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REVIEW

Treating Clostridioides difficile: Could Microbiota-based Live Biotherapeutic Products Provide the Answer?

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Abstract: *Clostridioides difficile* infection (CDI) is a pressing health care issue due to the limited effectiveness of current treatments and high recurrence rates. Current available antibiotic options for CDI disrupt the fecal microbiome which predisposes recurrent CDI. Fecal microbiota transplantation (FMT) has improved the outcomes of recurrent CDI, but concerns surrounding the safety and standardization of the product persist. Microbiota-based live biotherapeutic products (LBPs), are emerging as potential alternatives to FMT for CDI treatment. This review explores the potential of LBPs as safe and effective therapy for CDI. While preclinical and early clinical studies have shown promising results, further research is necessary to determine the optimal composition and dosage of LBPs and to ensure their safety and efficacy in clinical practice. Overall, LBPs hold great promise as a novel therapy for CDI and warrant further investigation in other conditions related to disruption of the colonic microbiota.

Keywords: *Clostridioides difficile*, microbiome, live biotherapeutic products, recurrence

Introduction

Clostridioides difficile, (C. difficile), is an anaerobic gram-positive, spore forming rod. It is the leading infectious cause of health-care associated diarrhea and an increasing cause of community onset diarrhea. *C. difficile* infection (CDI) has become an emerging global health threat due to its rapidly increasing frequency, significant morbidity, and mortality. According to the CDC, there were approximately 500,000 CDI cases per year associated with 15,000 to 30,000 deaths.[1–3] The CDC estimates that there were 223,900 hospitalizations from CDI and 12,800 deaths due to CDI in the United States in 2017.[4] CDI most commonly occurs following broad-spectrum antibiotic use which alters the composition of the gut microbiota and their metabolites which in turn predisposes recurrence of CDI. The microorganisms which colonize the human lower gastrointestinal tract provide a protective barrier which prevents *C. difficile* from causing infection. The outcome of the ingestion of *C. difficile* spores from the environment depends upon a variety of host factors which may impact its sporulation and conversion into the vegetative, toxin-producing state. The gut microbiota may inhibit CDI via modification of its environment, reducing its nutrient supply or the production of inhibiting compounds such as bile acids or bacteriocins. Unfortunately, our historical approach to management has been to treat CDI with more antibiotics, targeted at the pathogen. This approach further compounds the dysbiotic environment and provides the milieu for recurrent disease upon discontinuation of the antibiotic.

The human body is host to a diverse and intricate community of microorganisms collectively known as the microbiota. This community is comprised of a range of microorganisms, such as bacteria, fungi, archaea, protozoa, and viruses. When referring to these microorganisms, their genetic material, and the environment they inhabit within a specific ecosystem, the term "microbiome" is used. The microbiome is a complex and dynamic system that plays a critical role in maintaining the overall health of its host. It carries out numerous functions, including aiding in digestion, synthesizing essential compounds like vitamins, and providing protection against harmful pathogens.[5] This holobiont

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concept recognizes that the organisms do not exist as independent entities but rather are part of a larger community of microorganisms that collectively make up the host's microbiome.[6] The host actively shapes the microbiome, and this action creates powerful selective pressures that can impact the composition and function of the microbiota.[7] The healthy gut microbiota of humans is mainly composed of bacteria, Bacteroidetes and Firmicutes as the dominant phyla, while Proteobacteria and Actinobacteria are present in smaller amounts in the human stool. Any changes in this microbial structure can result in an imbalance known as dysbiosis. Dysbiosis can occur due to a reduction in beneficial microbes, an increase in pathogenic microbes, a loss of diversity, or a combination of these factors. This altered gut microbiota status can lead to changes in its metabolic, neurological, and immunological functions.

The mechanisms by which microbial dysbiosis leads to recurrent CDI is becoming better understood. Studies have identified the microbial species most important in preventing CDI recurrence,[8] including the impact of microbial diversity[9] and more recently the role of the gut metabolome.[10] Recent studies have shown a parallel between the patterns of microbial diversity and metabolome which seem to occur around two weeks after infection treatment. Specific metabolites associated with host inflammation appear to predict recurrent disease.[8]

Today's guideline-based treatment for the first episode of CDI is an antibiotic targeted at *C. difficile*: metronidazole, vancomycin, or fidaxomicin. However, administering vancomycin orally leads to additional alterations in the composition of the gut microbiome. These changes include a decrease in the levels of Firmicutes, Bacteroidetes, and Actinobacteria, as well as a significant rise in the presence of Proteobacteria. Compared to oral vancomycin, fidaxomicin has a more limited impact on the gut microbiota and less risk for *C. difficile* colonization.[11] Ridinilazole, (Summit Therapeutics, Inc.), an antimicrobial currently in development is a more targeted antibiotic designed specifically for treating CDI. In both phase 2 and 3 clinical trials, it has been found to be less disruptive to the gut microbiota than oral vancomycin.[12] In a study where ridinilazole was used for 10 days as an initial treatment for CDI, 73% of the patients who received it (compared to 70% who received vancomycin) achieved a lasting clinical response after 30 days, and their microbiome diversity was preserved unlike the vancomycin group.[13] However, it is important to note that a sustained response in 70% of patients implies that another 30% may experience a recurrence.

Unfortunately, the unintended consequences of these agents still result in an ongoing cycle of disruption to the gut microbiota and loss of its natural ability to prevent CDI. The failure to acknowledge the crucial role of the microbiota in preventing further episodes of CDI has contributed to a widespread recurrent disease, which disproportionately affects the most susceptible patients. Preventing recurrent CDI has become a major challenge. With an incidence of between 15 and 30% after a first episode and much higher rates with subsequent ones, patients become trapped in a frustrating and debilitating cycle of recurrence and antimicrobial management. We have learned that more targeted therapies which spare the anaerobic microbiota appear to be associated with less frequent recurrence – vancomycin less than metronidazole, fidaxomicin less than vancomycin. Though these more targeted and microbiota-sparing agents may limit the damage, they do not restore the microbiota to its premorbid state. Restoration of the essential microbes often takes between 1 and 2 months and most patients recur within that window.[14]

When the gut microbiota is in a state of dysbiosis and metabolic changes occur, it results in a reduced ability to resist colonization by *C. difficile*. It would then seem intuitive that one approach to preventing recurrence would be to restore the metabolic or microbial balance to the gut. The initial approach to this dates back centuries[15] and is most recently known as fecal microbiota transplantation (FMT). By directly introducing a diverse range of fecal microbiota into the gastrointestinal tract of the affected individual, FMT immediately restores the balance of the gut microbiota.

FMT can enrich the gut microbiome with commensal bacteria, such as Bacteroides, nontoxigenic *Clostridium*, and *Prevotella*, which have been associated with preventing recurrent CDI.[16] The current guidelines from the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) recommend FMT for patients who have suffered from multiple CDI recurrences and have not been cured with appropriate antibiotics.[17] However, the FMT process has limitations, including the need for a source of healthy human stool, risk of transmitting multidrug-resistant infections and potential for failure due to nonstandardized preparations.[18] This initial approach to microbiota restoration has appeared highly effective both in controlled and uncontrolled trials.[19] However, the heterogeneity of the process and samples and potential safety risks led the US Food and Drug Administration (FDA) to revise the policy that determines how FMT is regulated and enforced.[20] Instead, FDA has encouraged the development of

standardized, safe live biologic therapeutics.[21] These, live biological products (LBPs) are designed to interact with the host's microbiome and restore its balance to promote health which seems promising in CDI.

Live biotherapeutic products (LBPs) refer to biological products that contain live microorganisms such as bacteria or yeast, that are specifically designed to provide health benefits to the host. These microorganisms are derived from defined consortia of clonal bacterial isolates. The FDA defines, LBPs as biological products, other than vaccines, that contain live organisms and are intended for the prevention, treatment, or cure of diseases or medical conditions in humans.[21]

Review

LBPs are formulated to interact with the microbiome of the host and restore its natural balance, with the aim of promoting health and preventing recurrent CDI. There are ongoing studies exploring the potential use of LBPs for gastrointestinal disorders such as inflammatory bowel disease, irritable bowel syndrome, small intestinal bowel overgrowth and other similar conditions. The FDA classifies live biotherapeutic products as drugs and, as such, they must meet rigorous regulatory requirements. This includes ensuring that the contents of these products are highly standardized and that their production processes strictly adhere to good manufacturing practices. They may be administered orally, topically, or via other routes and are often made using strains of bacteria that have been specifically selected and optimized for therapeutic use. Their effectiveness is highly dependent on the specific strains of bacteria used in the product. Several of these LBPs are in development for preventing recurrent CDI; two products are now FDA approved, Rebyota™ (fecal microbiota live-jslm; RBX2660; RBL), and VOWST (SER-109).

REBYOTA (RBL)

Fecal microbiota, live-jslm – REBYOTA™, (previously known as RBX2660 and abbreviated as RBL), developed by Rebiotix, a Ferring company, is a novel LBP approved in November 2022 for treating recurrent CDI. This product contains 150 mL of microbiota suspension and has about 10^7 organisms per milliliter derived from healthy stool donors. It is available as a single-dose ready-to-use enema.

FDA approval of this product, RBL was based on the largest clinical microbiome-based therapeutics program. The PUNCH CD trial was an open-label, noncomparative study that showed FRBL to be safe and effective in preventing CDI recurrence for those experiencing more than one recurrent episode of CDI. After two doses of RBL, 87.1% (27/31) of patients had no further recurrences at week 8.[22] The Phase 2 open label clinical trial (PUNCH CD2) was conducted at 29 medical centers in the United States and Canada. The patients who had ≥2 previous recurrent CDI episodes and had completed standard of care antibiotic therapy, or ≥2 severe CDI episodes requiring hospitalization were included in this study. This trial compared the open label RBL group to a comparable historical control cohort. The recipients in the trial had 78.9% treatment success compared to 30.7% in the historic control group at week 8 ($P<0.0001$). RBL was well-tolerated with very few adverse effects which were mostly mild to moderate gastrointestinal symptoms. It has demonstrated long-term clinical success, with treatment responders remaining free of CDI in 97%, 95%, and 91% of cases at 6, 12, and 24 months, respectively. It has been also shown that restoration of the microbiota (rebiosis) occurs within one week after administration of RBL and remains stable for up to 24 months.[23,24]

RBL showed reduction in the CDI recurrence rates in both a Phase 2B randomized controlled trial (PUNCH CD2) and Phase 3 randomized controlled trial (PUNCH CD3) when compared to placebo. In the Phase 2B trial, recurrence rates were 18.8% for RBL compared to 51.8% for placebo.[25] In the Phase 3 trial, recurrence rates were 11.1% for RBL compared to 36.4% for placebo, with a *P*-value of 0.002. These results confirmed the efficacy of RBL in reducing the recurrence of CDI.[26]

VOWST™

VOWST, formerly SER-109 is an oral LBP developed by Seres Therapeutics and is now approved by the US FDA for prevention of rCDI in patients 18 years of age following antibiotic treatment for rCDI. It is composed of purified live Firmicutes bacterial spores derived from healthy human donor stool. It is given as an oral capsule formulation within 2–4 days of antibiotic treatment for rCDI. VOWST requires an initial dose of 10 ounces of magnesium citrate as a bowel

washout for the previously consumed antibiotics, which is then followed by three consecutive days of four capsules orally once daily on an empty stomach.

In the initial Phase 1 study, VOWST achieved 96.7% clinical resolution of CDI at week 8 and had a favorable safety profile.[27] In the Phase 2 multicenter, double-blind randomized placebo-controlled trial, the primary endpoint of reducing recurrent CDI rates was not met. This was thought to be related to underdosing of VOWST (1×10^8 spores) and the use of PCR testing for diagnosis in most subjects. A preplanned analysis showed a statistically significant reduction in recurrence rates among subjects ≥65 years who received VOWST compared to placebo (45.2% vs 80%, respectively; RR, 1.77; 95%CI: 1.11–2.81). Early engraftment of VOWST was also associated with nonrecurrence ($P<0.05$) and increased secondary bile acid concentrations ($P<0.0001$).[28]

In the Phase 2b/3 trial of VOWST, the number of spores in the formulation was increased 10-fold and trial design was adjusted to better detect clinical outcomes. In this trial, ECOSPOR III, VOWST given at four capsules daily for 3 days vs placebo showed favorable safety and efficacy in reducing the risk of CDI recurrences up to 8 weeks in a phase 3 double-blind, randomized placebo-controlled trial of 182 patients experiencing three or more episodes of CDI in the last 12 months.[29,30] The effectiveness of the treatment was maintained up to 24 weeks.[31] As with RBL, the shift toward the transplanted microbiota occurred early and was maintained, concomitant with improvements in bile acids and clinical outcomes.

CP101

For several years, a stool bank in the USA, Open Biome has studied the clinical applications of FMT. In collaboration with a former company, Crestovo, Open Biome created Finch Therapeutics to develop an oral live biologic product for CDI called CP101. CP101 is an oral capsule containing healthy donor stool which increases microbiome diversity and prevents CDI.[32] CP101 dosing consists of a single administration of 10 capsules without any bowel preparation. CP101 successfully completed two Phase II trials, PRISM3 and PRISM-EXT, which focused on patients experiencing recurrence after participating in PRISM3.[33,34] The PRISM3 trial was a randomized double-blinded, and placebo-controlled trial of CP101 following standard antimicrobial therapy in patients experiencing CDI recurrences. The trial enrolled patients with first recurrent CDI who were over 65 years old and had a high risk of recurrent CDI, or who had experienced two or more recurrent CDIs. A total of 198 patients were enrolled, and they were given oral CP101 or a placebo at least 2 days after completing their standard CDI treatment. The primary objective of sustained clinical cure, defined as no CDI recurrence at week 8 was significantly higher in the CP101 group (74.5%) versus placebo group (61.5%) and was maintained through week 24. In addition, within one week of administration, an increase in alpha diversity and microbiome diversity as measured by 16s rRNA gene sequencing was observed in CP101 recipients compared to the placebo group, and this effect persisted through 8 weeks. Out of the 20 patients who were given a second dose of CP101 after experiencing recurrence in PRISM3, 14 patients (70%) achieved successful treatment for a period of 8 weeks and overall, about 80% had clinical success in PRISM-EXT trial up to week 24. There were no reported safety issues during this treatment. Enrollment for the Phase III trial, PRISM4, was halted by FDA to find out more details on SARS-CoV-2 screening protocols of the donors and was briefly restarted in late 2022.[35] However, Finch closed the trial for financial reasons in January 2023.

VE303

VE303 is a unique live biotherapeutic product (LBP) developed by Vedanta Biosciences, a clinical-stage microbiome company that is focused on the discovery and development of drugs based on the human microbiome. VE303 is not made from human stool but rather consists of eight different strains of Clostridia bacteria that are commensals in the human gut. Unlike other LBPs that are derived directly from human stool, VE303 is produced ex vivo under conditions of Good manufacturing practices (GMP) that mimics the conditions of the human gut by growing the bacteria from bacterial cell banks. This process ensures that the bacteria are safe, effective, and consistent from batch to batch.

VE303 is administered orally and works by colonizing the gut and modulating the immune system to promote a healthy balance of bacteria in the gut and resisting *C. difficile* colonization. VE303 was associated with a higher conversion of primary and secondary bile acids as well as short-chain fatty acids in the gut in 14 days.

The phase 1 study of VE303 aimed to evaluate the safety and tolerability of the drug in healthy volunteers. The study included 39 human volunteers who received either oral vancomycin alone or in combination with VE303 at ascending doses. The results showed that VE303 was safe and well-tolerated at all doses tested. No serious adverse events were reported, and the drug did not affect the pharmacokinetics of vancomycin. The study also demonstrated that VE303 was able to suppress the growth of *C. difficile* to levels comparable with that of *Clostridium bifermentans*, a commensal bacterium with anti-*C. difficile* activity.[36] The safety and tolerability of VE303 were assessed in healthy volunteers with vancomycin-induced dysbiosis through a phase 1 dose-escalation study. In a dose-dependent manner, VE303 safely and effectively restored the microbiome composition that was disrupted by antibiotics, and was well-tolerated.[37] A randomized, double-blind, placebo-controlled, dose ranging phase 2 study was performed in which patients were assigned to high dose VE303, low dose VE303 or placebo given as capsules daily for two weeks It included a total of 79 adult patients with prior history of one or more episodes of CDI in the past 6 months or patients with primary CDI with high risk of recurrence (age 65 years and above with one or more risk factors to acquire CDI or age 75 and above). The 8-week CDI recurrence was 13.8% (86.2% effective) for high dose VE303, 37% (63% effective) for low dose VE 303 and 45.5% (54.5% effective) for placebo.[38]

RBX7455

RBX7455 manufactured by Rebiotix, a Ferring Company is a lyophilized, nonfrozen capsule-based therapy derived from the same process used to manufacture REBYOTA. It is administered as an oral capsule without need of bowel preparation. The safety and efficacy of RBX7455 were evaluated in phase 1 open label, single arm dose-ranging study. In this study, 30 adults ages 18 or more with one or more prior CDI episodes were included. The patients must have had a positive stool for PCR or EIA for *C. difficile* toxin and must have no active diarrhea following standard of care antibiotic treatment of CDI. Subjects who had prior treatment with FMT or RBL were excluded. The patients were divided into three dosing schedules (four capsules of RBX7455 twice daily for 4 days – Group 1; four capsules of RBX7455 twice daily for 2 days – Group 2; and two capsules twice daily for 2 days – Group 3). |Eight-week recurrence free was noted in 90% in Group 1, 80% in Group 2 and 100% in Group 3. Microbiome content at 6 months revealed an increase in the content of Bacteroides and nonpathogenic clostridia.[39,40]

Conclusions

Microbiota based LBP have now demonstrated both clinical efficacy and safety in preventing recurrent CDI in the populations studied. The next steps will include assessing their efficacy in more complicated patients with underlying diseases such as irritable bowel syndrome, inflammatory bowel disease and immunocompromised people and those with underlying comorbidities not assessed in prior trials. These LBPs have been studied following a treatment course with standard antibiotic therapy for the initial CDI. Questions regarding the timing of delivery of the LBPs, need for antimicrobial washout, and optimal route of delivery are still to be answered. These questions will need to be answered by further trials. These new agents mark the beginning of a new era of the use of microbiota therapeutics to address diseases triggered by dysbiosis. Since the microbiota of the gut play important roles in inflammatory, immunological, and neurologic conditions, their role as therapeutic agents will be worthy of far more exploration.

Disclosure

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References

1. Centers for Disease Control and Prevention. Clostridioides difficile infection. Available from: https://www.cdc.gov/media/releases/2015/p0225-clostridium-difficile.html. Accessed February 21, 2023.
2. Lessa FC, Mu Y, Bamberg WM, et al. Burden of Clostridium difficile infection in the United States. *N Engl J Med*. 2015;372(9):825–834. doi:10.1056/NEJMoa1408913
3. Hall AJ, Curns AT, McDonald LC, Parashar UD, Lopman BA. The roles of Clostridium difficile and norovirus among gastroenteritis-associated deaths in the United States, 1999–2007. *Clin Infect Dis*. 2012;55(2):216–223. doi:10.1093/cid/cis386

4. Centers for Disease Control and Prevention. CDC Healthcare Associated Infections: Clostridiodes difficile infection. Available from: https://www.cdc.gov/hai/organisms/cdiff/cdiff_infect.html. Accessed February 21 21, 2023.
5. Marchesi JR, Ravel J. The vocabulary of microbiome research: a proposal. *Microbiome*. 2015;3:31. doi:10.1186/s40168-015-0094-5
6. Gordon J, Knowlton N, Relman DA, Rohwer F, Youle M. Superorganisms and holobionts. *Microbe*. 2013;8(4):152–153.
7. Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. The evolution of the host microbiome as an ecosystem on a leash. *Nature*. 2017;548 (7665):43–51. doi:10.1038/nature23292
8. Dawkins JJ, Allegretti JR, Gibson TE, et al. Gut metabolites predict Clostridioides difficile recurrence. *Microbiome*. 2022;10(1):87. doi:10.1186/s40168-022-01284-1
9. Crobach MJT, Ducarmon QR, Terveer EM, et al. The bacterial gut microbiota of adult patients infected, colonized or noncolonized by Clostridioides difficile. *Microorganisms*. 2020;8(5):677. doi:10.3390/microorganisms8050677
10. Robinson JI, Weir WH, Crowley JR, et al. Metabolomic networks connect host-microbiome processes to human Clostridioides difficile infections. *J Clin Invest*. 2019;129(9):3792–3806. doi:10.1172/JCI126905
11. Ajami NJ, Cope JL, Wong MC, Petrosino JF, Chesnel L. Impact of oral fidaxomicin administration on the intestinal microbiota and susceptibility to Clostridium difficile colonization in mice. *Antimicrob Agents Chemother*. 2018;62(5). doi:10.1128/aac.02112-17
12. Thorpe CM, Kane AV, Chang J, Tai A, Vickers RJ, Snydman DR. Enhanced preservation of the human intestinal microbiota by ridinilazole, a novel Clostridium difficile-targeting antibacterial, compared to vancomycin. *PLoS One*. 2018;13(8):e0199810. doi:10.1371/journal.pone.0199810
13. Okhuysen PC, Ramesh M, Garey KW, et al. 730. A Phase 3, randomized, double-blind study to evaluate the efficacy and safety of ridinilazole compared with vancomycin for the treatment of Clostridioides difficile infection. *Open Forum Infect Dis*. 2022;9(Supplement_2). doi:10.1093/ofid/ofac492.021
14. Palleja A, Mikkelsen KH, Forslund SK, et al. Recovery of gut microbiota of healthy adults following antibiotic exposure. *Nat Microbiol*. 2018;3 (11):1255–1265. doi:10.1038/s41564-018-0257-9
15. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery*. 1958;44 (5):854–859.
16. Seekatz AM, Safdar N, Khanna S. The role of the gut microbiome in colonization resistance and recurrent Clostridioides difficile infection. *Therap Adv Gastroenterol*. 2022;15:17562848221134396. doi:10.1177/17562848221134396
17. 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 (7):987–994. doi:10.1093/cid/ciy149
18. Tariq R, Hayat M, Pardi D, Khanna S. Predictors of failure after fecal microbiota transplantation for recurrent Clostridioides difficile infection: a systematic review and meta-analysis. *Eur J Clin Microbiol Infect Dis*. 2021;40(7):1383–1392. doi:10.1007/s10096-021-04163-z
19. Quraishi MN, Widlak M, Bhala N, et al. Systematic review with meta-analysis: the efficacy of faecal microbiota transplantation for the treatment of recurrent and refractory Clostridium difficile infection. *Aliment Pharmacol Ther*. 2017;46(5):479–493. doi:10.1111/apt.14201
20. US Food and Drug Administration. Guidance for Industry: Enforcement policy regarding investigational new drug requirements for use of fecal microbiota for transplantation to treat Clostridoides difficile infection not responsive to standard treatments. Available from: https://www.fda.gov/media/86440/download. Accessed February. 21, 2023
21. US Food and Drug Administration. Guidance for Industry: Early clinical trials with live biotherapeutic products:chemistry, manufacturing, and control information. Available from: https://www.fda.gov/files/vaccines,%20blood%20%26%20biologics/published/Early-Clinical-Trials-With-Live-Biotherapeutic-Products–Chemistry–Manufacturing–and-Control-Information–Guidance-for-Industry.pdf. Accessed February 17, 2023.
22. 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. doi:10.1093/cid/civ938
23. 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(1):245. doi:10.1186/s12879-022-07256-y
24. Orenstein R. The role of microbiome-based therapeutics in Clostridioides difficile infection: durable, long-term results of RBX2660. *Infect Dis Ther*. 2023;12(1):1–7. doi:10.1007/s40121-022-00714-9
25. Dubberke ER, Orenstein R, Khanna S, Guthmueller B, Lee C. Final results from a Phase 2b randomized, placebo-controlled clinical trial of RBX2660: a microbiota-based drug for the prevention of recurrent Clostridioides difficile infection. *Infect Dis Ther*. 2023;12(2):703–709. doi:10.1007/s40121-022-00744-3
26. Khanna S, Assi M, Lee C, et al. Efficacy and safety of RBX2660 in PUNCH CD3, a Phase III, randomized, double-blind, placebo-controlled trial with a bayesian primary analysis for the prevention of recurrent Clostridioides difficile infection. *Drugs*. 2022;82(15):1527–1538. doi:10.1007/s40265-022-01797-x
27. Khanna S, Pardi DS, Kelly CR, et al. A novel microbiome therapeutic increases gut microbial diversity and prevents recurrent Clostridium difficile infection. *J Infect Dis*. 2016;214(2):173–181. doi:10.1093/infdis/jiv766
28. 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(12):2132–2140. doi:10.1093/cid/ciaa387
29. 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(3):220–229. doi:10.1056/NEJMoa2106516
30. Khanna S, Sims M, Louie TJ, et al. SER-109: an oral investigational microbiome therapeutic for patients with Recurrent Clostridioides difficile Infection (rCDI). *Antibiotics*. 2022;11(9):1234. doi:10.3390/antibiotics11091234
31. Sims MD, Khanna S, Feuerstadt P, et al. Safety and tolerability of SER-109 as an investigational microbiome therapeutic in adults with recurrent Clostridioides difficile infection: a Phase 3, open-label, single-arm trial. *JAMA Network Open*. 2023;6(2):e2255758. doi:10.1001/jamanetworkopen.2022.55758
32. Khanna S, Kelly CR, Louie T, et al. S131 CP101, an Investigational orally administered microbiome therapeutic, increases intestinal microbiome diversity and prevents recurrent C. difficile Infection: results from a randomized, placebo-controlled trial. *Off J Am Coll Gastroenterol*. 2021;116: S57. doi:10.14309/01.ajg.0000772996.83378.7c
33. Allegretti J, Kelly C, Louie T. An investigational oral microbiome drug, CP101, for the prevention of recurrent C. difficile infection: a randomized, placebo-controlled, multi-center trial (PRISM3). Late Break Abstr ACG. 2020;

34. Allegretti JR, Kelly CR, Fischer M, Cohen T, Budree S, Khanna S. Tu1519: CP101, an investigational microbiome therapeutic for the prevention of recurrent C. difficile infection: a combined analysis of the PRISM3 (Randomized Placebo-Controlled) and PRISM-EXT (Open-Label) trials. *Gastroenterology*. 2022;162(7):S-995-S–996.
35. ClinicalTrials.gov. A trial of CP101 for the Prevention of Recurrent CDI (PRISM4). Available from: https://clinicaltrials.gov/ct2/show/NCT05153499. Accessed February. 16, 2023
36. Dsouza M, Menon R, Crossette E, et al. Colonization of the live biotherapeutic product VE303 and modulation of the microbiota and metabolites in healthy volunteers. *Cell Host Microbe*. 2022;30(4):583–598.e8. doi:10.1016/j.chom.2022.03.016
37. Bobilev D, Bhattarai S, Menon R, et al. 1953. VE303, a rationally designed bacterial consortium for prevention of recurrent Clostridioides difficile (C. difficile) infection (rCDI), Stably restores the gut microbiota after vancomycin (vanco)-induced dysbiosis in Adult Healthy Volunteers (HV). *Open Forum Infect Dis*. 2019;6(Suppl2):S60. doi:10.1093/ofid/ofz359.130
38. Louie T, Golan Y, Khanna S, et al. VE303, a defined bacterial consortium, for prevention of recurrent Clostridioides difficile infection: a randomized clinical trial. *JAMA*. 2023;329(16):1356–1366. doi:10.1001/jama.2023.4314
39. Khanna S. Microbiota restoration for recurrent Clostridioides difficile: getting one step closer every day! *J Intern Med*. 2021;290(2):294–309. doi:10.1111/joim.13290
40. Khanna S, Pardi DS, Jones C, Shannon WD, Gonzalez C, Blount K. RBX7455, a non-frozen, orally administered investigational live biotherapeutic, is safe, effective, and shifts patients' microbiomes in a Phase 1 study for recurrent Clostridioides difficile infections. *Clin Infect Dis*. 2021;73(7):e1613–e1620. doi:10.1093/cid/ciaa1430