ORIGINAL ARTICLE

SER-109, an Oral Microbiome Therapy for Recurrent *Clostridioides difficile* Infection

Paul Feuerstadt, M.D., Thomas J. Louie, M.D., Bret Lashner, M.D., Elaine E.L. Wang, M.D., Liyang Diao, Ph.D., Jessica A. Bryant, Ph.D., Matthew Sims, M.D., Ph.D., Colleen S. Kraft, M.D., Stuart H. Cohen, M.D., Charles S. Berenson, M.D., Louis Y. Korman, M.D., Christopher B. Ford, Ph.D., Kevin D. Litcofsky, Ph.D., Mary-Jane Lombardo, Ph.D., Jennifer R. Wortman, M.Sc., Henry Wu, Ph.D., John G. Auniņš, Ph.D., Christopher W.J. McChalicher, B.Ch.E., Jonathan A. Winkler, Ph.D., Barbara H. McGovern, M.D., Michele Trucksis, M.D., Ph.D., Matthew R. Henn, Ph.D., and Lisa von Moltke, M.D.

ABSTRACT

BACKGROUND

Current therapies for recurrent *Clostridioides difficile* infection do not address the disrupted microbiome, which supports *C. difficile* spore germination into toxin-producing bacteria. SER-109 is an investigational microbiome therapeutic composed of purified Firmicutes spores for the treatment of recurrent *C. difficile* infection.

METHODS

We conducted a phase 3, double-blind, randomized, placebo-controlled trial in which patients who had had three or more episodes of *C. difficile* infection (inclusive of the qualifying acute episode) received SER-109 or placebo (four capsules daily for 3 days) after standard-of-care antibiotic treatment. The primary efficacy objective was to show superiority of SER-109 as compared with placebo in reducing the risk of *C. difficile* infection recurrence up to 8 weeks after treatment. Diagnosis by toxin testing was performed at trial entry, and randomization was stratified according to age and antibiotic agent received. Analyses of safety, microbiome engraftment, and metabolites were also performed.

RESULTS

Among the 281 patients screened, 182 were enrolled. The percentage of patients with recurrence of *C. difficile* infection was 12% in the SER-109 group and 40% in the placebo group (relative risk, 0.32; 95% confidence interval [CI], 0.18 to 0.58; P<0.001 for a relative risk of <1.0; P<0.001 for a relative risk of <0.833). SER-109 led to less frequent recurrence than placebo in analyses stratified according to age stratum (relative risk, 0.24 [95% CI, 0.07 to 0.78] for patients <65 years of age and 0.36 [95% CI, 0.18 to 0.72] for those ≥65 years) and antibiotic received (relative risk, 0.41 [95% CI, 0.22 to 0.79] with vancomycin and 0.09 [95% CI, 0.01 to 0.63] with fidaxomicin). Most adverse events were mild to moderate and were gastrointestinal in nature, with similar numbers in the two groups. SER-109 dose species were detected as early as week 1 and were associated with bile-acid profiles that are known to inhibit *C. difficile* spore germination.

CONCLUSIONS

In patients with symptom resolution of *C. difficile* infection after treatment with standard-of-care antibiotics, oral administration of SER-109 was superior to placebo in reducing the risk of recurrent infection. The observed safety profile of SER-109 was similar to that of placebo. (Funded by Seres Therapeutics; ECOSPOR III ClinicalTrials.gov number, NCT03183128.)

From Yale University School of Medicine, New Haven, and PACT Gastroenterology Center, Hamden — both in Connecticut (P.F.); the University of Calgary and Foothills Medical Centre, Calgary, AB, Canada (T.J.L.); Cleveland Clinic, Cleveland (B.L.); Seres Therapeutics, Cambridge, MA (E.E.L.W., L.D., J.A.B., C.B.F., M.-J.L., K.D.L., J.R.W., H.W., J.G.A., C.W.J.M., J.A.W., B.H.M., M.T., M.R.H., L.M.); Beaumont Hospital, Royal Oak, Royal Oak, and Oakland University William Beaumont School of Medicine, Rochester — both in Michigan (M.S.); Emory University, Atlanta (C.S.K.); the University of California, Davis, Davis (S.H.C.); the University at Buffalo and Veterans Affairs Western New York Healthcare System — both in Buffalo (C.S.B.); and Capital Digestive Care, Washington, DC (L.Y.K.). Dr. McGovern can be contacted at bmcgovern@serestherapeutics.com or at Seres Therapeutics, 200 Sidney St., Cambridge, MA 02139.

N Engl J Med 2022;386:220-9.
DOI: 10.1056/NEJMoa2106516

CLOSTRIDIOIDES DIFFICILE INFECTION HAS been estimated to be associated with more than 460,000 cases of illness and 20,000 deaths annually in the United States.[1,2] Clinical outcomes remain suboptimal because current therapies do not address fundamental aspects of the two-phase life cycle of this pathogen or disease pathogenesis.[3] Vancomycin and fidaxomicin lead to symptom relief by killing toxin-producing *C. difficile* bacteria that cause colonic inflammation and debilitating diarrhea.[4,5] However, antibiotic agents do not kill *C. difficile* spores, which can rapidly germinate into toxin-producing vegetative bacteria after treatment discontinuation.[3]

The main risk factor for *C. difficile* infection is exposure to broad-spectrum antibiotics, which leads to compositional and functional changes in the gastrointestinal microbiome.[6] Within healthy microbial communities, spore-forming bacteria of the phylum Firmicutes modulate the production and consumption of metabolites that are important to host defense and colonization resistance. For example, when concentrations of secondary bile acids exceed those of primary bile acids, *C. difficile* spore germination is inhibited. However, antibiotic-induced loss of beneficial Firmicutes bacteria leads to increases in primary bile-acid concentrations, which enables *C. difficile* spore germination and a cycle of recurrent disease.[6,7] Other demographic risk factors include older age, female sex, the presence of coexisting conditions, use of proton-pump inhibitors, and a history of recurrence.[8-11]

A durable clinical response is dependent on the resilience of the microbiome — that is, the recovery of these beneficial resident bacteria after discontinuation of antibiotic treatment. Most recurrences occur within days to a few weeks after completion of an antibiotic regimen, during a "window of vulnerability" when antibiotic-induced microbiome disruption facilitates *C. difficile* spore germination.[12] Antibiotics are necessary but not sufficient to achieve a sustained clinical response, thus highlighting the need for a two-pronged treatment approach that includes microbiome repair.[13,14]

Studies of fecal microbiota transplantation for recurrent *C. difficile* infection after antibiotic therapy have shown proof of concept that the recovery of bowel flora is associated with an improved clinical response.[15,16] However, the range of efficacy of fecal microbiota transplantation is wide, with lower cure rates observed in controlled trials than in open-label studies (i.e., 68% vs. 83%).[17] A more important issue is that fecal microbiota transplantation carries a risk of transmission of undetected or emerging pathogens that may lead to hospitalization or death.[18-21]

A Quick Take is available at NEJM.org

SER-109, an investigational oral microbiome therapeutic composed of live purified Firmicutes bacterial spores, was developed to reduce the risk of *C. difficile* infection recurrence.[14] We hypothesized that these spore-forming bacteria would compete metabolically with *C. difficile* for essential nutrients, modulate bile-acid profiles to reestablish resistance to colonization, or have both of these effects. Here, we report the 8-week efficacy and safety results from a phase 3 trial involving patients with recurrent *C. difficile* infection, along with supportive microbiome engraftment and metabolomic analyses.

METHODS

TRIAL DESIGN AND OVERSIGHT

We conducted ECOSPOR III (SER-109 versus Placebo in the Treatment of Adults with Recurrent *Clostridium difficile* Infection), a double-blind, placebo-controlled trial, in accordance with Good Clinical Practice guidelines at 56 U.S. and Canadian sites from July 2017 through September 2020. The protocol and amendments, available with the full text of this article at NEJM.org, were approved by institutional review boards at all trial sites. Written informed consent was obtained from trial participants at screening. The authors had full access to the data under statements of confidentiality. The authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol.

TRIAL PARTICIPANTS AND PROCEDURES

The trial consisted of a screening period of approximately 3 weeks before randomization and an 8-week efficacy period after randomization, followed by a 16-week follow-up period. The trial included patients 18 years of age or older who had had three or more episodes of *C. difficile* infection within 12 months, inclusive of the qualifying acute episode, which was defined as three or more unformed bowel movements over 2 consecutive days, a positive *C. difficile* toxin test, and resolution of symptoms while receiving 10 to 21 days of standard-of-care antibiotic therapy. Patients were required to test positive for *C. difficile* toxin by enzyme immunoassay at a local certi-

fied laboratory or by enzyme immunoassay or cell cytotoxicity neutralization assay at a central laboratory (Eurofins).

Patients were stratified according to age (<65 or ≥65 years) and antibiotic received for *C. difficile* infection (vancomycin or fidaxomicin) before randomization. Patients were randomly assigned in a 1:1 ratio to receive SER-109 (approximately 3×10^7 spore colony-forming units) or placebo administered as four matching oral capsules once daily over 3 consecutive days. Because vancomycin and fidaxomicin can persist for up to 5 to 7 days after discontinuation, 10 ounces of magnesium citrate was administered the night before treatment to limit inactivation of SER-109 dose species (i.e., species of bacteria present in SER-109).[12] Patients were monitored after randomization for 8 weeks for recurrence, which was defined as onset of three or more unformed bowel movements per day over 2 consecutive days, a positive *C. difficile* stool toxin assay (enzyme immunoassay or cell cytotoxicity neutralization assay), an assessment by the investigator that treatment was warranted, and persistence of diarrhea until antibiotic treatment was initiated. Adverse events were evaluated during clinic visits at weeks 2 and 8 and with telephone calls at weeks 1 and 3 through 7. Patients reported solicited adverse events using diary cards for 7 days after completion of the course of SER-109 or placebo. Although the trial had a planned sample size of 188 patients, the last patient underwent randomization on April 13, 2020, after 182 patients had been enrolled, because of the coronavirus disease 2019 (Covid-19) pandemic.

Stool specimens for whole metagenomic sequencing and targeted bile-acid analyses were obtained at baseline (within 3 days after completion of the antibiotic regimen) and at weeks 1, 2, and 8. Full details of the trial design are provided in the protocol and statistical analysis plan.

MANUFACTURE AND CHARACTERIZATION OF SER-109

The donor screening program and manufacturing program were reviewed by the Food and Drug Administration and are consistent with their recent publication.[22] Before donating stool, four donors underwent an extensive health examination, including personal and family medical history, laboratory chemical and hematologic screening, urinalysis, and viral, bacterial, and parasite testing of blood and stool; donated stool was obtained before December 1, 2019.[14] Donors completed physical examinations, questionnaires, and laboratory testing during and after the donation period before material was released for manufacturing. Capsules were produced in accordance with Good Manufacturing Practice regulations, including clearance of vegetative (non–spore form) bacteria, fungi, parasites, and viruses through solvent treatment and by sequential purification steps and bioburden testing.[14] The taxonomic composition of the bacteria identified in SER-109 is shown in Table S3 in the Supplementary Appendix, available at NEJM.org.

PRESPECIFIED EFFICACY, SAFETY, AND MICROBIOME END POINTS

The primary efficacy objective was to show the superiority of SER-109 as compared with placebo in reducing the risk of *C. difficile* infection recurrence up to 8 weeks after dosing (the primary efficacy end point). Patients whose condition did not meet the criteria for recurrent *C. difficile* infection were considered to have a sustained clinical response. The safety and the acceptability of the side-effect profile of SER-109 as compared with placebo were evaluated up to 8 weeks after dosing.

Exploratory microbiome-related analyses included evaluations of changes in species composition and bile-acid concentrations in the SER-109 group as compared with the placebo group from baseline to weeks 1, 2, and 8 after dosing. Engraftment was defined as the number of SER-109 dose species detected in post-treatment specimens that had not been present at baseline. Engraftment was evaluated with the use of species profiles generated from whole metagenomic sequencing, and stool bile-acid concentrations were measured by means of liquid chromatography–mass spectrometry.

STATISTICAL ANALYSIS

Efficacy analyses were performed in the intention-to-treat population, which included all patients who underwent randomization. Because of the logistics of delivering drug or placebo at some trial sites, the interactive response system was programmed in a blinded fashion to allow randomization to skip the original assignment on the basis of the available site supply, which occurred for six patients (see the Supplementary Appendix). However, in the intention-to-treat

analysis, these patients were analyzed according to the intended assignment rather than the drug received. Safety was assessed in the as-treated population.

Patients who were lost to follow-up, discontinued participation in the trial prematurely, or died without a recurrence of *C. difficile* infection before 8 weeks after treatment were defined as having a *C. difficile* infection recurrence. For the primary efficacy end point, two null hypotheses were tested sequentially at the two-sided 0.05 α level; if the null hypothesis that the relative risk would be greater than or equal to 1.0 was rejected, then a null hypothesis that the relative risk would be greater than or equal to 0.833 was tested. The primary efficacy analysis was performed with the use of the Cochran–Mantel–Haenszel test of the relative risk with SER-109 as compared with placebo, stratified according to age (<65 years or ≥65 years) and previous antibiotic regimen for the qualifying episode (vancomycin or fidaxomicin). For the primary end point, P values and confidence intervals are reported. For other end points, the confidence intervals are reported without adjustment for multiplicity. The logarithm of the Cochran–Mantel–Haenszel estimate of the common relative risk is approximately normal, and the variance estimate was based on the method of Greenland and Robins.[23]

In addition, as a post hoc analysis, primary efficacy was analyzed with the use of multiple imputation rather than with the imputation of *C. difficile* infection recurrence that was prespecified in the statistical analysis plan. The imputation was performed 100 times with the distribution within each group. The multivariate model for multiple imputation included age (<65 years or ≥65 years), sex (male or female), number of *C. difficile* infection episodes (two, three, or more than three), antibiotic regimen (vancomycin or fidaxomicin), and use of proton-pump inhibitors (yes or no). After each imputation, Cochran–Mantel–Haenszel methods were used to perform an efficacy analysis, and estimates from each imputation were combined with the use of Rubin's rule.[24]

Microbiome compositional and targeted metabolomic analyses for primary and secondary bile acids were performed in the safety population. Specimens that passed quality control (see the Supplementary Appendix) and paired baseline and post-treatment specimens were included in the analysis. The distributions of engraftment and secondary bile-acid measurements within each group are presented with nonparametric median and interquartile range statistics.

Table 1. Demographic and Clinical Characteristics of the Patients at Baseline (Intention-to-Treat Population).*

Characteristic	SER-109 (N=89)	Placebo (N=93)
Age — yr	65.6±16.5	65.5±16.7
Age group — no. (%)		
<65 years	41 (46)	38 (41)
≥65 years	48 (54)	55 (59)
Female sex — no. (%)	60 (67)	49 (53)
Race or ethnic group — no. (%)†		
Asian	1 (1)	0
Black	4 (4)	4 (4)
White	82 (92)	88 (95)
Other	2 (2)	1 (1)
Hispanic or Latino		
Yes	5 (6)	6 (6)
No	84 (94)	87 (94)
Episodes of *C. difficile* infection, including qualifying episode — no. (%)		
3	49 (55)	61 (66)
≥4	39 (44)	32 (34)
Missing data	1 (1)	0
Previous antibiotic regimen — no. (%)		
Vancomycin	64 (72)	69 (74)
Fidaxomicin	25 (28)	24 (26)

* Plus–minus values are means ±SD. Demographic characteristics (with the exception of sex) and risk factors for *Clostridioides difficile* infection were balanced between the groups.
† Race and ethnic group were reported by the patients.

RESULTS

PATIENT POPULATION

Of the 281 patients who were screened, 182 were enrolled, underwent randomization, and were included in the intention-to-treat efficacy and safety population. The mean age of the enrolled patients was 65.5 years, 93% were White, and 99% were outpatients. Demographic characteristics and risk factors for *C. difficile* infection were balanced between the groups, although women made up a larger percentage of patients in the SER-109 group than in the placebo group (67% and 53%, respectively) (Table 1). Of the 182 en-

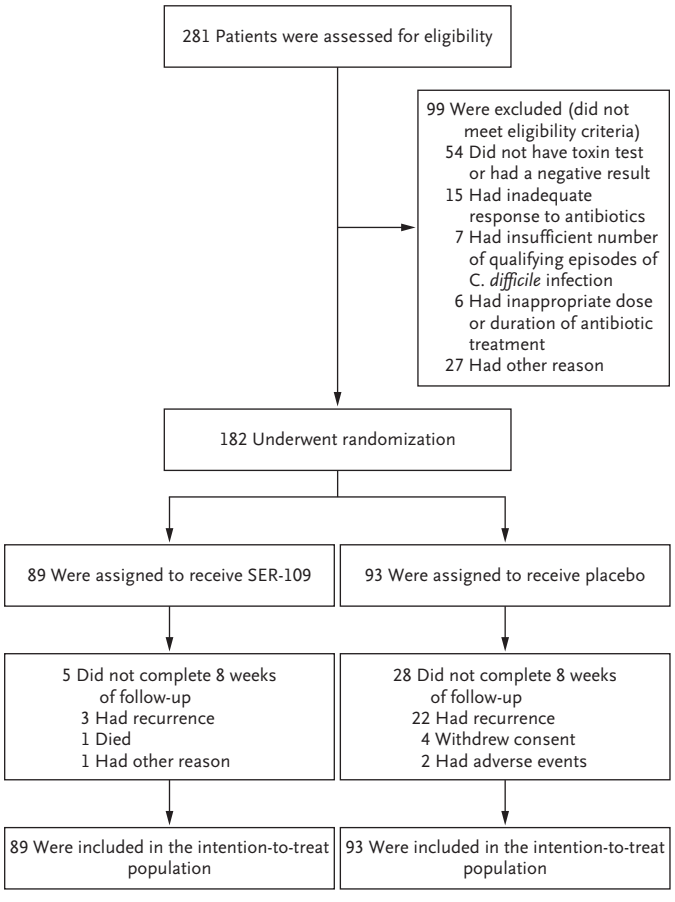

Figure 1. Screening, Randomization, and Follow-up through 8 Weeks.

The intention-to-treat population was evaluated in the analysis of the primary efficacy objective. Other reasons for exclusion before randomization included an inability to adhere to the protocol (5 patients), concurrent receipt of antibiotics for infection other than *Clostridioides difficile* (5), inability to receive SER-109 or placebo within 4 days after completion of antibiotic therapy (5), admission to an intensive care unit (4), investigator discretion (2), absence of informed consent (2), suspected toxic megacolon or small-bowel ileus (1), gastrointestinal surgery within 3 months (1), history of fecal microbiota transplantation (1), and patient preference to continue receiving probiotics (1). Three deaths occurred in the SER-109 group, all of which were reported by the investigator as being unrelated to SER-109; two of the patients had onset of fatal adverse events within the 8-week period after dosing, but only one of these two patients died during that period (see the Supplementary Appendix).

rolled patients, 149 (82%) completed 8 weeks of follow-up. Five of the 89 patients (6%) in the SER-109 group and 28 of 93 (30%) in the placebo group withdrew before week 8. The most common reason for withdrawal from the trial was recurrence of *C. difficile* infection, which was more common in the placebo group than in the SER-109 group (24% and 3%, respectively) (Fig. 1).

EFFICACY

The primary efficacy objective was met: SER-109 was found to be superior to placebo in reducing the risk of *C. difficile* infection recurrence. The percentage of patients with recurrence was significantly lower in the SER-109 group than in the placebo group (12% and 40%, respectively; relative risk, 0.32; 95% confidence interval [CI], 0.18 to 0.58; P<0.001 for both hypotheses tested) (Fig. 2A). In the analysis of the alternative metric of sustained clinical response, 88% of the SER-109 recipients were found to have a sustained clinical response, as compared with 60% of the placebo recipients (Fig. 2B).

SER-109 also led to lower percentages of patients with *C. difficile* infection recurrence than did placebo in the age-stratified analysis (relative risk, 0.24 [95% CI, 0.07 to 0.78] among patients <65 years of age and 0.36 [95% CI, 0.18 to 0.72] among those ≥65 years of age) and in the antibiotic-stratified analysis (relative risk, 0.41 [95% CI, 0.22 to 0.79] among patients who took vancomycin and 0.09 [95% CI, 0.01 to 0.63] among those who took fidaxomicin) (Fig. 2C and 2D). However, more patients were treated with vancomycin than with fidaxomicin.

In addition, multiple imputation was used to impute primary end-point data for 9 patients with missing data (3 of 89 in the SER-109 group and 6 of 93 in the placebo group). Estimates of primary efficacy when multiple imputation was used were consistent with those in the prespecified primary analysis (relative risk, 0.28; 95% CI, 0.14 to 0.56; P<0.001 for both hypotheses tested).

All four lots of SER-109 led to less frequent recurrence than did placebo. However, the power to detect differences among SER-109 lots was limited by the small numbers of patients who received each lot (range, 12 to 33 patients).

Most recurrence events occurred rapidly, with onset as early as day 4 after randomization. Of the 48 recurrences that occurred in the overall trial population by week 8, a total of 36 (75%) occurred within 2 weeks and 41 (85%) occurred within 4 weeks after administration of SER-109 or placebo.

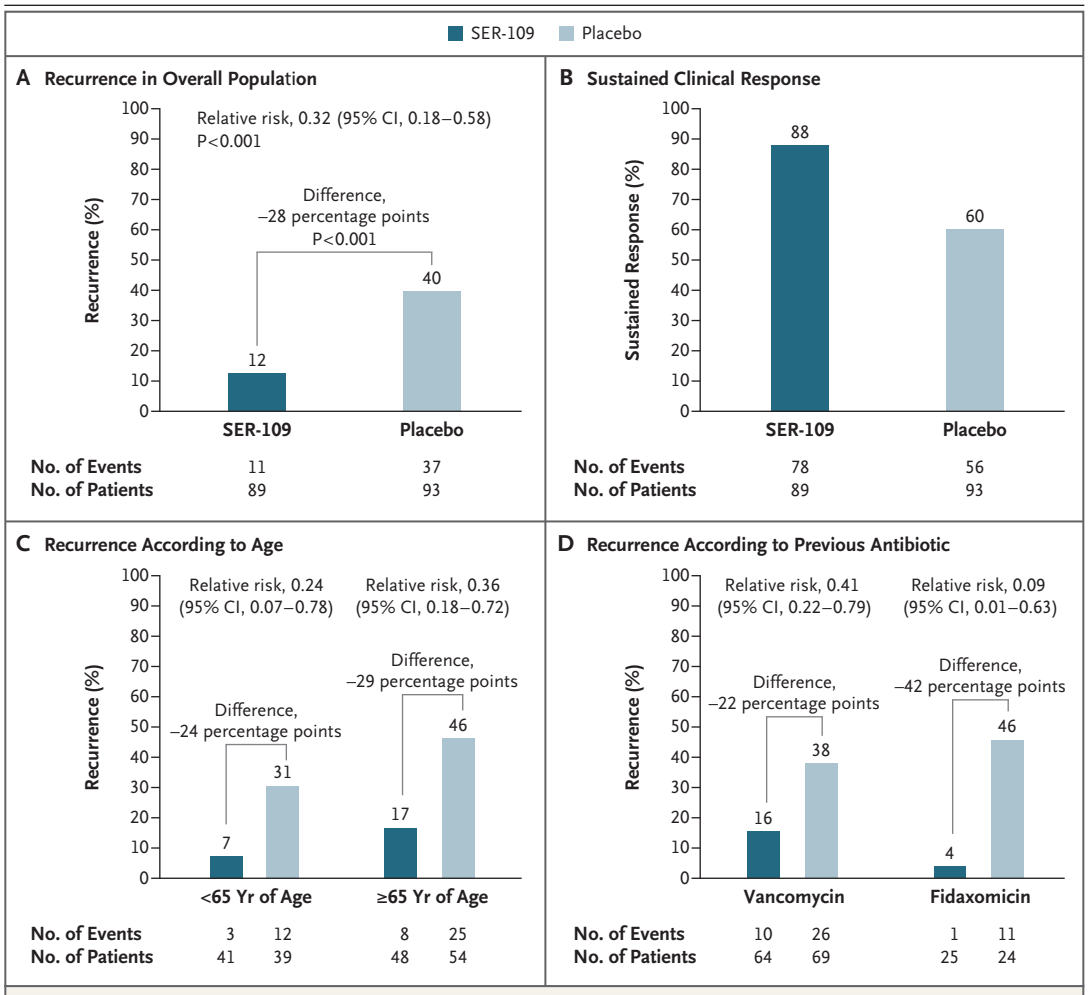

Figure 2. Recurrence of *C. difficile* Infection up to 8 Weeks (Intention-to-Treat Population).

End points up to 8 weeks after dosing are shown. The results of the primary efficacy analysis show superiority of SER-109 as compared with placebo in the reduction of the risk of *C. difficile* infection recurrence (Panel A). Results of analyses with stratification according to age (Panel C) and antibiotic received (Panel D) are also shown. Relative risk is defined as the percentage of patients with recurrence in the SER-109 group divided by the percentage in the placebo group; analyses were performed with the Cochran–Mantel–Haenszel estimate of the common relative risk, stratified according to age group (<65 years or ≥65 years) and antibiotic regimen received for the qualifying acute episode (vancomycin or fidaxomicin). Panel B shows the analysis of sustained clinical response, defined as an absence of recurrence treated with antibiotics through 8 weeks. Patients who were lost to follow-up, who left the trial prematurely, or who died without a recorded recurrence before the end of 8 weeks were assumed to have had a recurrence.

SAFETY

No serious adverse events that were assessed by the site investigator as being related to SER-109 were observed through week 8 (Table S1). Adverse events that were related or possibly related to SER-109 or placebo occurred in slightly more than half of the patients in each group (Table 2). The most common adverse events were gastrointestinal disorders, the majority of which were mild to moderate in nature. Three deaths occurred in the SER-109 group, none of which were deemed by the investigators, who were unaware of the trial-group assignments, to be drug-related (Table 2 and the Supplementary Appendix).

Table 2. Adverse Events through 8 Weeks (Safety Population).*

Adverse Event	SER-109 (N=90)	Placebo (N=92)
	no. of patients (%)	
Any adverse event	84 (93)	84 (91)
Adverse event related or possibly related to SER-109 or placebo	46 (51)	48 (52)
Serious adverse event†	7 (8)	15 (16)
Adverse event of special interest that occurred or worsened after initiation of SER-109 or placebo	1 (1)	1 (1)
Serious adverse event or an adverse event of special interest that occurred or worsened after initiation of SER-109 or placebo and was related or possibly related to SER-109 or placebo	0	0
Serious adverse event leading to withdrawal from the trial	0	1 (1)
Adverse event leading to death‡	2 (2)	0
Adverse events reported in ≥5% of patients		
Gastrointestinal disorders	79 (88)	80 (87)
Flatulence	63 (70)	70 (76)
Abdominal distension	49 (54)	49 (53)
Abdominal pain	46 (51)	56 (61)
Constipation	28 (31)	22 (24)
Diarrhea	22 (24)	20 (22)
Nausea	16 (18)	30 (33)
Vomiting	3 (3)	10 (11)
General disorders and administration site conditions	57 (63)	65 (71)
Fatigue	53 (59)	58 (63)
Chills	21 (23)	22 (24)
Metabolism and nutrition disorders	28 (31)	36 (39)
Decreased appetite	26 (29)	34 (37)
Infections and infestations	18 (20)	14 (15)
Urinary tract infections	6 (7)	1 (1)
C. difficile colitis	1 (1)	7 (8)
Musculoskeletal and connective-tissue disorders	7 (8)	5 (5)
Nervous system disorders	7 (8)	4 (4)
Injury, poisoning, and procedural complications	4 (4)	6 (7)
Respiratory, thoracic, and mediastinal disorders	4 (4)	6 (7)
Renal and urinary disorders	3 (3)	5 (5)

* Adverse events were coded with the use of the *Medical Dictionary for Regulatory Activities*, version 20.0. Adverse events of special interest included invasive infections such as bacteremia, meningitis, and abscess.
† Many of the serious adverse events were related to the primary end point of recurrent *C. difficile* infection, which was more common in the placebo group than in the SER-109 group (see Table S1).
‡ Three deaths occurred in the SER-109 group, all of which were reported by the investigator as being unrelated to SER-109; two of the patients had onset of fatal adverse events within the 8-week period after dosing, but only one of these two patients died during that period (see the Supplementary Appendix).

COMPOSITIONAL AND METABOLOMIC CHANGES IN THE MICROBIOME

Of the 182 patients, 29 were excluded from the metagenomic and metabolomic analyses because of missing specimens or protocol deviations. Engraftment of SER-109 dose species was seen by week 1 and persisted through week 8. The number of SER-109 dose species observed in the

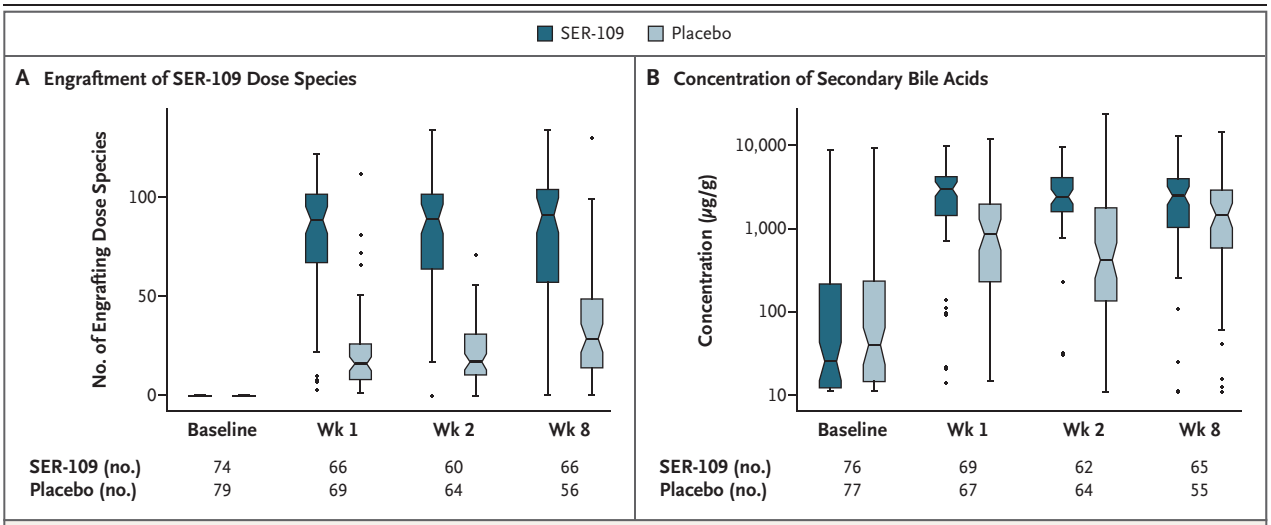

Figure 3. Compositional and Metabolomic Changes in the Microbiome after Receipt of SER-109 or Placebo.

Box plots show the median (central notched horizontal line) and interquartile range (shaded box) in each group at each time point; I bars indicate the most extreme non-outlier values (within 1.5 times the interquartile range), and plus signs indicate outlier values (outside 1.5 times the interquartile range). The number of engrafting dose species (Panel A) and the concentration of pooled secondary bile acids (Panel B) detected in patient stool specimens collected at baseline, week 1, week 2, and week 8 after receipt of SER-109 or placebo are shown. Species engraftment is defined as detection of species after treatment but not at baseline. The number of specimens available for analysis depended on the availability of baseline and post-treatment samples in addition to quality filtering. The figure shows observed data only. Multiple imputation was used in another analysis to impute any missing values (Fig. S2).

placebo group gradually increased after week 1 as a result of recovery of Firmicutes bacteria after discontinuation of antibiotic treatment; however, numbers of engrafting SER-109 dose species remained higher among SER-109 recipients through week 8 (Fig. 3). In parallel, greater increases in secondary bile acids from baseline were observed in the SER-109 group than in the placebo group at all time points through week 8 (Table S2). Compositional changes after dosing with SER-109 as compared with placebo showed declines in proinflammatory Enterobacteriaceae bacteria and increases in Firmicutes bacteria, such as those in the families Ruminococcaceae and Lachnospiraceae (Fig. S1).

DISCUSSION

Clinical outcomes in recurrent *C. difficile* infection may be improved by using a two-pronged treatment approach: antibiotics to kill toxin-producing bacteria followed by a microbiome therapeutic to inhibit *C. difficile* spore germination and bacterial replication through microbiome repair. In this trial, SER-109, an investigational oral live microbiome therapeutic, was superior to placebo in reducing the risk of *C. difficile* infection recurrence. The lower incidence of recurrence achieved with SER-109 administered after treatment with standard-of-care antibiotics represents a 68% lower risk than that with antibiotics alone and a number needed to treat of 3.6 to avoid one recurrence of *C. difficile* infection. Sustained clinical response occurred in a higher percentage of patients in the SER-109 group than in the placebo group (88% vs. 60%), which indicates the importance of microbiome repair in patients with a history of recurrence. Most recurrences of *C. difficile* infection during this 8-week time frame were observed within days to 2 weeks after the completion of antibiotic treatment, which highlights the need for early microbiome intervention.

The benefit of SER-109 as compared with placebo in patients with recurrent disease was also observed among age- and antibiotic-stratified groups. A reduction in the risk of recurrence among patients 65 years of age or older is clinically important, since patients in this age group are at increased risk for recurrent disease and hospital readmission.[25] SER-109 also reduced the risk of recurrence regardless of the selected anti-

biotic. Although fidaxomicin is viewed as less disruptive than vancomycin to microbial communities, the higher percentages of recurrence in the fidaxomicin–placebo subgroup highlight the paradox of treating an antibiotic-associated disease, rooted in microbiome disruption, with antibiotics alone.[26,27]

Through week 8, SER-109 had an observed safety profile similar to that of placebo. No serious adverse events or deaths were considered to be drug-related. This observed safety profile of SER-109 might be expected, since spore-forming Firmicutes bacteria are abundant in healthy microbiomes.[28] In addition, the SER-109 manufacturing process delivers the effective microbial components while mitigating risk of transmitting undetected or emerging pathogens, a difference from other fecal microbiota transplantation–like investigational products, which are reliant on donor screening alone.

Engraftment of spore-forming Firmicutes bacteria is a necessary first step for the production of microbe-associated metabolites, such as secondary bile acids, which inhibit *C. difficile* spore germination and vegetative growth.[29,30] These compositional and functional changes reflect the pharmacokinetics and pharmacodynamics of this microbiome therapeutic.[14] As expected, Firmicutes bacteria were gradually detected by week 8 among placebo recipients after discontinuation of antibiotic treatment.[31] However, microbiome recovery was slower and insufficient to prevent *C. difficile* infection recurrence among some patients in this group. In contrast, SER-109 led to faster engraftment, enabling a more sustained clinical response.

The durability of engraftment through week 8 is an advantage of SER-109, since Firmicutes bacteria are a dominant component of the healthy microbiome.[32] The bile-acid data support one potential mechanism of action for SER-109 and the validity of this purified spore–based therapeutic approach.[33] Additional functional pathways are also under investigation, since microbiome therapeutics have the potential to affect multiple disease-relevant pathways because of the multifunctional ways in which these microbes interact with each other and the host.[34] These clinical data will inform the design of investigational cultivated microbiome therapeutics for *C. difficile* infection and other diseases.

Limitations of this trial include the low representation of minority populations; it is unclear from the literature whether this is related to a low incidence of recurrent *C. difficile* infection among Black and Hispanic patients or due to other factors.[4,35] The absence of a stool specimen before antibiotic treatment limits our understanding of the full effect of SER-109 on the preantibiotic microbiome. Strengths of the trial include the stringent diagnosis of *C. difficile* infection, with toxin testing performed at trial entry and at a suspected recurrence, ensuring appropriate candidate selection and accuracy of the definition of recurrence.[36,37] The primary efficacy results were consistent whether the prespecified imputation of *C. difficile* infection recurrence or multiple imputation was used, most likely because of the low rates of loss to follow-up or missing data. The trial population was restricted to patients who were at risk for recurrence (i.e., those with a recent acute infection and no prolonged use of vancomycin), in order to avoid artificial inflation of efficacy.[38,39] Furthermore, the analysis of the primary end point included 8 weeks of follow-up; if a shorter time frame had been used, 15% of recurrences would have been missed.

In patients with recurrent *C. difficile* infection, achievement of a sustained clinical response can be made more likely with a two-pronged treatment paradigm of antibiotics followed by a microbiome therapeutic. SER-109 was superior to placebo in reducing the risk of recurrence, with an observed safety profile similar to that of placebo. We continue to collect data from our open-label study, SERES-013. Insights into the pharmacologic properties of this oral microbiome therapeutic have implications not only for treatment of recurrent *C. difficile* infection but also for other diseases with pathogenesis that may be rooted in microbiome disruption.[40]

Supported by Seres Therapeutics.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank the Seres staff, including Asli Memisoglu, Sc.D., for statistical support; Kelly Brady, M.S., Kostia Franklin, B.A., and Tyce Havens, B.S., for clinical operations; Ananya De, Ph.D., and Robert Stevens, Pharm.D., for preparation and quality review of earlier versions of the figures, tables, and manuscript; Sarah Garant, M.S.E., for manufacturing oversight; and Jennifer Mahoney, Ph.D., for donor operations oversight; and all the patients who participated in the trial.

REFERENCES

1. Guh AY, Mu Y, Winston LG, et al. Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. N Engl J Med 2020;382:1320-30.
2. Dubberke ER, Olsen MA. Burden of Clostridium difficile on the healthcare system. Clin Infect Dis 2012;55:Suppl 2: S88-S92.
3. Theriot CM, Young VB. Interactions between the gastrointestinal microbiome and Clostridium difficile. Annu Rev Microbiol 2015;69:445-61.
4. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. N Engl J Med 2011;364:422-31.
5. Louie TJ, Cannon K, Byrne B, et al. Fidaxomicin preserves the intestinal microbiome during and after treatment of Clostridium difficile infection (CDI) and reduces both toxin reexpression and recurrence of CDI. Clin Infect Dis 2012;55: Suppl 2:S132-S142.
6. Theriot CM, Bowman AA, Young VB. Antibiotic-induced alterations of the gut microbiota alter secondary bile acid production and allow for Clostridium difficile spore germination and outgrowth in the large intestine. mSphere 2016;1(1):e00045-15.
7. Sorg JA, Sonenshein AL. Chenodeoxycholate is an inhibitor of Clostridium difficile spore germination. J Bacteriol 2009; 191:1115-7.
8. 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;67:649-56.
9. Guh AY, Adkins SH, Li Q, et al. Risk factors for community-associated *Clostridium difficile* infection in adults: a case-control study. Open Forum Infect Dis 2017;4:ofx171.
10. Shields K, Araujo-Castillo RV, Theethira TG, Alonso CD, Kelly CP. Recurrent Clostridium difficile infection: from colonization to cure. Anaerobe 2015;34:59-73.
11. Claesson MJ, Jeffery IB, Conde S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178-84.
12. Abujamel T, Cadnum JL, Jury LA, et al. Defining the vulnerable period for re-establishment of Clostridium difficile colonization after treatment of C. difficile infection with oral vancomycin or metronidazole. PLoS One 2013;8(10):e76269.
13. 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;214:173-81.
14. 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.
15. Staley C, Hamilton MJ, Vaughn BP, et al. Successful resolution of recurrent Clostridium difficile infection using freeze-dried, encapsulated fecal microbiota; pragmatic cohort study. Am J Gastroenterol 2017;112:940-7.
16. Jiang Z-D, Jenq RR, Ajami NJ, et al. Safety and preliminary efficacy of orally administered lyophilized fecal microbiota product compared with frozen product given by enema for recurrent Clostridium difficile infection: a randomized clinical trial. PLoS One 2018;13(11):e0205064.
17. 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;68:1351-8.
18. Food and Drug Administration. Safety alert regarding use of fecal microbiota for transplantation and additional safety protections pertaining to SARS-CoV-2 and COVID-19. March 23, 2020 (https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-additional-safety-protections).
19. Food and Drug Administration. Safety alert regarding use of fecal microbiota for transplantation and risk of serious adverse events likely due to transmission of pathogenic organisms. March 12, 2020 (https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/safety-alert-regarding-use-fecal-microbiota-transplantation-and-risk-serious-adverse-events-likely).
20. Blaser MJ. Fecal microbiota transplantation for dysbiosis — predictable risks. N Engl J Med 2019;381:2064-6.
21. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-resistant *E. coli* bacteremia transmitted by fecal microbiota transplant. N Engl J Med 2019;381:2043-50.
22. Carlson PE Jr. Regulatory considerations for fecal microbiota transplantation products. Cell Host Microbe 2020;27:173-5.
23. Greenland S, Robins JM. Estimation of a common effect parameter from sparse follow-up data. Biometrics 1985;41:55-68.
24. Schafer JL, Graham JW. Missing data: our view of the state of the art. Psychol Methods 2002;7:147-77.
25. Zilberberg MD, Shorr AF, Micek ST, Doherty JA, Kollef MH. Clostridium difficile-associated disease and mortality among the elderly critically ill. Crit Care Med 2009;37:2583-9.
26. Bartlett JG, Chang TW, Gurwith M, Gorbach SL, Onderdonk AB. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. N Engl J Med 1978;298:531-4.
27. Crowther GS, Wilcox MH. Antibiotic therapy and Clostridium difficile infection — primum non nocere — first do no harm. Infect Drug Resist 2015;8:333-7.
28. Lopetuso LR, Scaldaferri F, Petito V, Gasbarrini A. Commensal Clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog 2013;5:23.
29. Allegretti JR, Kearney S, Li N, et al. Recurrent Clostridium difficile infection associates with distinct bile acid and microbiome profiles. Aliment Pharmacol Ther 2016;43:1142-53.
30. Ridlon JM, Harris SC, Bhowmik S, Kang D-J, Hylemon PB. Consequences of bile salt biotransformations by intestinal bacteria. Gut Microbes 2016;7:22-39.
31. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol 2008;6(11):e280.
32. Browne HP, Forster SC, Anonye BO, et al. Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation. Nature 2016;533:543-6.
33. Bryant JA, Diao L, O'Brien EJ, et al. Rapid conversion of primary to secondary bile acids in subjects with recurrent *Clostridioides difficile* infection (CDI) following SER-109, an investigational microbiome therapeutic. Presented at Digestive Disease Week, virtual, May 21–23, 2021 (https://www.serestherapeutics.com/wp-content/uploads/2021/07/Bryant-J-DDW-2021-Rapid-Conversion-of-Primary-to-Secondary-Bile-Acids.pdf).
34. Henn MR, O'Brien EJ, Diao L, et al. A phase 1b safety study of SER-287, a spore-based microbiome therapeutic, for active mild to moderate ulcerative colitis. Gastroenterology 2021;160(1):115-127.e30.
35. Johnson S, Louie TJ, Gerding DN, et al. Vancomycin, metronidazole, or tolevamer for Clostridium difficile infection: results from two multinational, randomized, controlled trials. Clin Infect Dis 2014;59:345-54.
36. Polage CR, Gyorke CE, Kennedy MA, et al. Overdiagnosis of Clostridium difficile infection in the molecular test era. JAMA Intern Med 2015;175:1792-801.
37. Sethi AK, Al-Nassir WN, Nerandzic MM, Bobulsky GS, Donskey CJ. Persistence of skin contamination and environmental shedding of Clostridium difficile during and after treatment of C. difficile infection. Infect Control Hosp Epidemiol 2010;31:21-7.
38. Kelly CR, Khoruts A, Staley C, et al. Effect of fecal microbiota transplantation on recurrence in multiply recurrent Clostridium difficile infection: a randomized trial. Ann Intern Med 2016;165:609-16.
39. Patron RL, Hartmann CA, Allen S, et al. Vancomycin taper and risk of failure of fecal microbiota transplantation in patients with recurrent Clostridium difficile infection. Clin Infect Dis 2017;65:1214-7.
40. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. Nat Med 2018;24:392-400.