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A Novel Microbiome Therapeutic Increases Gut Microbial Diversity and Prevents Recurrent *Clostridium difficile* Infection

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(See the editorial commentary by Vehreschild and Cornely on pages 169–70.)

Background. Patients with recurrent *Clostridium difficile* infection (CDI) have a ≥60% risk of relapse, as conventional therapies do not address the underlying gastrointestinal dysbiosis. This exploratory study evaluated the safety and efficacy of bacterial spores for preventing recurrent CDI.

Methods. Stool specimens from healthy donors were treated with ethanol to eliminate pathogens. The resulting spores were fractionated and encapsulated for oral delivery as SER-109. Following their response to standard-of-care antibiotics, patients in cohort 1 were treated with SER-109 on 2 consecutive days (geometric mean dose, 1.7×10^9 spores), and those in cohort 2 were treated on 1 day (geometric mean dose, 1.1×10^8 spores). The primary efficacy end point was absence of *C. difficile*–positive diarrhea during an 8-week follow-up period. Microbiome alterations were assessed.

Results. Thirty patients (median age, 66.5 years; 67% female) were enrolled, and 26 (86.7%) met the primary efficacy end point. Three patients with early, self-limiting *C. difficile*–positive diarrhea did not require antibiotics and tested negative for *C. difficile* at 8 weeks; thus, 96.7% (29 of 30) achieved clinical resolution. In parallel, gut microbiota rapidly diversified, with durable engraftment of spores and no outgrowth of non–spore-forming bacteria found after SER-109 treatment. Adverse events included mild diarrhea, abdominal pain, and nausea.

Conclusions. SER-109 successfully prevented CDI and had a favorable safety profile, supporting a novel microbiome-based intervention as a potential therapy for recurrent CDI.

Keywords. *Clostridium difficile* infection; microbiome; dysbiosis; vancomycin-resistant *Enterococcus*; *Clostridium difficile* treatment.

Clostridium difficile infection (CDI) and its attendant complications, including diarrhea, pseudomembranous colitis, and toxic megacolon, are associated with an estimated 29 000 annual deaths in the United States and is recognized by the Centers for Disease Control and Prevention as an urgent public health priority [1]. Antibiotic exposure is the leading risk factor for CDI, and the risk of recurrent disease is increased among elderly patients and following antibiotic reexposure. Antibiotic therapy for recurrent CDI contributes to persistent disruption of the gut microbiome, which is the first-line defense against colonization and infection by pathogens, including *C. difficile* [2–5]. The risk of recurrence increases to >60% following a second episode [3, 6, 7].

Research has focused on the potential role that the human microbiome plays in health and disease. In 2008, the National Institutes of Health supported the creation of the Human Microbiome Project to characterize the species composition and function of the healthy microbiome. In the gut, the 2 dominant phyla are Firmicutes (ie, gram-positive organisms, including Bacilli and Clostridia) and Bacteroidetes (ie, gram-negative anaerobes, including *Bacteroides*, *Parabacteroides*, and *Prevotella*) [8, 9]. In contrast, gram-negative Enterobacteriaceae, such as *Escherichia coli*, make up only a fraction of the healthy microbiome [8]. There is also significant intersubject variability at both the genus and species level, suggesting that the bacterial communities in any one individual are unique, mirroring the complex interplay of diet, host genetics, immune response, and microbial coadaptation. Despite this variation, there are common core species found in a majority of healthy individuals, and metabolic pathways are preserved due to functional redundancy [10]. Thus, a wide range of microbiomes defines a healthy state.

In states of disease, there are also broad patterns that define gut dysbiosis, such as a loss of microbial diversity and increasing

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representation of gram-negative facultative anaerobes, such as Enterobacteriaceae [11, 12]. Antibiotic-induced dysbiosis underlies colonization and invasion by *C. difficile*, while repair of the microbiome, through fecal microbiota transplantation (FMT), is associated with efficacy rates of 81%–90% for those with recurrent CDI [13–16]. FMT involves transferring minimally processed, uncharacterized fecal material from a healthy donor to a recipient [17].

FMT administration is often invasive and requires donor screening and stool preparation. Despite donor screening, stool preparations for FMT have the potential to transmit infections due to pathogens that are present at times outside the period of detectability or for which diagnostic tests are unavailable; there is also the possibility of unwitting transmission of emerging pathogens that have not been identified to date [18, 19]. While there have been recent reports of stool delivered via oral encapsulated FMT or stool enemas, the data demonstrate first-dose efficacy of approximately 52%–70%, which is significantly lower than that for other modes of administration, such as colonoscopy [14, 20, 21]. In recognition of FMT as an experimental biologic, the Food and Drug Administration issued guidance that this intervention should only be used for prevention of recurrent CDI and after receipt of informed consent. An alternative approach for achieving improved safety and convenience with comparable efficacy is urgently needed [22].

SER-109 is composed of approximately 50 species of Firmicutes spores derived from stool specimens from healthy donors. After demonstrating the preclinical efficacy of SER-109 in rodent CDI models, we formulated it for oral delivery in humans based on the hypothesis that spore-forming organisms would compete metabolically with *C. difficile* for essential nutrients and/or bile acids [23–27]. In addition, spore purification with ethanol reduces the risk of transmission of other potential pathogens [28]. This initial study was designed to evaluate the efficacy and safety profile of SER-109 for CDI prevention in patients with recurrent infections and to measure alterations in the gut microbiota.

METHODS

Study Design

This open-label, single-arm, descending-dose study evaluated the safety, efficacy, and engraftment of SER-109 formulated for oral delivery. The study was sponsored by Seres Therapeutics and conducted at 4 US medical centers: Massachusetts General Hospital (Boston, Massachusetts), Mayo Clinic (Rochester, Minnesota), Miriam Hospital (Providence, Rhode Island), and Emory University Hospital (Atlanta, Georgia). The protocol was developed by investigators at Seres Therapeutics and authors of the current study (E. L. H., D. S. P., and S. K.) and was approved by the institutional review boards of the participating medical centers.

Study Population

Eligible patients were 18–90 years old and had ≥3 laboratory-confirmed CDI episodes in the previous 12 months, had a life expectancy of ≥3 months, and gave informed consent to receive this donor-derived product. Patients were excluded for a history of acute leukemia; hematopoietic stem cell transplantation, chemotherapy within 2 months and an absolute neutrophil count of <1000 neutrophils/mm^3, a history of inflammatory or irritable bowel disease, colectomy, cirrhosis, pregnancy/lactation, severe acute illness unrelated to CDI, antibiotic exposure for a non-CDI indication within 14 days of screening, or prior FMT.

Eligible patients had a clinical response to antibiotic therapy for their current CDI episode immediately prior to dosing and were neither anticipated to require admission to an intensive care unit nor expected to need antibiotics within 6 weeks following study entry.

Screening of Donors

Seven adult donors of stool specimens gave informed consent, underwent a complete medical history and laboratory assessment, and were screened for blood-borne and fecal pathogens, consistent with published protocols [29, 30]. Donors were excluded for being older than 50 years, having a body mass index (BMI; calculated as the weight in kilograms divided by the height in meters squared) of >25, engaging in high-risk behaviors as per a modified American Association of Blood Banks blood donor questionnaire [31], having a history of acute/chronic gastrointestinal disorders, or using antibiotics (in the previous 6 months), immunosuppressive/antineoplastic agents, or cigarettes (Supplementary Materials).

Preparation of SER-109

SER-109 comprises Firmicutes spores fractionated from stool specimens obtained from healthy donors. Donor stool specimens were processed separately to make unique batches of SER-109. Upon collection, stool specimens were frozen at −80°C. Approximately 150 g was suspended and homogenized in normal saline and filtered through mesh screens. The slurry was centrifuged, and supernatant containing bacterial cells and spores was combined with 100% ethanol to 50% (wt/wt) and incubated at room temperature for 1 hour to reduce risk of pathogen transmission to the recipient [28]. The supernatant was pelleted by centrifugation, washed with saline to remove ethanol, resuspended with sterile glycerol, and filled into capsules (hypromellose DRcaps, Capsugel), which were stored at −80°C.

The product was characterized for spore concentration and absence of residual gram-negative bacteria. Spore content was determined by measuring the dipicolinic acid (DPA) content and normalizing against the DPA content of known numbers of spores representing 3 commensal species [32]. The absence of residual gram-negative bacteria was confirmed by selective plating on MacConkey lactose agar and Bacteroides bile esculin agar. No vegetative microbes were found in any SER-109 preparation within the limit of assay detection (<30 colony-forming units/mL).

Treatment Protocol

Two days prior to dosing, patients discontinued antibiotics for CDI. One day prior to dosing, patients underwent a bowel preparation (to minimize residual antibiotic in the gastrointestinal tract), followed by overnight fasting. Two sites used a regimen of 300 mL of magnesium-citrate (one with Dulcolax), and 2 sites used polyethylene glycol.

Part 1 enrolled 15 patients who each received 30 capsules of SER-109 (observed dose of 15 capsules on day 0 and day 1). The dose of spores varied between 3×10^7 and 2×10^{10}, based on natural variations in spore concentration among healthy donors. Based on initial efficacy, 15 additional patients were enrolled in part 2 and treated with SER-109 capsules containing a lower fixed dose of 1×10^8 spores (approximately 17-fold lower than the geometric mean dose administered in part 1 and 3-fold above the minimum dose shown to be effective). Depending on spore content, patients received an observed dose of 1–12 capsules on day 0.

Any patient whose diarrhea recurred between 1 and 8 weeks was eligible for another dose of SER-109, based on data from the conventional FMT literature showing efficacy of a second dose [13, 14]. If a patient elected to receive a second dose of SER-109, the time course of study events was restarted concurrent with the second dose of SER-109.

Adverse events and recurrence of CDI symptoms were monitored through phone calls (on day 4 and weeks 1, 2, and 4) and in-clinic visits (on weeks 8 and 24). Patients were asked to provide a stool sample on day 4 and on weeks 1, 2, 4, 8, and 24 after treatment for genomic and culture-based analysis.

Clinical Outcomes

The primary end point was prevention of recurrent CDI during the 8-week follow-up after SER-109. CDI recurrence was defined as a composite end point of >3 unformed bowel movements in a 24-hour period and laboratory confirmation of *C. difficile* in the stool. Safety was evaluated by monitoring adverse events and assessing changes in laboratory values, vital signs, and physical examination findings over a 24-week period after dosing.

Alterations in Gut Microbiota Composition

The impact of SER-109 on gut microbiota was determined by examining stool samples before and after treatment for (1) engraftment by spore-forming species and (2) augmentation (outgrowth) of commensal bacteria not found in SER-109. Alterations in composition were measured by 16S ribosomal RNA (rRNA) genomic and culture-based analysis of patient fecal samples (Supplementary Materials). Engraftment was defined by newly detected spore formers in the patient after treatment, which were present in SER-109 but not detectable in the patient before treatment. Augmented bacteria were defined as non–SER-109 organisms whose levels increased at least 10-fold after treatment.

Table 1. Patient Demographic Characteristics, by Cohort

Characteristic	Cohort 1 (n = 15)	Cohort 2 (n = 15)
Age, y		
Mean ± SD	64.7 ± 19.6	59.1 ± 15.3
Median (range)	71.0 (22–88)	58.0 (39–83)
Sex, no. (%)		
Female	10 (66.7)	10 (66.7)
Male	5 (33.3)	5 (33.3)
BMI,[a] median (range)	23.3 (18.2–42.9)	26.6 (20.3–36.7)
Recurrences, no., median (range)	3 (2, 6)	3 (2, 5)
Time from initial *C. difficile* diagnosis, wks, median (range)	23.1 (8.0–726)	34.3 (6.3–318)
Time from most recent *C. difficile* recurrence, wks, median (range)	4.0 (1.9–23.3)	4.0 (0.3–16.3)

Abbreviation: *C. difficile, Clostridium difficile.*

[a] Body mass index (BMI) is calculated as the weight in kilograms divided the height in meters squared.

RESULTS

Patient Population

Thirty patients were enrolled after therapeutic response to appropriate CDI antibiotics (ie, vancomycin [n = 23], fidaxomicin [n = 5], metronidazole [n = 1], and rifaximin [n = 1]) was documented (Table 1). Patients had a median age of 66.5 years (range, 22–88 years), and the majority of subjects (67%) were female. The median time from the initial *C. difficile* diagnosis to the most recent recurrence was 23.1 weeks in cohort 1 and 34.3 weeks in cohort 2. In the overall study population, the median number of CDI recurrences was 3 (range, 2–6 recurrences). Infecting *C. difficile* strains were identified in 10 patients and included types BI, Y, and DH (Supplementary Table 1).

Complete blood counts and a chemistry panel (including liver function tests and analysis of albumin and creatinine levels) were performed at week 8 (for 27 of 30 patients) and at week 24 or early termination for 20 of 30 patients. No significant changes in laboratory findings were observed, with the exception of those for 1 patient, who had an elevated white blood cell count at week 8 at the time of diagnosis of a urinary tract infection.

Clinical Outcomes

Of the 30 patients who received SER-109, 26 (86.7%) achieved the primary end point of no *C. difficile*–positive diarrhea up to 8 weeks following dosing, with similar outcomes in both dosing cohorts (Figure 1). Of the patients who met the primary end point, 1 required a second dose of SER-109 for recurrence of *C. difficile*–positive diarrhea on day 26, as per protocol. Four patients who did not meet the primary end point had early onset of symptoms at days 3, 5, 7, and 9 after administration of SER-109 and laboratory confirmation of *C. difficile*. One of these patients declined a second SER-109 dose and chose not to continue participating in the study. Notably, the other 3 patients were determined by their primary investigator to be recovering from a

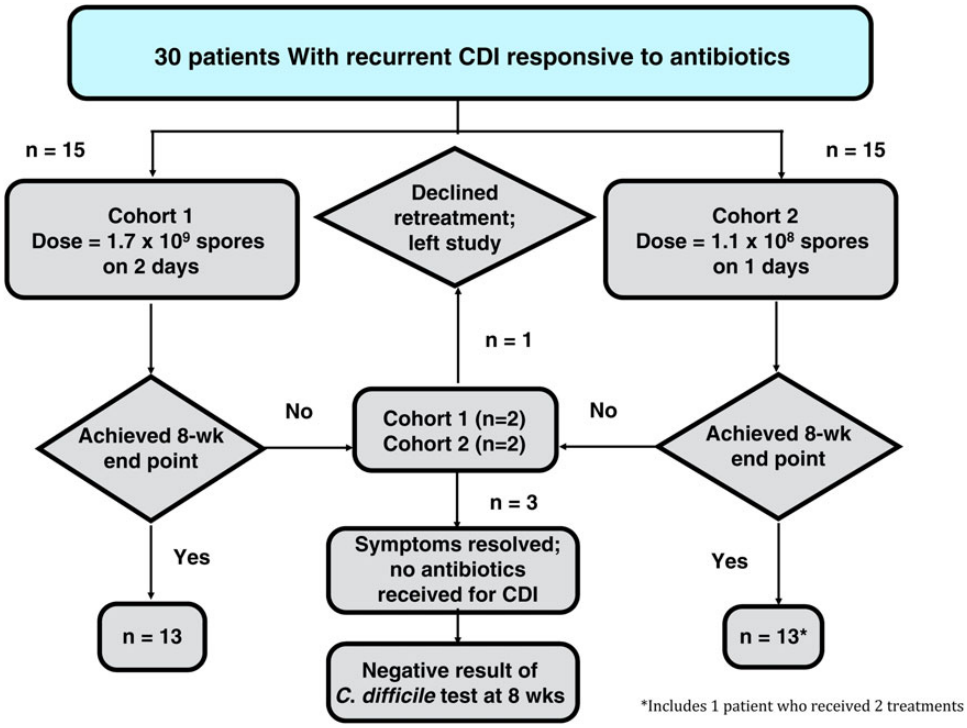

Figure 1. Patient flow chart and outcomes. Three patients tested positive for *Clostridium difficile* carriage on formed stool at week 8 but were asymptomatic through 24 weeks of follow-up, consistent with asymptomatic carriage/colonization, as is frequently observed for recovered patients [33]. Abbreviation: CDI, *C. difficile* infection.

self-limiting diarrheal episode at the time of stool submission for *C. difficile* testing. In each case, the investigators advised the patients to refrain from antibiotic use, and all symptoms resolved without any therapeutic intervention; stool samples from these 3 patients were negative for *C. difficile* carriage at 8 weeks, using a sensitive nucleic acid amplification test for detection of toxins A and B. Thus, 29 of 30 patients (96.7%) achieved clinical resolution of recurrent CDI following SER-109 administration.

During the safety phase (weeks 8–24), 1 patient discontinued the protocol for personal reasons, and 2 were lost to follow-up. The clinical response to SER-109 was durable over the safety period in 87% of the 23 patients who met the primary end point and remained in the study. Of the 3 patients who relapsed during the safety phase, one had received antibiotics for a non-CDI indication. Four other patients who were also exposed to antibiotics did not have a CDI recurrence.

SER-109 Sequencing and Characterization

To characterize SER-109 by using 16S rRNA sequencing, samples were sequenced to a depth of 22 500 operational taxonomic units (OTUs), at which point the rarefaction curves begin to plateau. As defined by 16S rRNA analysis, the spore community across all doses in this study comprised a mean (±SD) of approximately 50 ± 8 distinct OTUs.

Despite microbiome variation among the 7 donors, there was broad conservation of SER-109 at the taxonomic family level. In all 7 donors, the following families were detected by 16S sequencing: Clostridiaceae, Erysipelotrichaceae, Eubacteriaceae, Lachnospiraceae, Peptostreptococcaceae, and Ruminococcaceae. In 6 of 7 donors, the family Oscillospiraceae was demonstrated.

Safety Profile

SER-109 was well tolerated, and all patients took their full dose, with the exception of 1 patient. who did not take 1 capsule of the planned dose. Adverse events were observed in 12 of 15 patients (80%) in cohort 1 and 15 of 15 patients (100%) in cohort 2 (Table 2). Adverse events that were considered related to SER-109 occurred in 15 of 30 patients (50%), all of which were mild or moderate in severity and did not differ significantly by dosing group. The most common adverse events were diarrhea (n = 12), nausea (n = 9), and abdominal pain (n = 9). One severe adverse event and 7 serious adverse events were deemed by the investigators to be unrelated to the study drug (Table 2). There were no deaths and no significant changes in laboratory values (eg, glucose or lipid levels) or physical examination findings (eg, BMI) over 24 weeks.

Remodeling of the Gut Microbiota

Microbial diversity increased significantly at 8 weeks (as measured by the mean Chao1 index [±SD]) from 24 ± 8 at baseline to 31 ± 5 ($P < .01$; n = 24 paired samples). These changes were rapid and durable, occurring as early as day 4 and detectable

Table 2. Subjects With at Least 1 Advent Event (AE), by Cohort and AE Type

AE Type	Cohort 1, Subjects, No. (%) (n = 15)	Cohort 2, Subjects, No. (%) (n = 15)	Overall, Subjects, No. (%) (n = 30)
Any	13 (86.7)	15 (100.0)	28 (93.3)
TEAE	13 (86.7)	15 (100.0)	28 (93.3)
TEAE related to the study drug[a,b]	5 (33.3)	10 (66.7)	15 (50.0)
Serious TEAE[c]	2 (13.3)	2 (13.3)	4 (13.3)
SAE related to the study drug[a]	0	0	0
SAE leading to death	0	0	0

Abbreviations: SAE, serious adverse event; TEAE, treatment-emergent adverse event.

[a] Related AEs were those that were determined by the investigator to be either possibly or probably related to the study drug.

[b] There was 1 SAE of chest pain in a patient with preexisting coronary artery disease, which was judged by the investigator to be unrelated to the study drug.

[c] There were 7 SAEs documented in 4 patients that were considered by the investigators to be unrelated to study drug, including chest pain in a patient with a history of coronary artery disease (n = 1), staphylococcal foot infection in a patient with diabetes (n = 1), and hospital admission for chronic jaw and ear pain in a patient with multiple comorbidities. The remaining 4 SAEs occurred in 1 patient with a history of coronary artery disease, valvular heart disease, and atrial fibrillation and included 2 episodes of congestive heart failure, 1 episode of culture-negative hematuria, and *C. difficile*–positive diarrhea at week 10 following receipt of broad-spectrum antibiotics for a skin infection.

for up to 24 weeks, respectively (Figure 2*A* and Supplementary Figure 1). At week 8, engrafted spore-forming bacteria from SER-109 constituted, on average, 33% of the total gut microbial carriage (Supplementary Figure 2). In addition, the augmented non–SER-109 bacteria constituted, on average, 32% of the gut microbiota. For example, *Bacteroides* and *Parabacteroides*, dominant taxa in many healthy individuals [10], were augmented in 11 of 29 patients by 38-fold–2 100 000-fold, including 6 patients who had undetectable levels in their pretreatment specimen (Figure 3). In combination, engraftment and augmentation led to a restructuring of the microbiome to a state more closely reflective of healthy individuals (Figure 2*B*).

Restructuring of the microbiome was also observed within the family Enterobacteriaceae, which contains both common commensals and well-known pathogens (Figure 2*C*). For example, *Klebsiella* species were detected at baseline in 29 patients, 22 of whom harbored >1 species. However, after SER-109 administration, *Klebsiella* carriage decreased in 25 of 29 patients (92%) by week 4, with 18 of 29 (62%) showing at least a 100 fold decline in titer (Supplementary Table 2). As a consequence of this ecological succession, in 19 of 27 patients (70%), *E. coli* became the most abundant facultative gram-negative anaerobe (Figure 2*D*).

DISCUSSION

We report the use of a novel microbiome therapeutic, SER-109, to prevent CDI recurrence in patients with a history of multiple relapses. Per-protocol efficacy was observed in 26 of 30 patients (86.7%) over 8 weeks of follow-up. In addition, 3 of 4 patients who met the protocol definition of CDI recurrence had early self-limiting diarrhea, as has been commonly described in the literature [13, 34]. However, none of the 3 patients required antibiotics, and all subsequently tested negative for *C. difficile* at 8 weeks. Thus, clinical resolution of CDI was observed in 29 of 30 patients. Although our study is limited by the lack of a placebo arm, the single clinical recurrence of *C. difficile* diarrhea in this study contrasts starkly with the recurrence rates documented in the placebo arms of 3 randomized, controlled trials involving patients with similar demographic characteristics and histories of recurrent episodes of CDI [13, 35, 36]. The most recent of these trials demonstrated relapse rates of 69%–77% in the 2 vancomycin-containing treatment arms among patients with a median of 2–3 CDI episodes prior to enrollment [13].

Clinical improvement occurred in parallel with remodeling of the gut microbiome. Seeding of the microbiome with a purified fraction of spores led to marked diversification of commensal bacteria, including augmentation of species not present in SER-109. The engraftment and augmentation of commensals observed in recipients of SER-109 suggests that this spore-based approach provides the ecologic scaffolding needed to promote the regrowth of a diverse microbial community that is shaped by the individual host. In addition, colonization by Enterobacteriaceae such as *Klebsiella* species was reduced or eliminated, and *E. coli* emerged as the dominant gram-negative species in the majority of patients. These broad changes in relative abundance of *E. coli* versus other Enterobacteriaceae species are reflective of what is seen in the healthy gut microbiome [8].

A variety of methods have been assessed in animal models and humans to manipulate the microbiome, ranging from use of minimally processed stool specimens, as in FMT, to administration of defined compositions, ranging from a single spore species to combinations of microbes [23, 37–39]. In a recent phase 2 trial, the efficacy and safety of administering spores from a single nontoxigenic *C. difficile* (NTCD) strain was evaluated for the prevention of recurrent CDI in patients who had responded to antibiotics for a primary episode or first recurrence [37]. Colonization with NTCD spores was correlated with protection, which supports the use of a spore-based approach as a proof of concept for the prevention of CDI. However, overall only 69% of patients became colonized, and among noncolonized patients the rate of CDI recurrence was identical to that among placebo recipients. In addition, NTCD colonization was not detected in any patient at the week 26 follow-up examination.

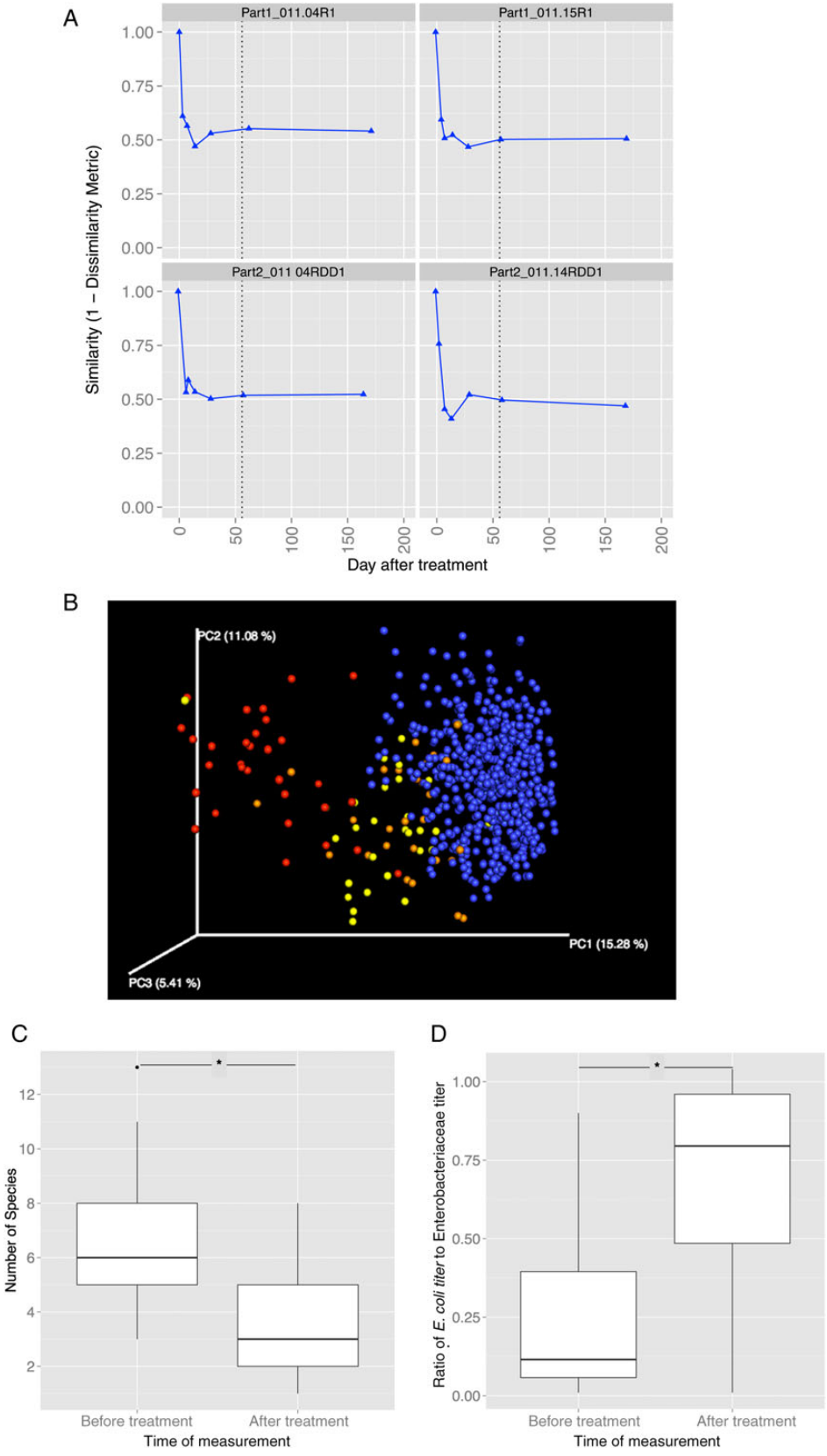

Figure 2. *A,* Change in the gastrointestinal microbiome of representative patients over time relative to the pretreatment state. Four representative patients are shown whose SER-109 dose spanned a 700-fold range (04-1 = 1.5×10^9 spores, 15-1 = 2.3×10^{10} spores, 04-2 = 8.6×10^7 spores, and 14-2 = 1.0×10^8 spores). The gastrointestinal microbiome shifted by 4 days after treatment with SER-109; the maximum dissimilarity was observed at 4 weeks and remained stable through 24 weeks. The vertical dotted black line in

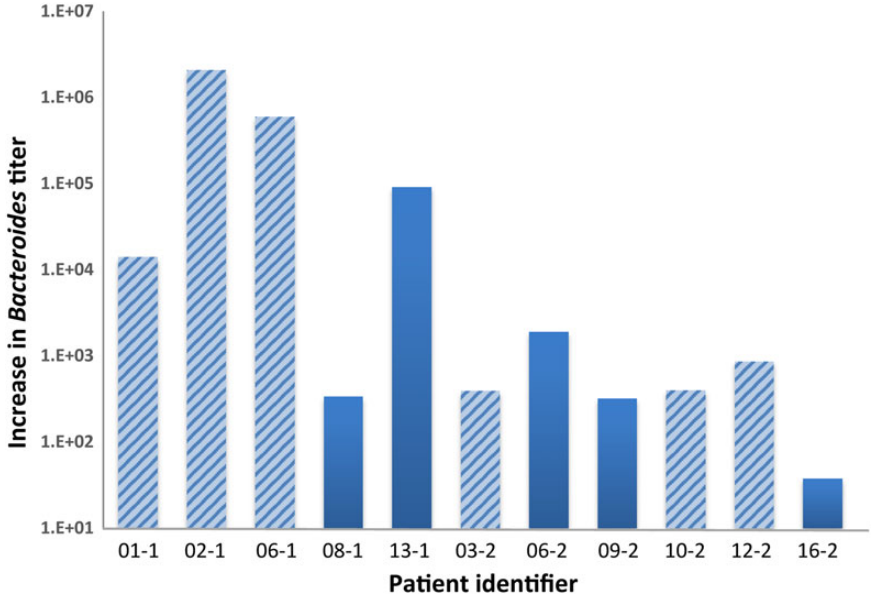

Figure 3. Change in titer in stool at 4 weeks after treatment, compared with before treatment, in patients who showed augmentation of the *Bacteroides* and *Parabacteroides* species. Stool samples were plated on selective agar medium as described in the Supplementary Materials, and colonies were picked and evaluated by full-length 16S sequencing to identify species. Hatched bars represent samples for which there were no detectable *Bacteroidetes* species in the pretreatment sample, and solid bars represent samples with detectable *Bacteroidetes* species in the pretreatment sample. Limits of detection were determined by non-*Bacteroidetes* bacteria that grew under the selective conditions. Patients with identifiers ending in "-1" were from cohort 1, and those with identifiers ending in "-2" were from cohort 2. The species detected in each patient are identified in Supplementary Table 2.

In contrast to the NTCD trial, in this present study of SER-109, engraftment was rapid, dose-independent, and durable over 24 weeks of follow-up, consistent with the fact that SER-109 is a community of live commensal spores that replicate in the gastrointestinal tract. Our findings are consistent with the theory that colonization resistance is driven not by individual populations, but by a consortium of organisms [40]. In fact, the entire spore ecology among healthy donors was represented, based on sequencing and microbiology studies showing that the SER-109 fractionation process does not deplete or enrich for specific spore species. Although the exact spore species may vary among donors, there is broad conservation based on phylogenetic relatedness and functional redundancy [41]. In addition, we could not discern any donor effect or the critical species required, owing to the high rates of clinical response. Finally, although the exact mechanism of action is not yet apparent in these exploratory analyses, it appears that increased microbial diversity, as seen in healthy individuals, restores the protective function of the microbiome against *C. difficile*.

SER-109 also had a favorable safety profile. No drug-related serious adverse events were observed over the 24-week observation period. Most adverse events were generally limited to transient and mild gastrointestinal symptoms, such as diarrhea, nausea, and abdominal discomfort, and more than half of the reported adverse events occurred within 3 days of dosing. Transient gastrointestinal symptoms have also been frequently reported in patients who clinically responded to FMT [13, 34]. No significant abnormal laboratory findings were observed in either dosing cohort.

Figure 2 continued. each plot demarks 8 weeks after treatment. Results for all patients are presented in Supplementary Figure 1. *B*, Microbial ecology compositional change after treatment with SER-109. Principal coordinates analysis showing similarity of healthy subjects characterized as part of the Human Microbiome Project (HMP), and patient microbial ecologies before and after treatment with SER-109, based on unweighted UniFrac measures of community dissimilarity (Supplementary Materials). Clinically qualified healthy donor samples are colored blue, and patient samples are color-coded by time point after treatment: pretreatment, red; 8 weeks, yellow; and 24 weeks, orange. For patients who did not provide an 8-week or 24-week sample, the sample provided closest to these time points is plotted. The microbiome is characterized using 16S V4 genomic data, and reads are taxonomically annotated at the resolution of phylogenetic clades (Supplementary Materials). HMP (n = 626) 16S data sets were acquired from the Short Read Archive of the National Center for Biotechnology Information (accession nos. SRP002395 and SRP002860). Significant differences between groups were modeled using ADONIS with the inclusion of pretreatment, 8 weeks, 24 weeks, and HMP as treatment groups (model $R^2 = 0.38$; $P < .005$; permutations = 200; Supplementary Materials). Treatment with SER-109 and the subsequent engraftment of SER-109 bacteria into the patient's gastrointestinal tract leads to a shift in the patient's microbiome away from a dysbiotic disease state towards a state that is more characteristic of healthy subjects. *C* and *D*, Change in Enterobacteriaceae counts and titers from the pretreatment (ie, baseline) time point to 4 weeks after treatment. Number of Enterobacteriaceae species observed before treatment and 4 weeks after treatment in each patient (*C*). Species were determined by plating on MacConkey lactose agar and performing 16S full-length sequencing as described in the Supplementary Materials. The difference between pretreatment and posttreatment values was highly significant ($P < .0001$, by the Wilcoxon signed rank test). Fraction of the total Enterobacteriaceae titer represented by *Escherichia coli* observed before treatment and 4 weeks after treatment (*D*). The change in *E. coli* titer as a fraction of the total Enterobacteriaceae titer was highly significant ($P = .0002$, by the Wilcoxon signed rank test).

A meta-analysis of 536 patients has demonstrated that FMT is effective in preventing further recurrences of CDI [42]. However, despite the availability of guidance documents, significant concerns exist regarding the lack of standardized protocols, unknowns regarding the optimal dose of stool to administer, and the potential for transmission of emerging pathogens [18, 30]. The use of a purified fraction of spores in SER-109 provides an opportunity to enhance safety, as processing conditions preserve spore viability while selectively killing vegetative bacteria, fungi, parasites, and viruses that could be present in asymptomatic donors [18, 19]. Spores can be purified from nonactive organic material present in unprocessed stool specimens, enabling removal of proinflammatory macromolecules (eg, lipopolysaccharide and flagella), which may activate innate immunity in the gut [43, 44]. Taken together, these elements enable the development of a safer and highly efficacious treatment. Furthermore, oral delivery is a more favorable route than invasive procedures, such as colonoscopy, particularly for elderly patients.

The lack of a placebo arm is a limitation of this study. However, patients were demographically and clinically representative of those enrolled in placebo-controlled trials involving recurrent CDI, where recurrence rates of 50%–77% have been documented [13, 35, 36]. An unknown regarding microbiome therapeutics, whether via FMT or SER-109, is the potential for long-term consequences on the human host [18, 45]; however, our 24-week safety phase is longer than many human trials involving alterations of the human microbiome, and a larger placebo-controlled trial is underway that will better define the efficacy and safety profile of SER-109. Finally, manufacturing changes have reduced the pill burden to a single dose of 4 oral capsules.

In conclusion, CDI has been recognized as an urgent threat owing to its rising incidence and associated morbidity and mortality [1, 46]. There is also a significant healthcare economic burden due to recurrent CDI, estimated to be $18 000 per recurrence, due to readmissions, increased length of stay, and the need for patient isolation [47–49]. Recurrent CDI is common because of the limited efficacy of current antibiotic regimens, which do not address dysbiosis, the root cause of this disease [5, 40]. Promising results from this initial study support further development of SER-109 as a novel biologic agent that restores the gut microbiome as a primary defense against potential pathogens, such as *C. difficile*.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Note

Potential conflicts of interest. S. K., D. S. P., C. R. K., C. S. K., T. D., and E. L. H. were investigators in the SER-001 trial. D. S. P. is a consulting member of the clinical advisory board at Seres Therapeutics, and his fees in that capacity are paid to Mayo Clinic Foundation, Rochester, rather than to him. M. R. H., M.-J. L., M. V., T. O., J. W., B. H. M., R. J. P., J. G. A., and D. N. C. are employees of and hold equity positions in Seres Therapeutics, which funded the trial. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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