OL RBX2660 treatment administration according to the same schedule as required for the blinded portion of the study.

PUNCH CD3 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. The protocol received Institutional Review Board approval before its commencement and was conducted under a Food and Drug Administration Investigational New Drug application.

Outcomes

The primary efficacy outcome was assessed in the modified intent-to-treat (mITT) population. The mITT population was defined as all randomized participants who successfully received blinded treatment but excluded participants who withdrew before treatment and participants who discontinued from the study before evaluation of treatment success/failure if the reason for exit was unrelated to CDI symptoms.

Treatment success was defined as the absence of CDI diarrhea through 8 weeks after completing blinded treatment. CDI diarrhea was defined as the passage of ≥3 unformed/loose stools (ie, Bristol Stool Scale type 6–7) in 24 or fewer consecutive hours for at least 2 consecutive days, and positive stool test for the presence of *C difficile* toxin documented at the time of the diarrhea. For the determination of study failures, a central laboratory was used to perform the C. DIFF QUIK CHEK COMPLETE test, a rapid enzyme immunoassay for glutamate dehydrogenase and toxins A and B. Treatment outcomes were based on endpoint adjudication committee determination. If treatment outcome was indeterminate, it was counted as treatment failure (nonsuccess) for analysis purposes.

Treatment-emergent adverse events (TEAEs) were summarized for the double-blind treatment period within 8 weeks and censored at treatment failure. The safety population included all participants for whom treatment was attempted.

Subgroup Analysis

Outcomes were assessed in older adults categorized by baseline Charlson Comorbidity Index (CCI) scores and by the presence of cardiac, renal, or GI disorders.

Older adults (65–74 years and ≥75 years of age) were categorized using the age-adjusted CCI, a commonly used index that incorporates a subset of 16 conditions and participant age to estimate 10-year survival based on comorbidity burden [22, 23]. First published in 1987, the CCI was designed to classify comorbid conditions that may increase the risk of mortality for use in longitudinal studies, and after widespread use is now

often considered the gold-standard measure of comorbidity burden in clinical research [23, 24].

The index weighs the following age groups and medical conditions to calculate a score [25]: age (50–59 years: 1 point; 60–69 years: 2 points; 70–79 years: 3 points; ≥80 years: 4 points); myocardial infarction (1 point); congestive heart failure (1 point); peripheral vascular disease (1 point); cerebrovascular accident or transient ischemic attack (1 point); dementia (1 point); chronic obstructive pulmonary disease (1 point); connective tissue damage (1 point); peptic ulcer disease (1 point); liver disease (mild: 1 point; moderate to severe: 3 points); diabetes mellitus (uncomplicated: 1 point; end-organ damage: 2 points); hemiplegia (2 points); moderate to severe chronic kidney disease (CKD) (2 points); solid tumor (localized: 2 points; metastatic: 6 points); leukemia (2 points); lymphoma (2 points); and AIDS (6 points) (Supplementary Table 1). Older adult participants were grouped according to total CCI scores of 3 to 4 (moderate) or ≥5 (severe), as calculated at screening for study entrance. Based on the CCI, the estimated 10-year survival of individuals with a total CCI score of 3 or 4 points is 77% and 53%, respectively; estimated 10-year survival decreases to 21% for individuals with CCI of 5 points and drops to 0% for individuals with CCI ≥7 points.

Older adults with cardiac, renal, or GI disorders were identified by searching preferred terms in the medical history at screening based on Medical Dictionary for Regulatory Activities, version 20.0. The top 3 preferred terms for each category of cardiac disorders, CKD, and GI disorders are shown in Table 1 (see Supplementary Table 2 for the full list of medical conditions).

Safety Oversight and Data Monitoring

To ensure the safety and well-being of participants throughout the study, an independent data and safety monitoring board assessed if there was probable cause that RBX2660 or the enema procedure contributed to a new intestinal infection in the stool of any participant or if any events of major significance (ie, death or other serious outcome) causally and plausibly connected with RBX2660 was in excess in 1 of the study arms.

Statistical Analysis

The primary outcome of PUNCH CD3 used a Bayesian analysis that integrated data from the previous phase 2b trial, PUNCH CD2 [21]. For this subgroup analysis, observed treatment success rates from PUNCH CD3 are reported (Bayesian statistics were not applied). The difference in treatment success rates between RBX2660 and placebo in each subgroup is presented in a forest plot with the 95% confidence interval (CI) calculated by an exact method [26].

Participant Consent

All enrolled participants provided informed consent before randomization and treatment.

RESULTS

PUNCH CD3 Outcomes

Of the 289 participants randomly assigned in PUNCH CD3, 267 were treated with blinded RBX2660 (n = 180) or placebo (n = 87). Bayesian hierarchical model estimates of treatment success rates in the mITT population were 70.6% with RBX2660 versus 57.5% with placebo, with a statistically significant posterior probability of superiority of 0.991 [21]. For participants who achieved treatment success at 8 weeks, most had a sustained clinical response through 6 months (RBX2660: 92.1% [n = 116/126]; placebo: 90.6% [n = 48/53]) [21]. Overall, RBX2660 was well tolerated with expected and manageable adverse events (AEs). These results have been previously published [21].

Demographics of Older Participants With Underlying Comorbidities

Among participants aged 65–74 years, 27 (placebo: n = 8; RBX2660: n = 19) had moderate CCI scores and 34 (placebo: n = 12; RBX2660: n = 22) had severe CCI scores (Supplementary Table 3). Among participants ≥75 years old, 10 (placebo: n = 2; RBX2660: n = 8) had moderate CCI scores and 41 (placebo: n

Table 1. Top 3 Medical Conditions in Older Adults With Cardiac Disorders, Chronic Kidney Disease, or Gastrointestinal Disorders

Cardiac Disorder Preferred Terms	Placebo, No.	RBX2660, No.	CKD Preferred Terms	Placebo, No.	RBX2660, No.	GI Disorder Preferred Terms	Placebo, No.	RBX2660, No.
Atrial fibrillation	5	12	CKD	4	17	Gastroesophageal reflux disease	11	37
Congestive heart failure	4	11	Renal failure	0	2	Diverticulum[a]	5	13
Coronary artery disease	4	10	Hemodialysis	1	1	Hemorrhoids[b]	5	8

Participants with cardiac disorders, CKD, or GI disorders were identified by searching preferred terms in the medical history at screening based on Medical Dictionary for Regulatory Activities (MedDRA), version 20.0.

Abbreviations: CKD, chronic kidney disease; GI, gastrointestinal.

[a]MedDRA preferred term representing diverticulosis.

[b]Either internal or external.

= 8; RBX2660: n = 33) had severe CCI scores. A total of 50 participants ≥65 years old (placebo: n = 14; RBX2660: n = 36) had a cardiac disorder, 23 (placebo: n = 4; RBX2660: n = 19) had CKD, and 73 (placebo: n = 18; RBX2660: n = 55) had a GI disorder (Supplementary Table 4). Across all subgroups, older participants were mostly White females who had experienced at least 3 CDI episodes (ie, at least 2 recurrences) before RBX2660 treatment.

Treatment Success in Older Participants With Underlying Comorbidities

In the total population, RBX2660 treatment was successful across a range of participant ages and comorbidity burdens (Figure 1). RBX2660 treatment success in older adults aged 65–74 years with moderate or severe CCI scores was consistent with the total RBX2660-treated population (treatment success: all participants: 71% [n = 126/177]; moderate CCI: 74% [n = 14/19]; severe CCI: 73% [n = 16/22]), and a greater percentage of RBX2660-treated participants remained CDI-free through 8 weeks following treatment compared with placebo-treated participants in these subgroups (Figure 2). Among RBX2660-treated participants ≥75 years old, 50% (n = 4/8) with moderate CCI scores and 64% (n = 21/33) with severe CCI scores achieved treatment success. RBX2660 treatment success in older adults aged ≥65 years with cardiac disorders (69% [n = 25/36]), CKD (68% [n = 13/19]), and GI disorders (67% [n = 37/55]) was similar to the total RBX2660-treated population (Figure 3).

Following confirmed treatment failure, 65 participants in the total population received OL RBX2660, of whom 37 (57%) achieved treatment success within 8 weeks [21]. OL RBX2660 was efficacious in older adults with underlying comorbidities who experienced CDI recurrence after blinded treatment (Supplementary Table 5). Treatment success was achieved by 50% (blinded placebo + OL RBX2660: n = 3/7; blinded RBX2660 + OL RBX2660: n = 5/9), 50% (blinded placebo + OL RBX2660: n = 0/1; blinded RBX2660 + OL RBX2660: n = 3/5), and 55% (blinded placebo + OL RBX2660: n = 4/7; blinded RBX2660 + OL RBX2660: n = 7/13) of older adults ≥65 years of age with cardiac disorders, CKD, and GI disorders, respectively, after their second treatment course.

Safety Outcomes

In the total safety population, 87 participants received placebo and 180 participants received RBX2660 (Table 2). TEAEs were reported by 52% (n = 94/180) of RBX2660-treated participants compared with 44% (n = 38/87) of placebo-treated participants. The increased incidence of TEAEs after RBX2660 treatment was largely due to mild events by maximum severity: 40% of RBX2660-treated participants experienced mild events by maximum severity compared with 30% of placebo-treated

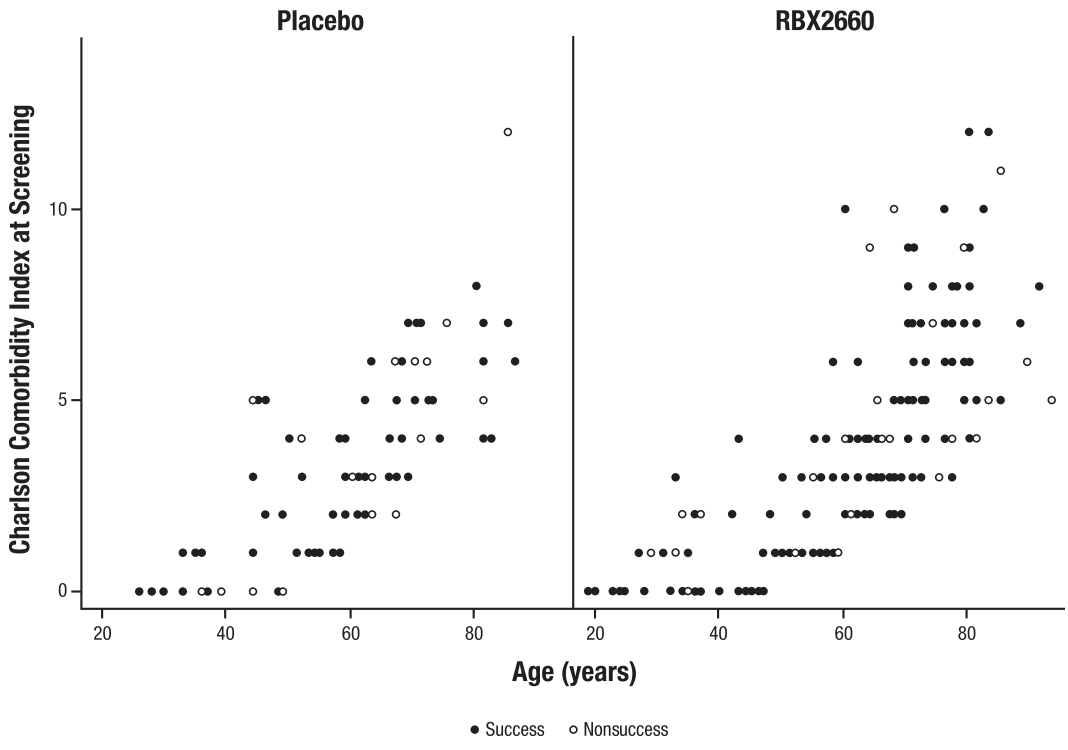
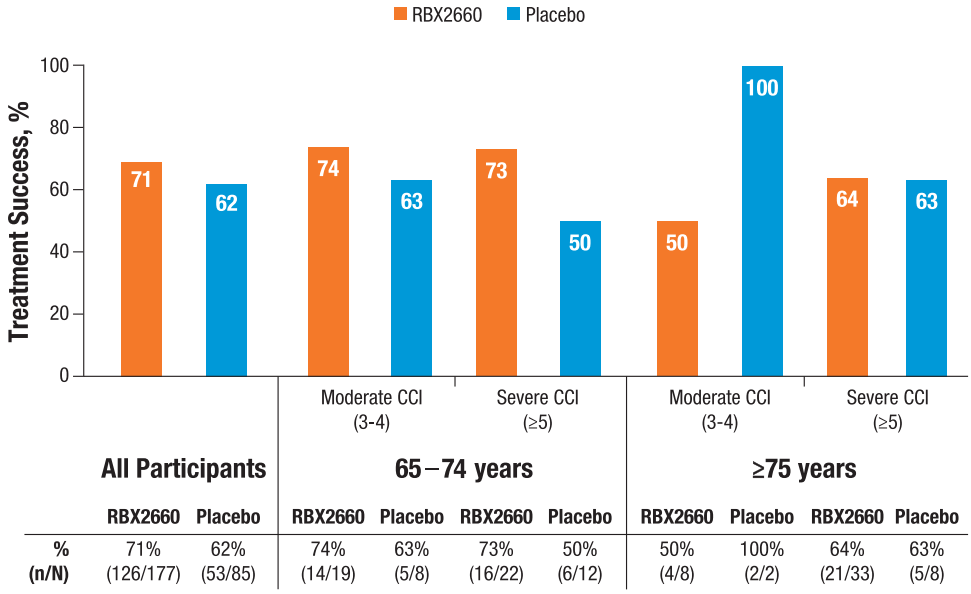


Figure 1. Summary of treatment success in participants with recurrent *Clostridioides difficile* infection by age and baseline Charlson Comorbidity Index (CCI) score (modified intent-to-treat population). PUNCH CD3 participants with treatment success or treatment failure at week 8 are plotted according to age and baseline CCI score. Each circle represents a unique participant. Nonsuccess includes treatment failures and indeterminants.



	All Participants		65–74 years				≥75 years			
	RBX2660	Placebo	RBX2660	Placebo	RBX2660	Placebo	RBX2660	Placebo	RBX2660	Placebo
% (n/N)	71% (126/177)	62% (53/85)	74% (14/19)	63% (5/8)	73% (16/22)	50% (6/12)	50% (4/8)	100% (2/2)	64% (21/33)	63% (5/8)

Figure 2. Summary of treatment success in older adults with recurrent *Clostridioides difficile* infection categorized by baseline Charlson Comorbidity Index (CCI) score (modified intent-to-treat population). The percentage of RBX2660-treated and placebo-treated participants achieving treatment success at week 8 are shown for the total population and across age and CCI score subgroups. The low number of participants in some subgroups (eg, ≥75 years old with moderate CCI) may limit data interpretation.

participants [21]. Through 6 months of follow-up of blinded treatment, only 1 RBX2660-treated participant discontinued because of TEAEs [21]. Serious adverse events (SAEs) were infrequent and reported in a similar percentage of placebo- and RBX2660-treated participants. The reported SAEs are summarized in Supplementary Table 6. No reported events of major complication (eg, death, toxic megacolon, or colonic perforation) from the presenting CDI episode were reported after treatment with RBX2660 or placebo [21].

When grouped by CCI scores, 32% to 70% of RBX2660-treated older adults with comorbidities had TEAEs (65–74 years old with moderate CCI: 32% [n = 6/19]; 65–74 years old with severe CCI: 70% [n = 16/23]; ≥75 years old with moderate CCI: 50% [n = 4/8]; ≥75 years old with severe CCI: 50% [n = 17/34]) (Table 2).

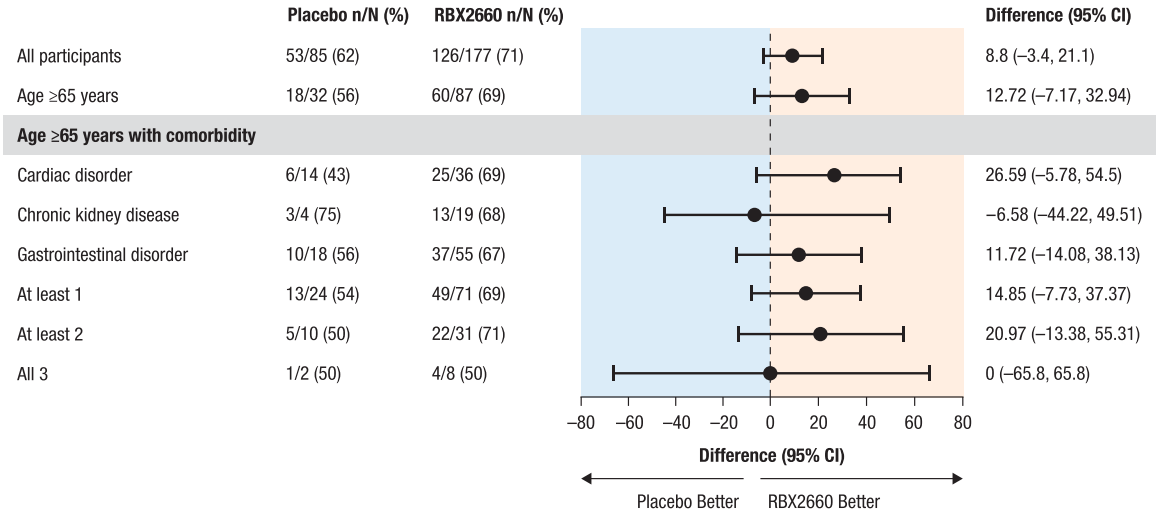


Figure 3. Summary of treatment success in older adults with recurrent *Clostridioides difficile* infection with cardiac disorders, chronic kidney disease (CKD), and/or gastrointestinal (GI) disorders (modified intent-to-treat population). The forest plot depicts the differences in the 8-week treatment success rates between placebo and RBX2660 in the total population and across subgroups. At least 1: participants with ≥1 comorbidity among cardiac disorder, CKD, and GI disorder; At least 2: participants with ≥2 comorbidities among cardiac disorder, CKD, and GI disorder.

Table 2. Summary of Treatment-Emergent Adverse Events Within 8-Week Follow-up of Blinded Treatment (Safety Population)

	All Participants		RBX2660-Treated Older Adults With Comorbidities									
			65–74 y		≥75 y		≥65 y					
	Placebo	RBX2660	Moderate CCI (3–4)	Severe CCI (≥5)	Moderate CCI (3–4)	Severe CCI (≥5)	Cardiac Disease	CKD	GI Disorder	At least 1[a]	At least 2[b]	All 3
Events	n = 87	n = 180	n = 19	n = 23	n = 8	n = 34	n = 38	n = 19	n = 57	n = 73	n = 33	n = 8
All TEAEs	38 (43.7)	94 (52.2)	6 (31.6)	16 (69.6)	4 (50.0)	17 (50.0)	23 (60.5)	13 (68.4)	29 (50.9)	39 (53.4)	19 (57.6)	7 (87.5)
Mild	26 (29.9)	72 (40.0)	3 (15.8)	13 (56.5)	2 (25.0)	12 (35.3)	16 (42.1)	9 (47.4)	21 (36.8)	28 (38.4)	13 (39.4)	5 (62.5)
Moderate	23 (26.4)	50 (27.8)	5 (26.3)	9 (39.1)	2 (25.0)	11 (32.4)	15 (39.5)	7 (36.8)	20 (35.1)	24 (32.9)	13 (39.4)	5 (62.5)
Severe	5 (5.7)	13 (7.2)	1 (5.3)	3 (13.0)	0 (0.0)	3 (8.8)	3 (7.9)	2 (10.5)	7 (12.3)	7 (9.6)	3 (9.1)	2 (25.0)
Potentially life-threatening	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)	1 (2.6)	0 (0.0)	1 (1.8)	1 (1.4)	1 (3.0)	0 (0.0)
Serious AEs	4 (4.6)	9 (5.0)	0 (0.0)	2 (8.7)	0 (0.0)	4 (11.8)	4 (10.5)	2 (10.5)	6 (10.5)	6 (8.2)	4 (12.1)	2 (25.0)
AEs leading to discontinuation	0 (0.0)	1 (0.6)[c]	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)[c]	1 (2.6)[c]	0 (0.0)	1 (1.8)[c]	1 (1.4)[c]	1 (3.0)[c]	0 (0.0)
TEAEs leading to death	0 (0.0)	1 (0.6)[c]	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)[c]	1 (2.6)[c]	0 (0.0)	1 (1.8)[c]	1 (1.4)[c]	1 (3.0)[c]	0 (0.0)

Percentage was calculated using the number of participants in the column heading as the denominator. TEAEs were defined as any adverse events occurring on or after the blinded treatment. For TEAEs counted during the blinded treatment period, TEAEs are reported with an onset date on or after the date of the blinded treatment through the 8-week timeframe or up to the time the participant received an unblinded treatment.

Abbreviations: AE, adverse event; CCI, Charlson Comorbidity Index; CKD, chronic kidney disease; GI, gastrointestinal; TEAE, treatment-emergent adverse event.

[a]At least 1: participants with ≥1 comorbidity among cardiac disorder, CKD, and GI disorder.

[b]At least 2: participants with ≥2 comorbidities among cardiac disorder, CKD, and GI disorder.

[c]One death due to cardiorespiratory arrest in a participant ≥75 years old with a history of cardiac and GI disorders and severe CCI score was deemed related to a preexisting condition and unrelated to RBX2660 or the administration procedure.

TEAE incidence was 61% (n = 23/38), 68% (n = 13/19), and 51% (n = 29/57) in RBX2660-treated older adults with cardiac disorders, CKD, and GI disorders, respectively. Most participants experienced mild or moderate TEAEs; few experienced severe or potentially life-threatening TEAEs. GI disorders were the only AEs reported in ≥5% of participants in the overall population (Supplementary Table 7) and were reported in a similar percentage of participants across subgroups. There was 1 death within 8 weeks of blinded treatment in the group of older adults. The participant was ≥75 years old with a history of cardiac and GI disorders. Upon review, the cause of death was determined to be related to a preexisting cardiac condition and not related to RBX2660 or its administration.

DISCUSSION

In this subgroup analysis of PUNCH CD3, RBX2660 treatment was consistently efficacious and safe in older adults with rCDI and common comorbidities. Similar to the total RBX2660-treated participant population, most TEAEs experienced by older adults with comorbidities were mild to moderate in severity. SAEs were infrequent, and none were related to RBX2660 or its administration. The 1 participant death during the 8 weeks of safety follow-up after blinded treatment was determined to be unrelated to treatment.

As previously addressed [21], placebo rates in PUNCH CD3 were higher than expected. It is important to clarify that all participants were treated with standard-of-care antibiotics for the enrolling CDI episode and had to show control of CDI symptoms the 2 days before washout in order to receive blinded treatment. Furthermore, approximately one-third of PUNCH CD3 participants were enrolled after a first recurrent episode (ie, 2 episodes of CDI), meaning the participants may have had a lower risk of recurrence because they likely had less severe dysbiosis compared with participants in other trials where ≥2 rCDI episodes (ie, 3 episodes of CDI) were required for enrollment [27].

Underlying comorbidities may not only impact patient prognosis but may also alter treatment and outcomes. Using the age-adjusted CCI, we found that RBX2660 remained efficacious and well tolerated by older adults with a moderate or high comorbidity burden.

Based on Medicare fee-for-service claims data from 2009 to 2017, 25% of older patients aged ≥65 years with rCDI died within 1 year of experiencing CDI (CDI-related mortality). This mortality rate is nearly 10 times that of CDI-related mortality after a first episode of CDI (2.7%) [17]. Additionally, patients with rCDI-related mortality were shown to have a higher burden of comorbid conditions and higher CCI scores, compared with patients who survived rCDI. In another study, a higher comorbidity burden was also found among CDI patients ≥65 years old who developed sepsis, compared with those who did not develop sepsis ($P < .0001$) [28]. These data underscore the importance of reducing rCDI in high-risk populations and highlight the need for treatment that is superior to standard-of-care antibiotics alone.

The combination of higher comorbidity burden and worse clinical outcomes from CDI/rCDI in older patients is critical to address. CDI incidence increases with age, and older adults are 3 times more likely to develop complicated CDI (eg, fulminant colitis and admission to an intensive care unit) [29]. Moreover, the growing number of older patients with comorbidities is increasing clinical challenges and complexity of medical decision making. Between 2013 and 2018, the prevalence of cardiovascular disease (including coronary heart disease, heart failure, stroke, and hypertension) in older adults 60–79 years old was a striking 78% among males and 75% among females. In addition, the US Centers for Disease Control and Prevention estimates that 38% of older adults have CKD (stages 1–4) [30, 31].

Several trials have associated CDI-related mortality with cardiopulmonary disease. One assessment of 374 747 cases of CDI from the 2011 Nationwide Inpatient Sample database found cardiopulmonary disease (including myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, and chronic obstructive pulmonary disease) to be an independent predictor of CDI-associated mortality (odds ratio, 1.46 [95% CI, 1.38–1.56]) [14]. Another study of patients requiring total colectomy for CDI found underlying cardiopulmonary disease also to be a covariate associated with increased mortality (odds ratio, 2.0; $P = .001$) [32].

CDI is more common in patients with CKD than in the general population and is associated with an increased risk of severe CDI and risk for recurrence [33]. A recent retrospective study of patients hospitalized with CDI reported CKD to be an independent predictor for 30-day all-cause readmissions. Another systematic review found that impaired renal function was associated with an increased risk of mortality in those with CDI [34].

Underlying GI comorbidities could also complicate rCDI treatment and outcomes. Prevalence estimates for gastroesophageal reflux disease (GERD), a common GI disorder in the United States, range from 18% to 28%. In a population-based survey, 35% of participants who had experienced GERD symptoms were on current therapy, 55% of whom were on proton pump inhibitors (PPIs) [35]. The association of PPI use and CDI has been documented in systematic reviews and meta-analyses that identified PPI use as a risk factor for CDI recurrence [29, 36–38]. Although the association remains controversial, in the current study, GERD was the most common GI disorder reported, and many participants were on PPI therapy. A history of diverticulitis, the second most commonly reported GI disorder among PUNCH CD3 participants, is associated with poor outcomes in patients with CDI, including increased length of hospital stay and mortality [39].

The route of administration of microbiota-based live biotherapeutic products in older patients with medical complexities and comorbid conditions warrants consideration. Administration issues that should be considered in older patients include aspiration risk and inability to swallow a high number of capsules or to withstand the rigors of bowel preparation [40]. Similar to the total PUNCH CD3 participant population, this subgroup analysis showed that RBX2660 and its administration were well tolerated in older adults with medical complexities, suggesting that this product may be an impactful novel treatment for patients with minimal to severe comorbid disease.

Like any study, our analysis has some limitations. PUNCH CD3 was not powered to detect significant differences in the subgroups presented here. Our conclusions are therefore limited by the small number of participants in some subgroups, particularly the placebo-treated participants ≥75 years old with moderate CCI (n = 2) and placebo-treated participants with CKD (n = 4). Additionally, patients with IBD and IBS were excluded from PUNCH CD3, which limits the broad generalizability of these data; however, a more real-world, open-label study (PUNCH CD3-OLS) that includes participants with IBD or IBS is ongoing, and efficacy and safety will be assessed in subgroups similar to those presented here.

This post hoc subgroup analysis of PUNCH CD3 showed that RBX2660 is efficacious and safe in a broad range of older adults with rCDI with varying comorbidity burden. RBX2660 was well tolerated across subgroups independent of comorbidities, with primarily mild to moderate TEAEs, no treatment-related SAEs, and only 1 AE-related study discontinuation. RBX2660 administration after standard-of-care antibiotic treatment could improve the outcomes of a potentially complex and vulnerable patient population and reduce the overall burden on patients and the healthcare system.

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

Author contributions. M. A. performed the statistical analysis. All authors contributed to concept and design; acquisition, analysis, and/or interpretation of data; drafting of the manuscript; and critical revision of the manuscript for important intellectual content.

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Data sharing. The datasets generated and/or analyzed during the current study are not publicly available but may be available from the corresponding author on reasonable request.

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Potential conflicts of interest. G. T. has served as a consultant for Ferring Pharmaceuticals, Dynavax Therapeutics, and Spero Pharmaceuticals. P. F. has served as a consultant/advisory board member and speaker for/received honoraria from Ferring Pharmaceuticals, Seres Therapeutics, and Takeda Pharmaceuticals, and served as a consultant for Merck & Co, Inc. S. J. has served as a consultant for Ferring Pharmaceuticals. S. N. is an employee of Ferring Pharmaceuticals. M. A. is a former employee of Ferring Pharmaceuticals. A. H. and L. B. are employees of Rebiotix Inc, a Ferring Company. L. A.-P. reports 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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