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Live Biotherapeutic Products Comparison

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Abstract: This comprehensive analysis highlights the emerging microbiota-based therapies—Rebyota, Vowst, VE303, and CP101—that are being used to manage recurrent *Clostridium difficile* infection, particularly following antibiotic failure. The Firmicutes/Bacteroidetes ratio is emphasized as a key biomarker for gut health, with therapies focusing on restoring this balance to enhance clinical outcomes. Factors affecting treatment choice include patient's ability to tolerate administration and specific dietary influences on microbiome composition. Although notable progress has been made, including efficacy results demonstrating improved treatment outcomes associated with established and emerging microbiome therapies, uncertainties regarding optimal applications persist, necessitating ongoing research to refine these therapeutic strategies. Understanding variations in composition, administration routes, and individual patient factors will remain critical in clinical decision-making as the landscape of microbiota-based therapies evolves.

Keywords: Recurrent CDI; LBP's; firmicutes; bacteroides

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Recurrent *Clostridioides difficile* infections (rCDIs) pose a public health threat, with researchers seeking ways to restore the gut microbiome after it has been disrupted by antibiotics or chemotherapy [1]. The first-line treatment for primary *C difficile* infections is also an antibiotic, with commonly prescribed options including fidaxomicin or vancomycin. Disappointingly, however, these drugs are associated with a higher risk of rCDI [2].

Traditionally, the last resort and lifesaving treatment for rCDI is fecal microbiota transplantation (FMT) using donor stool. Historically, this was the only available approach, but it was a non-standardized method with unknown long-term effects [3]. A multi-omics approach was used to study FMT for rCDI. The collection of longitudinal stool samples was performed before FMT and at 2 weeks, 2 months, and 6 months post-FMT. Metagenomics, metabolomics, and lipidomics were combined to form a multi-omics approach. This study attempts to standardize FMTs mechanism

and to determine what creates a successful FMT [4]. Safety was the primary concern, since there is no clear documentation as to whether an FMT can transmit infectious diseases. Hence, live biotherapeutic products (LBPs) such as CP101, Rebyota and Vowst were developed [5]. Vowst and Rebyota are currently approved by the Food and Drug Administration (FDA). CP101 clinical results fell short in their phase 3 trial, but all the FDA approved products and CP101 showed more uniform consistency from batch to batch. Another potential option in the clinical pipeline is VE303, a defined microbial consortium that is still under research and development. The goal is that these targeted strains in the consortium will lead to higher engraftment rates and better outcomes.

Clinical trials and papers investigated how these therapies restore gut diversity. They focused not only on bacterial populations but also on the metabolites and bile acids that play a role in controlling *C difficile* infections. Rebyota and Vowst both went through phase 3 trials and achieved good safety profiles. The known side effects were mild gastrointestinal (GI) disturbance without any major drug-related adverse events (AEs) [6]. This GI disturbance is a known AE related to improving microbial diversity and, overall, the engraftment process. However, a question arises as to how engraftment causes a GI disturbance in the short term.

Overall, the research surrounding LBPs reveals a significant push to standardize and understand how microbiome alterations can finally break the vicious cycle of rCDI. It is evident that all 4 therapies aim to regenerate the gut microbiota, and they do so with varying degrees of efficacy, accessibility, and predictability.

Firmicutes/Bacteroidetes Ratio

Restoring a balanced Firmicutes/Bacteroidetes (F/B) ratio is the goal of effective microbiome therapies.

- VE303 and Vowst directly increase Firmicutes, raising the F/B ratio.
- FMT and Rebyota, while variable depending on donor and recipient profiles, generally normalize the F/B ratio by restoring taxonomic diversity.
- In irritable bowel syndrome, a reduction in the elevated F/B ratio post-FMT correlates with symptom relief, suggesting disease-specific targets for modulation.

As a biomarker for gut health, inflammation, and metabolic balance, the F/B ratio has been a consistent factor in the following:

- A higher F/B ratio is associated with obesity, diabetes mellitus, and metabolic syndrome, while a lower F/B ratio is linked to inflammatory bowel disease (IBD) and non-alcoholic fatty liver disease [2].
- In breast cancer, a lower F/B ratio (mean 2.0) was observed compared to healthy individuals (mean 5.7), suggesting its potential role as a risk factor or biomarker for breast cancer development [7].
- The F/B ratio varies with age. It was observed to be low in infants (0.4), high in adults (10.9), and low again in the elderly (0.6), suggesting that it might reflect both developmental and degenerative stages of the microbiota ecosystem [6].
- The F/B ratio was proposed as a monitoring tool for inflammation and therapeutic response in diseases like IBD. For instance, the ratio increased after infliximab (Remicade) or vedolizumab (Entyvio) treatment in Crohn's disease and ulcerative colitis, corresponding with clinical improvement [8].
- Vegetarian diets were associated with a higher F/B ratio, while meat-based diets were associated with a lower ratio, indicating that dietary habits influence microbiome balance and, potentially, disease risk [9].

Comparative Review: Rebyota vs Vowst vs VE303 vs CP101

When discussing the management of rCDI, especially after standard antibiotic treatments fail, the newer microbiota-based therapies, Rebyota, Vowst, CP101 and the investigational VE303, are not interchangeable therapeutically. Therefore, understanding their compositions, mechanisms, costs, storage requirements, and routes of administration is important when making clinical decisions or trying to understand this evolving field.

Rebyota

Composition

Rebyota is a fecal microbiota suspension derived from human donor stool. It includes whole stool, which means that it contains a wide array of bacteria, not just spores or selected strains. Because it is whole stool, Rebyota will always display an appropriate balance of the bacterial phyla, Bacteroidetes and Firmicutes. These 2 phyla have shown to be critical in terms of replenishing the microbiome, eliminating dysbiosis, and bile acid conversion.

Rebyota has shown efficacy in rCDI, a condition marked by microbiota depletion. Although specific data on F/B ratio alterations were not reported, Rebyota's engraftment and the shift toward donor-like microbiota imply an ecological restoration, likely influencing the F/B ratio favorably, in line with improved colonization resistance [4].

Efficacy

PUNCH CD3 and CD3-OLS inclusion required that participants were over the age of 18, with medically documented recurrent CDI that included a primary episode followed by at least 1 recurrence or 2 severe CDI hospitalizations within the last year. In addition, all patients had to have adequate control of their diarrhea prior to administration. In the PUNCH CD3 and CD3-OLS studies, Rebyota achieved approximately 73% treatment success in immunocompromised patients and 76% in immunocompetent patients in terms of preventing rCDI at 8 weeks [10–13]. At 6 months, in the modified analysis of only patients who were recurrence-free, over 88% and 91% patients, respectively, showed a sustained response for the immunocompromised and immunocompetent subgroups. The results are notable even among mildly to moderately immunocompromised individuals. Rebyota's safety profile was favorable, with no systemic infections reported and most AEs classified as mild to moderate GI symptoms [11–13].

In the ROAR registry, which included 76 patients who received fecal microbiota, live-jslm (RBL) within 30 days of completing antibiotics, treatment success over a period of 8 weeks was observed in 82.9% of patients, with AEs occurring in 23.7% and serious AEs in 7.9%. Only 3.9% of AEs were considered related to RBL. The study also included a notable real-world population, with 31.6% being immunosuppressed, underscoring the treatment's applicability across vulnerable subgroups [14].

Complementing this, a phase 3b study demonstrated a notably higher treatment success rate of 95% over the same 8-week period when RBL was administered via colonoscopy, with only 9.8% of patients reporting mild, primarily GI AEs [15]. This trial enrolled 41 adults, most of whom were white females with a history of multiple CDI episodes. Administration via colonoscopy allowed for targeted delivery and mucosal assessment [15].

Mechanism

This treatment relies on the traditional "brute force" approach. It introduces a diverse living microbial community, which aims to re-establish colonization, thereby providing resistance against *C difficile*.

The definite bacterial components responsible are not extracted or singled out. It is more of a broad attempt to restore a whole, functional ecosystem.

Cost and Storage

Rebyota is stored frozen and requires a cold-chain infrastructure. Administration is clinic-based, since it is delivered as a 150 mL enema [7]. Treatment is generally expensive given the complexity of stool processing and donor screening. It has an estimated cost of US $9150 per treatment [9].

Administration Route

Rebyota is administered via rectal enema in an office setting. No bowel preparation is necessary prior to treatment [4]. The route of administration may be unpleasant or impractical for many patients because of issues with comfort and access. Recently, data were published regarding colonoscopy-delivered Rebyota [15]. Although this has been an off-label method of administration since the product came to market, this is the first publication regarding this route of administration. For patients who have no other alternatives, this colonoscopy-based method provides an additional option.

Vowst

Composition

Vowst, initially known as SER-109, is a more refined product than Rebyota. It does not involve the whole stool but rather specific isolates of Firmicutes spores from donor feces. By focusing only on spores, use of Vowst attempts to minimize the risk of transmitting unwanted pathogens through treatment. It also aims to target the types of bacteria that are most helpful in restoring colonization resistance against *C difficile*.

While the F/B ratio was not explicitly measured in trials, the deliberate use of Firmicutes spores directly and substantially elevates the F/B ratio, correcting the Firmicutes depletion caused by antibiotic use, a known risk factor for CDI.

Efficacy

A phase 3 randomized, placebo-controlled trial demonstrated that SER-109 significantly reduced rates of rCDI in patients with at least 1 recurrence. It demonstrated that SER-109 reduced CDI recurrence by restoring colonization resistance, primarily through the engraftment of Firmicutes species that metabolize bile acids and outcompete *C difficile*. Patients receiving SER-109 had a recurrence rate of 11.1% compared to 37.3% in the placebo group at 8 weeks, which was statistically significant. Additionally, SER-109 was well tolerated, with a safety profile comparable to placebo [7].

Mechanism

The addition of *Firmicutes* spores can modulate bile acid metabolism. This shifts the bile acid pool toward the desired secondary bile acids that inhibit *C difficile* spore germination. Furthermore, these spores help regenerate the short-chain fatty acids (SCFAs) that maintain gut health. The long-term impact on restoring a microbiome that is targeted toward only Firmicutes is currently unknown. In addition, we do not know the impact this product has on the F/B ratio within the colonic flora.

Cost and Storage

Vowst is stored at refrigerator temperatures. Since it is available as an oral capsule formulation, patients can administer this treatment at home. Pricing is estimated at US $17,500 per treatment course [7]. The higher pricing is likely due to the additional processing and standardization steps that are required to isolate and purify the spores prior to administration.

Administration Route

Vowst is available as an oral capsule formulation. Typical dosing schedule is 4 capsules daily for 3 consecutive days. The regimen is to begin 2–4 days after the last antibiotic dose has been completed. Magnesium citrate is given 1 day prior to administration as a bowel preparation. This bowel prep can occasionally be an issue for patients; since diarrhea is frequent symptom of rCDI, stimulating this can be anxiety-inducing. It is still unknown whether a bowel prep is needed if the washout period (2–4 days) would be increased to the longer duration of potentially 4–7 days.

VE303

Composition

VE303 is different from the 2 FDA-approved LBPs. It has a defined consortium of 8 spore-forming bacterial strains. These strains are grown from pure clonal cell banks [13,16]. This means it is entirely manufactured and not derived from stool. VE303 is the first major move toward a "designed" or synthetic microbiome therapy rather than donor-dependent therapy. This treatment, however, is still under evaluation.

Although the F/B ratio is not directly quantified, VE303 includes multiple strains from the Firmicutes phylum, such as *Blautia*, *Dorea*, and *Flavonifractor*. Their engraftment is expected to increase the F/B ratio, which aligns with recovery from dysbiosis and suppression of pro-inflammatory Bacteroidetes overgrowth commonly observed in CDI.

Efficacy

In a phase 2 CONSORTIUM trial, high-dose VE303 reduced rCDI by over 80% compared to placebo. Inclusion for this study was defined as those at high risk of recurrent CDI and who had at least 1 documented *C difficile* bout which responded to standard-of-care antibiotics. Clinical efficacy was tightly linked to strain colonization, particularly VE303-08, and patients with $\geqslant$5 colonizing strains had significantly higher recurrence-free probability. VE303 also accelerated microbiome recovery post-antibiotics, evidenced by increased production of SCFAs, secondary bile acids, and bile salt hydrolase genes, all of which enhance colonization resistance to *C difficile* [13,17].

Mechanism

Each of the 8 strains included in its composition were selected for specific properties. These include bile acid transformation, competition for *C difficile* nutrients, and production of SCFAs. In phase 2 studies, it was found that not all strains engraft well, and only one, VE303-08, showed a strong link to a positive clinical response [13,16].

Storage

Since it is still in development, VE303 is not available outside of clinical studies. It is formulated as a lyophilized consortium. Enteric-coated capsules contain equal colony-forming units of each strain drug substance. Both individual strain drug substances with the drug product are stable at the recommended storage temperature between refrigerator temperature (2 °C to 8 °C) or frozen between −15 °C and −25 °C [7].

Administration Route

This treatment is available as an oral capsule formulation. The dosing regimen involves taking 10 capsules once daily for 14 days [1]. There is no bowel prep necessary. Phase 2 data showed that prolonged dosing is important to ensure adequate engraftment. Compliance may become its real challenge because of the sheer number of capsules required to be taken daily during the 2-week regimen.

CP101

Composition

CP101 is a fecal microbiota suspension derived from a human donor stool. Although similar in composition to Rebyota, this treatment is produced in an oral capsule formulation.

Efficacy

A multicenter, randomized, double-blind, placebo-controlled phase 2 trial evaluated the efficacy and safety of CP101, a full-spectrum oral microbiome therapeutic, for the prevention of rCDI in adults with a history of recurrence. The study included 198 participants, randomized to receive either CP101 or placebo following standard-of-care antibiotics [1].

Patients eligible to participate in this study exhibited an adequate response to standard-of-care therapy, plus at least 1 recurrence of CDI, and were at least 65 years old, or patients who reported at least 2 recurrences and were over 18 years of age. At week 8, 74.5% of participants in the CP101 group were free of CDI recurrence compared to 61.5% in the placebo group, a statistically significant difference (p = 0.0488). This benefit was sustained through week 24, with 73.5% of CP101-treated participants remaining recurrence-free versus 59.4% in the placebo arm (p = 0.0347). Subgroup analysis showed similar efficacy among participants experiencing their first CDI recurrence and those with 2 or more prior recurrences, indicating that CP101 may be effective regardless of recurrence history [1,16].

The safety profile of CP101 was comparable to that of placebo. Through week 8, 93.3% of CP101 recipients and 91.9% of those receiving placebo experienced treatment-emergent AEs, most of which were mild GI symptoms. By week 24, treatment-emergent AEs were reported in 94.2% of CP101 participants and 93.9% in the placebo group. No treatment-related serious adverse events or deaths occurred in the CP101 group throughout the study period [1,17].

Importantly, severely immunocompromised individuals were excluded from the trial. These included patients undergoing initiation or escalation of immunosuppressive therapies, individuals with HIV infection and CD4 counts below 200 cells/mm^3, and those with neutropenia [1].

Storage

CP101 is stored refrigerated; any additional information is unknown, as it has not been brought to market.

Administration Route

The administration route of CP101 is 10 capsules taken orally for 14 days on an empty stomach. The total daily dose is to be taken within a 60-minute period of time.

Factors to Consider When Choosing Therapies

Selecting the correct therapy in today's landscape of LBPs is difficult and involved careful precision. Some factors for consideration include:

- An inability to take oral capsules (consider Rebyota)
- An inability to ambulate onto an exam table for a rectal enema (consider VE303, CP101 or Vowst)
- An inability to retain a rectal enema (consider VE303, CP101 or Vowst)
- An inability to tolerate bowel prep (consider VE303, Rebyota, or CP101)
- An inability to perform a washout prior to starting treatment (consider VE303)

Table: Comparative Summary of Characteristics

Feature	Rebyota	Vowst	VE303	CP101
Composition	Fecal microbiota suspension from human donor stool; whole stool processed under GMP	Purified Firmicutes spores isolated from donor feces via ethanol treatment	Defined consortium of 8 spore-forming bacterial strains from pure clonal cell banks	Fecal microbiota suspension from human donor stool; whole stool processed under GMP
Mechanism	Broad-spectrum microbial restoration; re-establishes colonization resistance	Targets bile acid metabolism; modulates bile acids to inhibit *C difficile* and regenerate SCFAs	Engineered mechanism; bile acid transformation, nutrient competition, SCFA production; only some engraft	Broad-spectrum microbial restoration; re-establishes colonization resistance
Cost and storage	~US $9150 per treatment; stored frozen in ultra-cold conditions (−80 °C); clinic-based cold-chain required	~US $17,500 per course; stored refrigerated; patient self-administered	Not priced yet; lyophilized; can be stored frozen or refrigerated	Unknown

Table: *Cont.*

Feature	Rebyota	Vowst	VE303	CP101
Administration route	Rectal enema or colonoscopy-delivered; no bowel prep needed	Oral capsules; 4 per day for 3 days; starts 2–4 days post-antibiotics; bowel prep with magnesium citrate	Oral capsules; 10 per day for 14 days; no bowel prep mentioned; compliance may be an issue	Oral capsules; 10 capsules as a single dose
Insurance coverage	Buy/bill product through medical benefit	Filled through pharmacy benefit	Unknown; likely pharmacy benefit	Unknown
FDA status	Approved	Approved	Phase 3 clinical trial—not yet approved	Phase 3 clinical trial— not yet approved

Abbreviations: *Clostridioides difficile*, *C difficile*; FDA, Food and Drug Administration; GMP, good manufacturing practice; SCFAs, short-chain fatty acids.

Conclusion

Clostridioides difficile infection treatment is still an evolving landscape. Rebyota, Vowst, and VE303 represent distinct attempts to restore a damaged gut microbiome. Each of these products offers a different operating principle, from broad, natural restoration with Rebyota to the more targeted, spore-based approach of Vowst, to the engineered accuracy and precision of VE303.

The importance of the F/B ratio from a Firmicutes-only compound like Vowst or VE303 is still unknown. An imbalance in this ratio has been linked to inflammatory bowel disease, metabolic syndromes, and various other health conditions. The benefits of eliminating *C difficile* from colonizing the GI tract likely outweigh any risk regarding the ratio.

Regardless, all products aim to reduce recurrence, but differences in composition and future data regarding long-term sustained clinical cure will likely determine which product will be utilized. The products' therapeutic positions in terms of the number of previous episodes are still unknown and are individualized to each patient. As our experience matures, all products will likely have a place in therapy as the importance of individualized care will be needed to provide our patients with the best possible potential outcomes.

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