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COMMENTARY

The Role of Microbiome-Based Therapeutics in *Clostridioides difficile* Infection: Durable, Long-Term Results of RBX2660

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ABSTRACT

A recently published manuscript described findings from a phase 2 open label study of the microbiota-based live biotherapeutic product RBX2660 in patients with two or more previous recurrent *Clostridioides difficile* infection (rCDI) episodes, and described long-term safety and sustained treatment success through 24 months. As previous studies have typically focused on short-term clinical outcomes, these new data provide insight into the tolerability, safety, and efficacy of RBX2660 over the long term. When microbiota-based products were first evaluated, the long-term efficacy and safety were principal concerns of the United States Food and Drug Administration. Microbiota-based live biotherapeutic products (LBPs) represent an emerging approach to the management of CDI and perhaps other gastrointestinal and medical conditions whose pathogenesis is defined by microbial dysbiosis. RBX2660 is a human-derived, broad consortium microbiota-based LBP that consists of a population of microbes obtained from healthy stool donors and may reflect the symbiotic nature of a healthy colonic microbiome. RBX2660 is rectally administered and does not require sedation or special preparation of the recipient. Potential advantages of the rectal administration of RBX2660 include the ease of administration and lack of need for any bowel preparation, which may benefit those who are frail, have swallowing issues, or cannot take bowel laxative preparations. In this multicenter prospective trial of rCDI, patients who achieved treatment success 8 weeks after receiving RBX2660 continued to have a sustained clinical response over the course of long-term follow-up, with more than 90% of treatment responders remaining CDI-free at 6, 12, and 24 months. Following receipt of RBX2660, the gut microbiota of those with treatment success were restored from a dysbiotic state to become more diverse and similar to RBX2660 composition. The restoration of the microbiota occurred as early as 7 days after RBX2660 administration and remained stable through the 24-month analysis. No new adverse outcomes were observed during the prospective assessment, and the safety profile of RBX2660 was consistent with previous studies. Based on the clinical

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studies, RBX2660 will most likely benefit those with ≥ 1 rCDI episode or those who are at a high risk of subsequent rCDI, such as patients who have comorbid conditions including renal disease, heart disease, or inflammatory bowel disease, or who are immunosuppressed.

Keywords: *Clostridioides difficile*; *Clostridium difficile*; Fecal microbiota transplantation; Recurrent *C. difficile* infection; Microbiota; Live biotherapeutic products

Key Summary Points

Several microbiota-based live biotherapeutic products (LBPs) have now completed phase 2 and 3 clinical trials and appear to offer a therapeutic advance for the management of recurrent *Clostridioides difficile* infection (rCDI). The long-term outcomes of these therapies are only now being described.

Data were recently published from a phase 2 safety and efficacy study of RBX2660, as a treatment for patients with ≥ 2 previous rCDI episodes who had completed standard-of-care antibiotic therapy, or severe CDI requiring hospitalization. RBX2660 was 79% successful in preventing recurrence of CDI at 8 weeks.

More than 90% of patients treated with RBX2660 who had treatment success at 8 weeks continued to be CDI-free at 6, 12, and 24 months.

The gut microbiota in patients who had treatment success were restored from dysbiosis, became more diverse and more similar to RBX2660 composition, and reflected a healthier microbiome to that observed prior to RBX2660 administration.

Based on the clinical trial data, LBPs will most likely benefit those who have had ≥ 1 rCDI episode or those who are at high risk of rCDI after an initial episode.

INTRODUCTION

A divergence from the normal microbiota composition, generally referred to as dysbiosis, has been associated with a range of gastrointestinal (GI) and non-GI diseases. Restoring the gut microbiota to a more diverse, healthier, and balanced composition, termed eubiosis, represents a novel therapeutic target to combat many conditions known to be influenced by the microbiome, such as infections caused by the healthcare-associated pathogen *Clostridioides difficile*. Microbiota-based therapeutics are an emerging therapy for preventing recurrence of *C. difficile* infection (CDI) [1]. However, there are few long-term safety or efficacy data for these new therapies. It is important to determine whether these products maintain their efficacy beyond the 8-week efficacy end point, typically used by the Centers for Disease Control and Prevention and the United States Food and Drug Administration (US FDA), to look for recurrences [2].

A recently published manuscript described findings from a phase 2 open label study of the microbiota-based live biotherapeutic product (LBP) RBX2660 in patients with ≥ 2 previous recurrent CDI (rCDI) episodes or ≥ 2 episodes of severe CDI requiring hospitalization, and was among the first studies to document long-term safety concomitant with sustained treatment success through 24 months [3]. Studies of other therapeutics for CDI have typically used the 8-week end point, though some have shown effectiveness at week 12/90 days [4–6]. The 2-year follow-up in this study is important because the safety and long-term impact on the gut microbiome has not previously been defined.

In an era of increasing microbial resistance, a shift has occurred from the 1950s paradigm of striking back at microbes with antimicrobial agents, to recognition of the value of diverse microbial communities to support health. Fecal microbiota transplantation (FMT), defined as the delivery of stool from a healthy donor to a recipient with the goal of mitigating disease by modifying the structure and/or function of the gut microbiome, has been reconsidered as a

treatment strategy for dysbiosis since it first appeared in Western medicine in 1958 [7–9]. The development of LBPs stemmed from the concept of FMT, and are defined by the US FDA as a biological product containing live organisms, such as bacteria, that are applicable to the prevention, treatment, or cure of a disease [10]. The best example of this is the role that microbiota-based LBPs play in the reduction of rCDI [11, 12]. The relationship between intestinal microbial diversity in a healthy state, and dysbiosis resulting in rCDI risk, has led to the development of new therapeutic approaches that include LBPs, which have demonstrated efficacy in reducing future CDI recurrences [13, 14].

EMERGING DATA

Much of the data on LBPs for rCDI have demonstrated short-term treatment success at 8 weeks as required for clinical trials, but long-term outcomes have been unknown. A patient with rCDI wants to know that the therapy they receive will not just alleviate their symptoms by 8 weeks but provide a long-term solution to the problem of recurrence. It appears that the greatest risk of recurrence occurs in the first 3 weeks after an episode of CDI, and that restoration of microbial diversity is associated with a continued CDI-free state. A recent study provides some of that data. The long-term safety and efficacy of RBX2660, a biologically sourced, rectally-administered, broad consortium microbiota-based LBP as treatment in patients with $\geq$ 2 previous rCDI episodes who had completed standard-of-care antibiotic therapy, or $\geq$ 2 severe CDI episodes requiring hospitalization, were recently published [3]. In this multicenter open label phase 2 study using historical controls as a comparison group, patients with rCDI who received RBX2660 achieved 79% treatment success at week 8 (absence of CDI diarrhea without the need for retreatment for 8 weeks after completing study treatment), compared with 31% in a matched historical control group ($p < 0.0001$; chi-squared test), with a subsequent recurrence rate of $\sim$21%. This recurrence rate was found to be substantially lower than the 50–65% recurrence rate previously reported in the literature for patients with at least one prior rCDI episode [15–17]. Newer studies using tailored, emerging rCDI therapies have shown lower recurrence rates after a prior rCDI episode of $\sim$20% [18–20].

Patients treated with RBX2660 who met the primary efficacy end point of treatment success at 8 weeks continued to demonstrate a sustained clinical response, and were CDI-free in 97%, 95%, and 91% of treatment responders at 6, 12, and 24 months, respectively [3]. Patients were to receive up to two doses of RBX2660, and of the 149 treatment recipients, 143 received two doses. A phase 3 trial of a single dose of RBX2660 demonstrated efficacy of 70% at week 8 [21] Many studies investigating microbiota-based LBPs utilize a relatively conservative follow-up period, with few describing long-term outcomes [22–24].

The primary efficacy results correlate well with similar studies of other microbial therapeutics. In a phase 2 trial of SER-109, an oral capsule formulation of Firmicutes spores, 44% of those receiving the product achieved clinical success at 8 weeks [25]. A later phase 3 trial of SER-109, given as four capsules/day for 3 consecutive days, was 88% effective at 8 weeks [26]. In this trial, 75% of recurrences occurred within 2 weeks and 85% within 4 weeks of administration. Microbiota restoration was also seen in this trial as early as week 1 and persisted through week 8, but longer-term data are not yet available. A recent phase 2 trial of another oral, full-spectrum microbiota product in capsule formulation, CP101, ten capsules once, demonstrated a 75% efficacy at week 8 [27]. Two phase 3 studies on the monoclonal antibody against *C. difficile* toxin B, bezlotoxumab, demonstrated 12-week recurrence rates of 16–17% [28].

The greatest insight into microbiota-based therapeutics comes from FMT trials. In a meta-analysis of 13 randomized, controlled trials of 610 patients treated with FMT for rCDI, clinical cure rates of 76% and 89% were reported after single and multiple FMTs, respectively [29]. The clinical cure rate in the pooled placebo groups of the meta-analysis was 43% [29], which is similar to that observed in many other

previously described trials of microbiota-based therapeutics. The degree of heterogeneity among study designs evaluating FMT is substantial, even in randomized controlled trials, and include different routes of administration, treatment formulations, and antibiotic pretreatment, all of which can potentially affect the study outcome [30]. Moreover, the interpretation of existing publications evaluating FMT as a treatment option for CDI is confounded by the abundance of nonrandomized trials, most of which fail to clearly describe their methodology, and hence may be subject to bias [31].

In the recently published RBX2660 phase 2 trial, the lack of rCDI was supported by the gut microbiome analysis in those patients who received RBX2660 [3]. Following receipt of RBX2660, the gut microbiota in patients who had treatment success were restored from dysbiosis, became more diverse and more similar to RBX2660 composition, and reflected a healthier microbiome from what was seen prior to RBX2660 administration. These microbiome profiles were consistently similar to the profile of RBX2660 from 7 days after receiving the LBP and remained stable through 24 months. Similar results were seen in a small randomized study of FMT in patients with ≥ 1 rCDI, in which the gut microbiome became significantly more diverse after FMT and were statistically indistinguishable from the diversity of the donor gut microbiome [32].

RBX2660 is a biologically sourced, rectally administered, broad consortium microbiota-based LBP that consists of a population of microbes obtained from healthy donors and may reflect the symbiotic nature of the healthy microbiome. RBX2660 undergoes comprehensive pathogen testing, is collected from rigorously screened, healthy donors, and processed to a stable, cryopreserved liquid suspension of $> 10^7$ live organisms/ml. In contrast to unregulated FMT, the efficacy and safety of RBX2660 has been evaluated in double-blind, randomized, placebo-controlled phase 2b and phase 3 trials. To date, more than 700 patients have received at least one dose of RBX2660.

The gut microbiota profiles of the few patients who did not have treatment success, i.e., those who had a recurrence during the study, remained largely dysbiotic despite receiving RBX2660. Nonresponders showed less reduction of Gammaproteobacteria and Bacilli and restoration of Clostridia and Bacteroidia after treatment. The cause of these treatment failures may be due to a profound loss of diversity prior to treatment, host immune factors, or possibly slow transit and residual antibiotic effect from prior therapy. As real-time microbiome analysis is not yet routinely performed, we do not have a marker to determine the likelihood of success at the point of care, but this may be accessible in the future.

The safety profile of RBX2660 was consistent with its previous studies, showing that most adverse events were mild or moderate in severity and GI in nature, such as local irritation or bloating [33, 34]. Overall, 123/149 patients reported a treatment-emergent adverse event (TEAE), with 84% of those TEAEs being mild to moderate in severity. Two participants reported serious adverse events assessed as possibly related to RBX2660, however, these events were also deemed related to pre-existing conditions and CDI.

Who could be considered for this therapy?

Based on the clinical trials data, microbiota-based LBPs could be considered for patients who have had ≥ 1 rCDI episode or those who are at a high risk of rCDI after an initial episode, such as patients who have comorbid conditions including renal disease, heart disease, or inflammatory bowel disease, or who are immunosuppressed. Future microbiome analytics might help predict who would benefit from microbiota-based LBPs prior to giving prolonged therapies with antimicrobials.

A potential advantage of RBX2660 is that it does not require bowel preparation prior to administration, which may benefit those who have a contraindication or intolerance to such procedures, especially in the setting of recent acute CDI. Additionally, RBX2660 is a rectally administered microbiota suspension that is not limited by swallowing issues, drug–drug interactions, emesis or aspiration concerns, a fasting window before or after administration, and does not require sedation as would be needed with colonoscopic administration, which can be

riskier in older adults. Rectal administration is not as messy and unpleasant as an enema, and there is no concern over treatment retention. One possible limitation of rectal administration is that RBX2660 must be administered by a healthcare provider in the inpatient/outpatient setting.

FMT has been included in clinical practice guidelines for CDI since 2011, but its accessibility has been a major challenge [11]. Current guidelines from the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA) suggest FMT as a treatment option for the second or subsequent recurrence of CDI [35]. European Society of Clinical Microbiology and Infectious Diseases clinical guidelines suggest FMT as a treatment option for multiple rCDI and severe/complicated refractory CDI [36].

For patients to realize the treatment benefit of microbiota-based LBPs and the healthcare system to lessen its burden of CDI, new therapies will need to become more accessible. Real-world claims analyses of adults in the USA with CDI demonstrate rates of FMT of approximately 0.7–1.1% in all patients, with a greater proportion of patients with rCDI having FMT (up to 10% of patients with ≥ 3 rCDI episodes) [17, 37]. FMT uptake for rCDI did increase under the US FDA Enforcement Discretion Policy when samples were available from stool banks [37, 38].

CONCLUSION

Microbiota-based LBPs represent a novel approach to the management of GI and other medical conditions whose pathogenesis is defined by microbial dysbiosis. The potential application of LBPs could expand over time to include preventive approaches in high-risk patients who require antimicrobials. If LBPs continue to demonstrate consistently high rates of clinical efficacy and long-term safety, they may be considered a reasonable treatment option earlier in the course of CDI for patients at risk of antimicrobial failure.

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REFERENCES

1. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474:1823–36.
2. Centers for Disease Control and Prevention. Clostridioides difficile infection (CDI) tracking. https://www.cdc.gov/hai/eip/cdiff-tracking.html. Accessed January 15, 2020.
3. Orenstein R, Dubberke ER, Khanna S, et al. Durable reduction of *Clostridioides difficile* infection recurrence and microbiome restoration after treatment with RBX2660: results from an open-label phase 2 clinical trial. BMC Infect Dis. 2022;22:245.
4. Hengel RL, Ritter TE, Nathan RV, et al. Real-world experience of bezlotoxumab for prevention of Clostridioides difficile infection: a retrospective multicenter cohort study. Open Forum Infect Dis. 2020;7:ofaa097.
5. Escudero-Sánchez R, Ruiz-Ruigómez M, Fernández-Fradejas J, et al. Real-world experience with bezlotoxumab for prevention of recurrence of *Clostridioides difficile* infection. J Clin Med. 2021;10:2.
6. Johnson TM, Molina KC, Howard AH, et al. Real-world comparison of bezlotoxumab to standard of care therapy for prevention of recurrent Clostridioides difficile infection in patients at high risk for recurrence. Clin Infect Dis. 2022;74:1572–8.
7. Kelly CR, Kim AM. The AGA's fecal microbiota transplantation national registry: an important step toward understanding risks and benefits of microbiota therapeutics. Gastroenterology. 2017;152:681–4.
8. US Food and Drug Administration, Center for Biologics Evaluation and Research, US Food and Drug Administration, Center for Biologics Evaluation and Research. Enforcement policy regarding investigational new drug requirements for use of fecal microbiota for transplantation to treat *Clostridium difficile* infection not responsive to standard therapies: draft guidance for industry. https://www.fda.gov/media/96562/download
9. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44:854–9.
10. United States Food and Drug Administration. Early clinical trials with live biotherapeutic products: Chemistry, manufacturing, and control information: Guidance for industry. https://www.fda.gov/files/vaccinesbloodbiologics/published/Early-Clinical-Trials-With-Live-Biotherapeutic-Products-Chemistry-Manufacturing-and-Control-Information-Guidance-for-Industry.pdf. Published 2016.
11. Chaar A, Feuerstadt P. Evolution of clinical guidelines for antimicrobial management of Clostridioides difficile infection. Ther Adv Gastroenterol. 2021;14:1–16.
12. Wang J-W, Kuo C-H, Kuo F-C, et al. Fecal microbiota transplantation: review and update. J Formos Med Assoc. 2019;118:S23–31.
13. Smits WK, Lyras D, Lacy DB, Wilcox MH, Kuijper EJ. *Clostridium difficile* infection. Nat Rev Dis Prim. 2016;2:16020.
14. Sehgal K, Khanna S. Gut microbiome and *Clostridioides difficile* infection: a closer look at the microscopic interface. Ther Adv Gastroenterol. 2021;14:1–9.
15. Kelly CP, LaMont JT. *Clostridium difficile*—more difficult than ever. N Engl J Med. 2008;359:1932–40.
16. Lessa FC, Mu Y, Bamberg WM, et al. Burden of *Clostridium difficile* infection in the United States. N Engl J Med. 2015;372:825–34.
17. Feuerstadt P, Nelson WW, Teigland C, Dahdal DN. Clinical burden of recurrent *Clostridioides difficile* infection in the medicare population: a real-world claims analysis. Antimicrob Steward Heal Epidemiol. 2022;2: e60.
18. Cornely OA, Miller MA, Louie TJ, Crook DW, Gorbach SL. Treatment of first recurrence of *Clostridium difficile* infection: fidaxomicin versus vancomycin. Clin Infect Dis. 2012;55:S154–61.
19. Gerding DN, Kelly CP, Rahav G, et al. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection in patients at increased risk for recurrence. Clin Infect Dis. 2018;67(5):649–56. https://doi.org/10.1093/cid/ciy171.
20. Madoff SE, Urquiaga M, Alonso CD, Kelly CP. Prevention of recurrent *Clostridioides difficile* infection: a systematic review of randomized controlled trials. Anaerobe. 2020;61: 102098.
21. Blount K, Walsh D, Gonzalez C, Shannon B. Treatment success in reducing recurrent *Clostridioides difficile* infection with investigational live biotherapeutic RBX2660 was associated with microbiota

restoration: consistent evidence from a Phase 3 clinical trial. Open Forum Infect Dis. 2021;8: S624–5.

22. Jalanka J, Hillamaa A, Satokari R, Mattila E, Anttila VJ, Arkkila P. The long-term effects of faecal microbiota transplantation for gastrointestinal symptoms and general health in patients with recurrent *Clostridium difficile* infection. Aliment Pharmacol Ther. 2018;47(3):371–9. https://doi.org/10.1111/apt.14443.

23. Perler BK, Chen B, Phelps E, et al. Long-term efficacy and safety of fecal microbiota transplantation for treatment of recurrent *Clostridioides difficile* infection. J Clin Gastroenterol. 2020. https://doi.org/10.1097/MCG.0000000000001281.

24. Saha S, Mara K, Pardi DS, Khanna S. Long-term safety of fecal microbiota transplantation for recurrent *Clostridioides difficile* infection. Gastroenterology. 2021;160:1961–9.

25. McGovern BH, Ford CB, Henn MR, et al. SER-109, an investigational microbiome drug to reduce recurrence after *Clostridioides difficile* infection: lessons learned from a Phase 2 trial. Clin Infect Dis. 2021;72:2132–40.

26. Feuerstadt P, Louie TJ, Lashner B, et al. SER-109, an oral microbiome therapy for recurrent *Clostridioides difficile* infection. N Engl J Med. 2022;386:220–9.

27. Khanna S, Kelly CR, Louie T, et al. (2021) CP101, an investigational orally administered microbiome therapeutic, increases intestinal microbiome diversity and prevents recurrent *C. difficile* infection: results from a randomized, placebo-controlled trial. Am J Gastroenterol. 2021;116:S57.

28. Wilcox MH, Gerding DN, Poxton IR, et al. Bezlotoxumab for prevention of recurrent *Clostridium difficile* infection. N Engl J Med. 2017;376:305–17.

29. Tariq R, Pardi DS, Bartlett MG, Khanna S. Low cure rates in controlled trials of fecal microbiota transplantation for recurrent *Clostridium difficile* infection: a systematic review and meta-analysis. Clin Infect Dis. 2019;68(8):1351–8. https://doi.org/10.1093/cid/ciy721.

30. Feuerstadt P, Aroniadis OC, Svedlund FL, et al. Heterogeneity of randomized controlled trials of fecal microbiota transplantation in recurrent *Clostridioides difficile* infection. Dig Dis Sci. 2022. https://doi.org/10.1007/s10620-021-07141-9.

31. Bafeta A, Yavchitz A, Riveros C, Batista R, Ravaud P. Methods and reporting studies assessing fecal microbiota transplantation a systematic review. Ann Intern Med. 2017;167(1):34–9. https://doi.org/10.7326/M16-2810.

32. Van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. N Engl J Med. 2013;368(5): 407–15. https://doi.org/10.1056/NEJMoa1205037.

33. Dubberke ER, Lee CH, Orenstein R, Khanna S, Hecht G, Gerding DN. Results from a randomized, placebo-controlled clinical trial of a RBX2660—a microbiota-based drug for the prevention of recurrent *Clostridium difficile* infection. Clin Infect Dis. 2018;67:1198–204.

34. Orenstein R, Dubberke E, Hardi R, et al. Safety and durability of RBX2660 (microbiota suspension) for recurrent *Clostridium difficile* infection: results of the PUNCH CD study. Clin Infect Dis. 2016;62(5): 596–602. https://doi.org/10.1093/cid/civ938.

35. McDonald LC, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults and children: 2017 update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018;66:e1–48.

36. van Prehn J, Reigadas E, Vogelzang EH, et al. European Society of Clinical Microbiology and Infectious Diseases: 2021 update on the treatment guidance document for *Clostridioides difficile* infection in adults. Clin Microbiol Infect. 2021;27: S1–21.

37. Feuerstadt P, Boules M, Stong L, et al. Clinical complications in patients with primary and recurrent *Clostridioides difficile* infection: a real-world data analysis. SAGE Open Med. 2021;9:1–8.

38. US Food and Drug Administration. Enforcement policy regarding investigational new drug requirements for use of fecal microbiota for transplantation to treat *Clostridium difficile* infection not responsive to standard therapies. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/enforcement-policy-regarding-investigational-new-drug-requirements-use-fecal-microbiota-0. Accessed January 15, 2020.

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