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Safety and Tolerability of SER-109 as an Investigational Microbiome Therapeutic in Adults With Recurrent *Clostridioides difficile* Infection
A Phase 3, Open-Label, Single-Arm Trial

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Abstract

IMPORTANCE A safe and effective treatment for recurrent *Clostridioides difficile* infection (CDI) is urgently needed. Antibiotics kill toxin-producing bacteria but do not repair the disrupted microbiome, which promotes spore germination and infection recurrence.

OBJECTIVES To evaluate the safety and rate of CDI recurrence after administration of investigational microbiome therapeutic SER-109 through 24 weeks.

DESIGN, SETTING, AND PARTICIPANTS This phase 3, single-arm, open-label trial (ECOSPOR IV) was conducted at 72 US and Canadian outpatient sites from October 2017 to April 2022. Adults aged 18 years or older with recurrent CDI were enrolled in 2 cohorts: (1) rollover patients from the ECOSPOR III trial who had CDI recurrence diagnosed by toxin enzyme immunoassay (EIA) and (2) patients with at least 1 CDI recurrence (diagnosed by polymerase chain reaction [PCR] or toxin EIA), inclusive of their acute infection at study entry.

INTERVENTIONS SER-109 given orally as 4 capsules daily for 3 days following symptom resolution after antibiotic treatment for CDI.

MAIN OUTCOMES AND MEASURES The main outcomes were safety, measured as the rate of treatment-emergent adverse events (TEAEs) in all patients receiving any amount of SER-109, and cumulative rates of recurrent CDI (toxin-positive diarrhea requiring treatment) through week 24 in the intent-to-treat population.

RESULTS Of 351 patients screened, 263 were enrolled (180 [68.4%] female; mean [SD] age, 64.0 [15.7] years); 29 were in cohort 1 and 234 in cohort 2. Seventy-seven patients (29.3%) were enrolled with their first CDI recurrence. Overall, 141 patients (53.6%) had TEAEs, which were mostly mild to moderate and gastrointestinal. There were 8 deaths (3.0%) and 33 patients (12.5%) with serious TEAEs; none were considered treatment related by the investigators. Overall, 23 patients (8.7%; 95% CI, 5.6%-12.8%) had recurrent CDI at week 8 (4 of 29 [13.8%; 95% CI, 3.9%-31.7%] in cohort 1 and 19 of 234 [8.1%; 95% CI, 5.0%-12.4%] in cohort 2), and recurrent CDI rates remained low through 24 weeks (36 patients [13.7%; 95% CI, 9.8%-18.4%]). At week 8, recurrent CDI rates in patients with a first recurrence were similarly low (5 of 77 [6.5%; 95% CI, 2.1%-14.5%]) as in patients with 2 or more recurrences (18 of 186 [9.7%; 95% CI, 5.8%-14.9%]). Analyses by select baseline characteristics showed consistently low recurrent CDI rates in patients younger than 65 years vs 65 years or older (5 of 126 [4.0%; 95% CI, 1.3%-9.0%] vs 18 of 137 [13.1%; 95% CI, 8.0%-20.0%]) and patients enrolled

(continued)

Key Points

Question What is the tolerability profile and rate of recurrent *Clostridioides difficile* infection (CDI) after administration of investigational microbiome therapeutic SER-109 following standard-of-care antibiotic treatment?

Findings In this phase 3, open-label, single-arm trial of 263 adults with a history of CDI, SER-109 was well tolerated in a population with prevalent comorbidities. Overall, 8.7% of patients had recurrent CDI at week 8 and 13.7% at week 24; the rate of recurrent CDI was low regardless of number of prior recurrences, demographics, or diagnostic approach.

Meaning These data support the tolerability and potential benefit of SER-109 in an expanded patient population with recurrent CDI in community practice.

➕ **Supplemental content**

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Abstract (continued)

based on positive PCR results (3 of 69 [4.3%; 95% CI, 0.9%-12.2%]) vs those with positive toxin EIA results (20 of 192 [10.4%; 95% CI, 6.5%-15.6%]).

CONCLUSIONS AND RELEVANCE In this trial, oral SER-109 was well tolerated in a patient population with recurrent CDI and prevalent comorbidities. The rate of recurrent CDI was low regardless of the number of prior recurrences, demographics, or diagnostic approach, supporting the beneficial impact of SER-109 for patients with CDI.

TRIAL REGISTRATION ClinicalTrials.gov identifier: NCT03183141

Introduction

Clostridioides difficile infection (CDI) frequently occurs in vulnerable patient populations, including older patients, those who are immunocompromised, and those with comorbidities, including malignant neoplasm, chronic kidney disease, and other medical conditions.[1-6] The leading risk factor for CDI is prior exposure to broad-spectrum antibiotics, which disrupt the gastrointestinal microbiome, a diverse ecosystem that provides essential functions for the host and serves as the primary defense against potential pathogens, such as *C difficile*.[7-9] In the past 2 decades, CDI has also affected younger patients in the community without traditional risk factors.[10] *Clostridioides difficile* infection is associated with excess health care burden, with higher hospitalization and mortality rates among inpatients diagnosed with CDI compared with those without a CDI diagnosis.[11]

Clostridioides difficile infection differs from most other infections in that it often recurs despite appropriate antibiotic treatment, highlighting the limitations of current standard therapeutic approaches.[12] Vancomycin and fidaxomicin achieve high stool drug concentrations and have excellent bactericidal activity against *C difficile* bacteria.[13,14] Antibiotic treatment commonly leads to resolution of diarrhea in 3 to 5 days in conjunction with rapid decline of stool toxin concentration.[14] Despite these excellent pharmacologic profiles, approximately 15% to 25% of patients with primary CDI experience recurrence after antibiotic treatment completion due to persistence of *C difficile* spores and alterations to the normal gut flora.[15,16] Patients with recurrent infection are at increased risk of subsequent episodes, with recurrence rates of 40% or more due to persistent or worsening antibiotic-mediated microbiome disruption and dysfunction.[17-19]

Thus, although antibiotics are necessary to kill the toxin-producing bacteria, microbiome repair is the foundation for functional restoration and is critical to achieve a sustained clinical response.[7,16] Most patients with resilience of their microbiome following primary CDI will recover without further CDI episodes. However, patients with persistent microbiome disruption after completion of CDI-targeted antibiotic treatment may have recurrence of CDI due to spore germination and replication of toxin-producing *C difficile* bacteria.[16,20] This recurrent cycle is facilitated by modulation of bile acid pathways favorable to *C difficile* when Firmicutes bacteria are depleted by antibiotics.[21,22] An increased risk of recurrence is associated with many factors that have also been linked to microbiome disruption: older age, comorbidities, proton pump inhibitor use, spectrum of activity of the inciting antibiotic or recurrent antibiotic exposure, immunosuppression, and most importantly, a history of recurrence.[10,12,23]

Some of us previously reported that SER-109, an investigational, oral microbiome therapeutic composed of purified Firmicutes spores, was superior to placebo in reducing risk of recurrent CDI at 8 weeks in patients with 2 or more CDI recurrences, the primary end point (12% vs 40%, respectively; relative risk, 0.32; 95% CI, 0.18-0.58).[17] We report the results of ECOSPOR IV, a phase-3, open-label, single-arm trial of SER-109 in an expanded patient population with a history of CDI.

Methods

Study Design, Oversight, and Participants

ECOSPOR IV was a phase-3, open-label, single-arm trial (NCT03183141) performed in accordance with Good Clinical Practice guidelines at 72 US and Canadian sites from October 2017 to April 2022 (trial protocol and statistical analysis plan in Supplement 1). The trial was conducted to meet the US Food and Drug Administration safety database requirements. The protocol and amendments were reviewed and approved by central and local investigational review boards. Written informed consent was obtained at screening. This trial followed the Consolidated Standards of Reporting Trials (CONSORT) reporting guideline.

The trial was conducted in 2 cohorts of adults aged 18 years or older. Cohort 1 included rollover patients from the ECOSPOR III trial[17] who had a CDI recurrence diagnosed by toxin enzyme immunoassay (EIA) within 8 weeks after receipt of either SER-109 or placebo. Cohort 2 included de novo patients with at least 1 CDI recurrence (ie, $\geq$2 CDI episodes inclusive of the current episode). Recurrence of CDI was defined as (1) 3 or more unformed stools per day for 2 consecutive days, (2) any positive result of a *C difficile* stool test for toxin production (ie, EIA for toxin or cell cytotoxicity neutralization assay) or a polymerase chain reaction (PCR) assay for detection of a toxin gene from a local or central laboratory, and (3) a response to CDI antibiotic treatment, defined as 10 to 42 days of vancomycin, 125 mg 4 times daily, or 10 to 25 days of fidaxomicin, 200 mg twice daily, including prolonged tapered antibiotic regimens. A full list of inclusion and exclusion criteria are included the eMethods in Supplement 2. The study duration for both cohorts was approximately 27 weeks, including a 3-week screening period, an 8-week primary efficacy period from initiation of treatment on day 1, and a 16-week follow-up period.

Study Procedures and Assessments

Patient race and ethnicity data were based on self-report or health records and collected to assess whether outcomes were similar among all races and ethnicities, because this is an innovative therapy. Categories for race were American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, and White, and categories for ethnicity were Hispanic or Latino or not Hispanic or Latino. All patients received a single daily dose of SER-109 with a target of 3×10^{7} spore colony–forming units per dose in 4 capsules, administered over 3 consecutive days. The first dose of study drug was administered within 4 days following antibiotic treatment completion. Patients were instructed to take 10 oz of magnesium citrate 1 day prior to treatment initiation to ensure washout of residual antibiotic from the gastrointestinal tract. Age-adjusted Charlson Comorbidity Index scores were based on medical history terms coded in MedDRA, version 23.1 (eMethods in Supplement 2), in which a higher total score indicates higher risk of mortality.[24] An on-study CDI recurrence was defined as 3 or more unformed stools per day for 2 consecutive days with a positive *C difficile* stool toxin test result (EIA or cell cytotoxicity neutralization assay) and a decision based on clinical assessment by the investigator that antibiotic treatment was needed. Patients who were lost to follow-up, terminated the trial prematurely, or died were imputed as a recurrence. If a patient had 1 missing criterion (eg, toxin test result) but the other 2 criteria were documented (eg, diarrhea, decision to treat), recurrence was imputed.

All treatment-emergent adverse events (TEAEs), serious AEs (SAEs), and AEs of special interest (AESIs), predefined as any invasive infection (eg, bacteremia, abscess, or meningitis), were collected weekly via scripted telephone calls from the time of initiation of SER-109 up to week 8. From weeks 8 to 24, all treatment-emergent SAEs and AESIs, their related data, and any antibiotic medication and the corresponding indication were collected every 4 weeks via weekly scripted telephone calls.

End Points

Safety and tolerability of SER-109 were evaluated up to 24 weeks. The secondary end point was CDI recurrence as determined by toxin assay up to week 4, 8, 12, and 24 after initiation of treatment. Patients who did not have a recurrence were considered to have a sustained clinical response.

Statistical Analysis

Descriptive statistics were reported for all end points. Analysis of safety was conducted for all patients who received any amount of study drug (ie, SER-109). Analyses of CDI recurrence rates were performed using the intent-to-treat population. Both populations included all enrolled patients.

The number and percentage of patients defined as having CDI nonrecurrence (ie, sustained clinical response) and CDI recurrence outcomes were summarized with 95% CIs using the Clopper-Pearson exact method. The number and percentage of patients with CDI recurrence up to week 8 were summarized with the corresponding 95% CIs for select baseline characteristics, including age (<65 years or ≥65 years); number of prior CDI episodes, including the qualifying episode (2 or ≥3); antibiotic regimen for the qualifying episode (vancomycin, fidaxomicin); sex (male, female); and diagnostic assay used for the qualifying episode (PCR alone vs toxin with or without PCR). SAS, version 9.4 (SAS Institute Inc), was used for the analyses.

Results

Study Population

A total of 351 patients were screened and 263 enrolled (cohort 1: n = 29; cohort 2: n = 234) (**Figure 1**). The mean (SD) age of patients was 64.0 (15.7) years; 180 (68.4%) were female, and 83 (31.6%) were male. One patient (0.4%) was American Indian or Alaska Native, 5 (1.9%) were Asian, 14 (5.3%) were Black or African American, none were Native Hawaiian or other Pacific Islander, and 243 (92.4%) were White; 20 patients (7.6%) were Hispanic or Latino and 243 (92.4%) were not Hispanic or Latino.

Figure 1. Flow Diagram of Cohorts 1 and 2
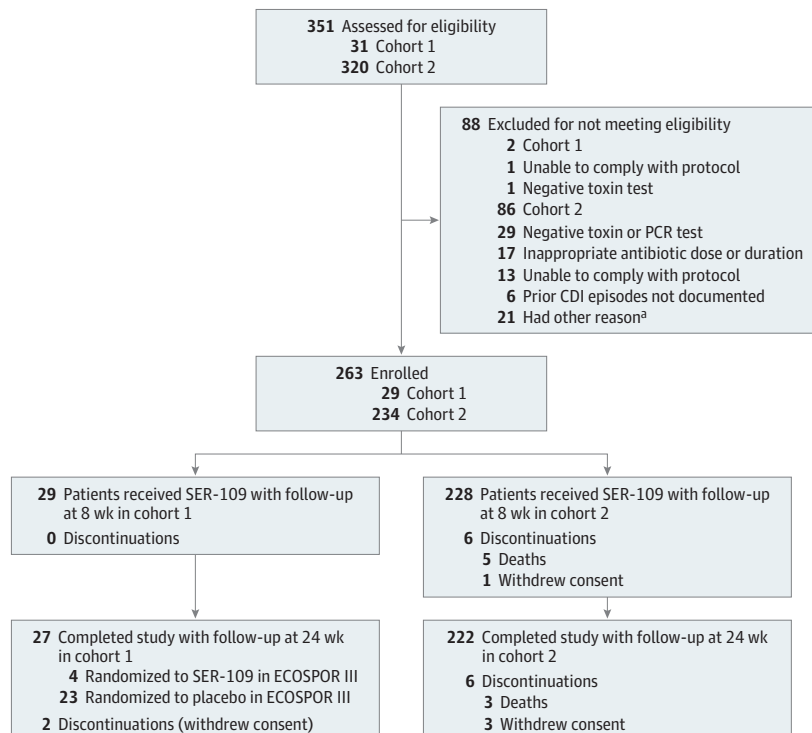
a Other reasons for exclusion included inadequate response to antibiotics (n = 4), could not receive treatment within 4 days of antibiotic completion (n = 3), currently taking or expected to take other antibiotics (n = 3), absolute neutrophil count less than 500 cells/μL (to convert to ×10^9/L, multiply by 0.001; n = 2), history of fecal microbiota transplantation (n = 1), investigator decision due to concurrent medical risks (n = 1), unable to stop loperamide (n = 1), did not meet unformed stool requirement (n = 1), received human monoclonal antibody (n = 1), known or suspected toxic megacolon (n = 1), and other (n = 3). CDI indicates *Clostridioides difficile* infection; PCR, polymerase chain reaction.

Overall, 191 patients (72.6%) received vancomycin for the qualifying CDI episode. Seventy-seven patients (29.3%) (all in cohort 2) were enrolled with their first recurrence (eg, 2 prior CDI episodes inclusive of the current episode), and 186 (70.7%) were enrolled with 2 or more recurrences (eg, ≥3 prior CDI episodes inclusive of the current episode); 69 patients (26.4%) were enrolled based on PCR diagnostics alone (**Table 1**). All patients received the study drug, so the number of patients in the intent-to-treat and safety populations were the same (n = 263). Two hundred sixty-one patients (99.2%) took all capsules, and the retention rate of enrolled patients was 95% (249 of 263); patient disposition is shown in the flow diagram (Figure 1).

Comorbidities were prevalent in the overall population, with a mean (SD) Charlson Comorbidity Index score of 3.8 (2.2). Common comorbidities included cardiac disorders (82 patients [31.2%]), neoplasms (56 [21.3%]), type 2 diabetes (28 [10.6%]), chronic obstructive pulmonary disease (26 [9.9%]), chronic kidney disease (25 [9.5%]), and hepatobiliary disorders (23 [8.7%]).

Safety

Overall, 141 patients (53.6%) experienced TEAEs, the majority of which were mild to moderate and resolved without sequelae (**Table 2**). No TEAEs led to study withdrawal. Invasive infections (AESIs) were reported in 17 patients (6.5%) (Table 2 and eTable 1 in Supplement 2). Thirty-three patients (12.5%) experienced SAEs, and 8 patients (3.0%) had a fatal outcome; none of these events were deemed by the investigators to be related to SER-109. There were various causes of death, including

Table 1. Demographic and Baseline Characteristics in the Intent-to-Treat and Safety Analysis Populations

Characteristic	Patients, No. (%)				
	Cohort 1[a]				
	SER-109 (n = 4)	Placebo (n = 25)	Total (n = 29)	Cohort 2 (n = 234)	Total (N = 263)
Age, mean (SD), y	85.0 (11.8)	69.5 (11.4)	71.7 (12.5)	63.1 (15.8)	64.0 (15.7)
Age group, y					
<65	0	8 (32.0)	8 (27.6)	118 (50.4)	126 (47.9)
≥65	4 (100)	17 (68.0)	21 (72.4)	116 (49.6)	137 (52.1)
Sex					
Female	2 (50.0)	16 (64.0)	18 (62.1)	162 (69.2)	180 (68.4)
Male	2 (50.0)	9 (36.0)	11 (37.9)	72 (30.8)	83 (31.6)
Ethnicity					
Hispanic or Latino	0	0	0	20 (8.5)	20 (7.6)
Not Hispanic or Latino	4 (100)	25 (100)	29 (100)	214 (91.5)	243 (92.4)
Race					
American Indian or Alaska Native	0	0	0	1 (0.4)	1 (0.4)
Asian	0	0	0	5 (2.1)	5 (1.9)
Black or African American	0	0	0	14 (6.0)	14 (5.3)
Native Hawaiian or other Pacific Islander	0	0	0	0	0
White	4 (100)	25 (100)	29 (100)	214 (91.5)	243 (92.4)
CDI episodes, No.[b]					
2	0	0	0	77 (32.9)	77 (29.3)
≥3	4 (100)	25 (100)	29 (100)	157 (67.1)	186 (70.7)
Antibiotic regimen for qualifying CDI episode					
Vancomycin	4 (100)	18 (72.0)	22 (75.9)	169 (72.2)	191 (72.6)
Fidaxomicin	0	7 (28.0)	7 (24.1)	65 (27.8)	72 (27.4)
Defining test for qualifying CDI episode[c]					
PCR alone	1 (25.0)[d]	0	1 (3.4)	68 (29.3)	69 (26.4)
Toxin with or without PCR	3 (75.0)	25 (100)	28 (96.6)	164 (70.7)	192 (73.6)

Abbreviations: CDI, *Clostridioides difficile* infection; PCR, polymerase chain reaction.

[a] Randomized treatment arm in ECOSPOR III.[17]

[b] Including qualifying episode. All cohort 1 patients were included in the category of 3 or more episodes.

[c] Two patients were enrolled in cohort 2 on the basis of a positive loop-mediated isothermal amplification assay result and are not included in the table.

[d] This patient was enrolled based on local testing results because the investigator verbally indicated that the toxin test result was positive. However, the source data, which were not available for some time after the decision to enroll was made, were from PCR results.

those due to preexisting conditions, with no apparent trends (a summary of TEAEs leading to death is given in eTable 2 in Supplement 2).

The most common TEAEs (occurring in ≥5% in any cohort) were gastrointestinal disorders (diarrhea, flatulence, nausea, abdominal pain, and abdominal distension), urinary tract infections (UTIs), and fatigue. There were 13 UTIs reported (4.9% of patients), of which 3 were SAEs (1.1%). Additionally, there were 2 urosepsis events (0.8%), each considered SAEs and invasive infections (AESIs). No UTIs or urosepsis events were related to SER-109 per the investigators, and the organisms identified from available urine culture specimens were known uropathogens not representative of SER-109 species.

One female patient had an adverse drug reaction to SER-109, including mild facial flushing, elevated temperature, and moderate throat and jaw tightness. This patient had a history of drug reactions to medications, including fidaxomicin, clindamycin, nitrofurantoin, prednisone, amoxicillin-clavulanate, penicillin, and azithromycin.

CDI Recurrence Rates and Subgroup Analyses

Of the 263 patients, 23 (8.7%; 95% CI, 5.6%-12.8%) had CDI recurrence up to week 8 (4 of 29 [13.8%; 95% CI, 3.9%-31.7%] in cohort 1 and 19 of 234 [8.1%; 95% CI, 5.0%-12.4%] in cohort 2) (**Table 3**). Of these 23 recurrences, 16 (69.6%) were confirmed and 7 (30.4%) were imputed due to loss to follow-up, early termination from the trial, or death (n = 4) or to missing components for defining recurring CDI (n = 3) (eTable 3 in Supplement 2).

By week 24, 36 patients (13.7%; 95% CI, 9.8%-18.4%) had a CDI recurrence. Of these 36 recurrences, 22 (61.1%) were confirmed and 14 (38.9%) were imputed recurrences due to loss to follow-up, early termination from the study, or death (n = 8) or to missing components (n = 6) (eTable 3 in Supplement 2). Sustained clinical response rates at weeks 8 and 24 were 91.3% (95% CI, 87.2%-94.4%) and 86.3% (95% CI, 81.6%-90.2%), respectively.

Analyses by select baseline characteristics, including age, antibiotic regimen for the qualifying episode, sex, or diagnostic test used for the qualifying episode also showed consistent low rates of recurrent CDI up to week 8 in all subgroups, ranging from 4.0% to 13.1% (**Figure 2**). Recurrent CDI

Table 2. Summary of Treatment-Emergent Adverse Events in the Safety Analysis Population

Characteristic	Patients, No. (%)[a]				
	Cohort 1[b]				
	SER-109 (n = 4)	Placebo (n = 25)	Total (n = 29)	Cohort 2 (n = 234)	Total (N = 263)
Any TEAE	4 (100)	15 (60.0)	19 (65.5)	122 (52.1)	141 (53.6)
Most frequently reported TEAEs by preferred term[c]					
Diarrhea	1 (25.0)	9 (36.0)	10 (34.5)	50 (21.4)	60 (22.8)
Flatulence	0	4 (16.0)	4 (13.8)	16 (6.8)	20 (7.6)
Nausea	0	3 (12.0)	3 (10.3)	17 (7.3)	20 (7.6)
Abdominal pain	1 (25.0)	2 (8.0)	3 (10.3)	15 (6.4)	18 (6.8)
Urinary tract infection	0	0	0	13 (5.6)	13 (4.9)
Fatigue	0	3 (12.0)	3 (10.3)	9 (3.8)	12 (4.6)
Abdominal distension	1 (25.0)	3 (12.0)	4 (13.8)	7 (3.0)	11 (4.2)
Related or possibly related TEAEs	1 (25.0)	4 (16.0)	5 (17.2)	27 (11.5)	32 (12.2)
Mild to moderate TEAEs	3 (75.0)	14 (56.0)	17 (58.6)	98 (41.9)	115 (43.7)
Serious TEAEs	1 (25.0)	0	1 (3.4)	32 (13.7)	33 (12.5)
TEAEs related or possibly related to study drug	0	0	0	0	0
Treatment-emergent AESIs[d]	1 (25.0)	0	1 (3.4)	16 (6.8)	17 (6.5)
TEAEs leading to withdrawal	0	0	0	0	0
TEAEs leading to death[e]	0	0	0	8 (3.4)	8 (3.0)

Abbreviations: AESI, adverse event of special interest; TEAE, treatment-emergent adverse event.

[a] Number of patients in the safety population who were in the study at the beginning of the specified time interval. Data presented are by patient. All TEAEs were collected and summarized from the time of enrollment up to week 8.

[b] Randomized treatment arm in ECOSPOR III.[17]

[c] Experienced by 5% or more in any cohort.

[d] Included in eTable 1 in Supplement 2.

[e] Included in eTable 2 in Supplement 2.

rates were lower in patients younger than 65 years vs 65 years or older (5 of 126 [4.0%; 95% CI, 1.3%-9.0%] vs 18 of 137 [13.1%; 95% CI, 8.0%-20.0%]). CDI recurrence rates at week 8 in patients with a first recurrence (ie, 2 prior CDI episodes) were similarly low (5 of 77 [6.5%; 95% CI, 2.1%-14.5%]) as in those with 2 or more recurrences (ie, ≥3 prior CDI episodes; 18 of 186 [9.7%; 95% CI, 5.8%-14.9%]) (Figure 2). By the alternative metric, sustained clinical response rates were also similar in patients with a first recurrence (72 [93.5%; 95% CI, 85.5%-97.9%]) vs those with 2 or more recurrences (168 [90.3%; 95% CI, 85.1%-94.2%]). Of note, CDI recurrence rates in patients enrolled based on PCR results alone were numerically lower (3 of 69 patients [4.3%; 95% CI, 0.9%-12.2%]) compared with those for patients enrolled based on a positive toxin EIA result (20 of 192 patients [10.4%; 95% CI, 6.5%-15.6%]). Among those who received vancomycin or fidaxomicin prolonged or tapered regimens prior to SER-109 administration, we observed CDI recurrence in 0 of 15 patients and 1 of 18 patients (5.6%; 95% CI, 0.1%-27.3%), respectively, compared with 22 of 230 patients (9.6%; 95% CI, 6.1%-14.1%) who did not receive prolonged or tapered regimens for the qualifying episode (eTable 4 in Supplement 2).

Discussion

In this study of 263 patients with a history of recurrent CDI, the safety profile of SER-109 was consistent with data observed among patients treated with SER-109 in the ECOSPOR III placebo-controlled randomized clinical trial.[17] Most adverse events were mild to moderate in intensity and affected the gastrointestinal tract. None of the deaths or serious TEAEs was deemed treatment related, and no pattern was observed in the cause of death. Further, none of the confirmed infections observed among the patients was related to SER-109 species.

The observed safety profile of SER-109 might be expected since spore-forming Firmicutes are normally abundant in the microbiome of healthy individuals and are thought to play a role in gut homeostasis.[25] These safety attributes of SER-109 are important to the patient populations at risk of CDI since CDI is a common infection among elderly individuals, immunosuppressed individuals, and other vulnerable populations with multiple comorbidities, who are at increased risk of poor clinical outcomes.[26] The mean Charlson Comorbidity Index score in the overall population was 3.8, indicative of high mortality risk.[24] The SER-109 manufacturing process delivers a purified consortium of Firmicutes spores, which play a key role in inhibiting *C difficile* while mitigating risk of transmitting undetected or emerging pathogens through inactivation of potential pathogens in donor product.[27,28] These defining features distinguish this purified consortium from fecal microbiota transplantation (FMT) and FMT-like products, which would be subject to transmission of undetected and emerging pathogens.[29]

The recurrence rate in the overall study population was 8.7%, corresponding to a sustained clinical response rate at 8 weeks of 91.3%, which was durable over 24 weeks. Recurrence rates were low regardless of diagnostic approach or number of preceding CDI episodes. In contrast, CDI

Table 3. Cumulative *Clostridioides difficile* Infection Recurrence Rates by Time in the Intent-to-Treat Population

| Time after SER-109 treatment, wk | Patients, No. (%) [95% CI][a] | | | | |
| | Cohort 1[b] | | | Cohort 2 (n = 234) | Total (N = 263) |
	SER-109 (n = 4)	Placebo (n = 25)	Total (n = 29)		
4	0 (0) [0.0-60.2]	4 (16.0) [4.5-36.1]	4 (13.8) [3.9-31.7]	10 (4.3) [2.1-7.7]	14 (5.3) [2.9-8.8]
8	0 (0) [0.0-60.2]	4 (16.0) [4.5-36.1]	4 (13.8) [3.9-31.7]	19 (8.1) [5.0-12.4]	23 (8.7) [5.6-12.8]
12	0 (0) [0.0-60.2]	5 (20.0) [6.8-40.7]	5 (17.2) [5.8-35.8]	23 (9.8) [6.3-14.4]	28 (10.6) [7.2-15.0]
24	1 (25.0) [0.6-80.6]	5 (20.0) [6.8-40.7]	6 (20.7) [8.0-39.7]	30 (12.8) [8.8-17.8]	36 (13.7) [9.8-18.4]

[a] Patients who were lost to follow-up, terminated the study prematurely, or died without a recorded recurrence before the end of the time interval were assumed to have had a recurrence. The handling of other types of missing data are described in the statistical analysis plan.

[b] Randomized treatment arm in ECOSPOR III.[17]

recurrence rates reported in the literature range from 20% to 36% in those with first recurrence to 40% or greater for 2 or more recurrences.[30] Thus, it is noteworthy that in the ECOSPOR IV study population with prevalent comorbidities and multiple risk factors for recurrent CDI, on-study recurrence rates ranged from 6.5% to 9.7% regardless of CDI history or demographics.
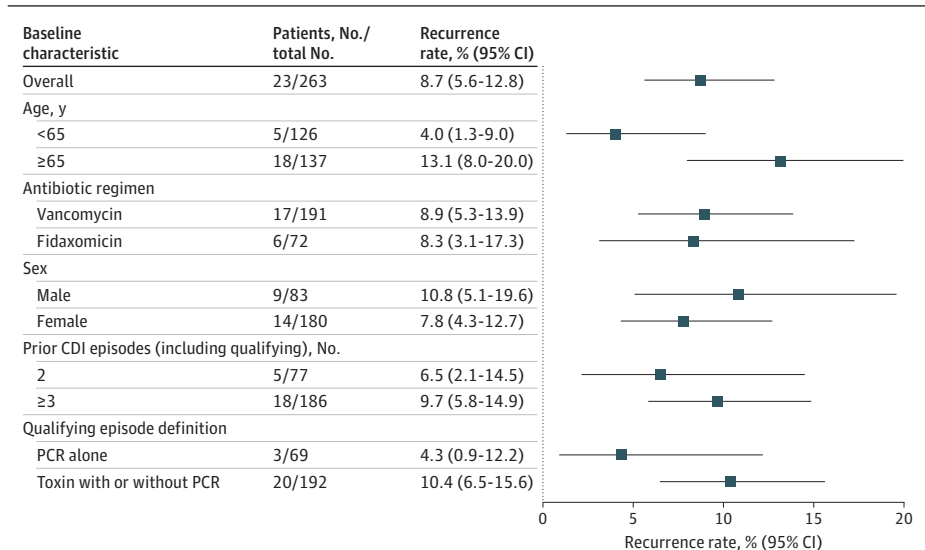
No firm conclusions can be drawn on efficacy from this open-label trial alone, which was mainly conducted for determination of safety. However, these data are consistent with the ECOSPOR III data, which demonstrated the superiority of SER-109 compared with placebo in reducing CDI recurrence rates.[17] Recurrent CDI is the signature event that identifies patients who may benefit from microbiome therapy to restore the functional deficiencies in the gastrointestinal microbiome that fuel recurrence.[22] Patients with their first CDI recurrence were included in this trial since treatment failure after antibiotics alone is suggestive of underlying microbiome disruption. Earlier intervention at the time of first recurrence may prevent hospitalizations and associated morbidity and additional health care costs.[11,31]

In clinical decision-making, the selection of a highly sensitive assay (ie, PCR) or a highly specific assay (ie, EIA toxin) for diagnosis of *C difficile* is based on the clinician's assessment of the prior probability of disease.[32,33] Additionally, some clinicians may only have access to PCR testing based on local laboratory protocols, limiting diagnostic choice. In this open-label safety study, we enrolled patients who were diagnosed by either assay to evaluate the safety of SER-109 in all patients with suspected recurrent CDI who may be treated in the community regardless of diagnostic method.

Treatment with SER-109 led to low rates of recurrence regardless of diagnostic approach for study entry, with rates numerically lower in the PCR testing cohort. Although we cannot rule out the possibility that a minority of patients with a positive PCR assay result had colonization alone, the ECOSPOR IV study requirements of symptom resolution after antibiotic treatment before study drug administration likely reduced that possibility. Of note, the placebo-controlled trial ECOSPOR III required toxin testing at study entry and at suspected recurrence to accurately assess the primary efficacy end point with this investigational agent.[17] We propose that toxin testing should be the gold standard for clinical trials evaluating efficacy of investigational agents, while the diagnosis of CDI in the community should be guided by clinical acumen, assay availability, and test interpretation.[28,34,35]

We permitted enrollment of patients who had taken vancomycin or fidaxomicin long-pulse or taper regimens, as suggested by major guideline panels.[36,37] A recent meta-analysis reported wide-ranging efficacy rates of 26% to 100%,[38] with marked heterogeneity between studies in treatment

Figure 2. Forest Plot of *Clostridioides difficile* Infection (CDI) Recurrence Rates up to 8 Weeks After Treatment as Determined by a Toxin Assay by Subgroup in the Intent-to-Treat Population

Baseline characteristic	Patients, No./total No.	Recurrence rate, % (95% CI)
Overall	23/263	8.7 (5.6-12.8)
Age, y		
<65	5/126	4.0 (1.3-9.0)
≥65	18/137	13.1 (8.0-20.0)
Antibiotic regimen		
Vancomycin	17/191	8.9 (5.3-13.9)
Fidaxomicin	6/72	8.3 (3.1-17.3)
Sex		
Male	9/83	10.8 (5.1-19.6)
Female	14/180	7.8 (4.3-12.7)
Prior CDI episodes (including qualifying), No.		
2	5/77	6.5 (2.1-14.5)
≥3	18/186	9.7 (5.8-14.9)
Qualifying episode definition		
PCR alone	3/69	4.3 (0.9-12.2)
Toxin with or without PCR	20/192	10.4 (6.5-15.6)

Patients who were lost to follow-up, terminated the study prematurely, or died without a recorded recurrence before the end of the time interval were assumed to have had a recurrence. The handling of other types of missing data are described in the statistical analysis plan (Supplement 1). The 95% CIs and recurrence rates (proportion of patients with CDI recurrence) were calculated using the Clopper-Pearson exact method. PCR indicates polymerase chain reaction.

regimens and duration of follow-up and varying definitions for recurrence. In the current study, it is possible that long-pulse or taper regimens contributed to the favorable outcomes observed in this small cohort, although recurrence rates did not differ from the larger study population. In light of published data showing rapid reduction of stool toxin concentrations following CDI-targeted antibiotic treatment, the rationale for longer antibiotic treatment regimens has considerably weakened, particularly with emerging understanding of the harmful effects of antibiotics on microbiome diversity, concerns about the emergence of vancomycin-resistant *Enterococcus*, and the cost of prolonged regimens.[15,39,40]

The efficacy and safety data from the ECOSPOR III[17] and ECOSPOR IV trials reinforce advantages of using a microbiome therapeutic composed of purified Firmicutes spores for the treatment of recurrent CDI. The use of Firmicutes spores (1) achieves efficient drug delivery within the acidic environment of the stomach; (2) enables a low pill burden because spores can germinate, multiply, and replicate into metabolically active bacteria within the gastrointestinal tract; and (3) allows for specific inactivation of nonspore microorganisms during manufacturing, mitigating risks to patients.[27,28] Spore-forming Firmicutes are thought to restore epithelial barrier integrity, decrease colonic inflammation, and modulate bile acid concentrations important to colonization resistance.[21,22,41]

Strengths and Limitations

Strengths of this trial include enrollment of patients with first recurrence, the broad diagnostic eligibility criteria, and allowance of longer antibiotic treatment regimens, typical of community practice. In this expanded population of patients, SER-109 was associated with low rates of CDI recurrence in a population at high risk of recurrent disease and was well tolerated.

Limitations include the open-label design of this study, in which all patients received SER-109, limiting any conclusions on efficacy. However, the safety and recurrence rate data are consistent with those of the double-blind, placebo-controlled ECOSPOR III trial.[17] We were not able to detect any difference in recurrence rates based on race and ethnicity due to the highly prevalent enrollment of White patients, consistent with other clinical trials. It is unclear whether this factor was due to lower risk of CDI among Black patients or lack of outreach and opportunity for racial and ethnic minority populations.

Conclusions

In this phase 3, open-label, single-arm trial of patients with a history of recurrent CDI, SER-109 was well tolerated and the overall rate of recurrent CDI was low, consistent with the ECOSPOR III placebo-controlled randomized clinical trial.[17] The baseline prevalence of multiple comorbidities was high, reflective of expanded populations with recurrent CDI.[6] With this potential first-in-class purified microbiome therapeutic, the rate of recurrent CDI after symptom resolution after antibiotic treatment was low at week 8 and durable through week 24 regardless of the number of recurrences before study entry. Earlier treatment with SER-109 at the time of first recurrence may be associated with reduced morbidity from recurrent CDI.[11,31] These data support an important role for SER-109 as part of a paradigm shift in the clinical management of recurrent CDI.

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SUPPLEMENT 1.
Trial Protocol and Statistical Analysis Plan

SUPPLEMENT 2.
eMethods. Study Inclusion and Exclusion Criteria, Prohibited Concomitant Medications and Procedures, and Charlson Comorbidity Index
eTable 1. Adverse Events of Special Interest
eTable 2. Patients With Treatment-Emergent Adverse Events Leading to Death
eTable 3. Confirmed and Imputed Recurrent *Clostridioides difficile* Infections by Time Point, Intent-to-Treat Population
eTable 4. Recurrent *Clostridioides difficile* Infections by Antibiotic Regimen, Prolonged or Tapered and Not Prolonged or Tapered
eReference

SUPPLEMENT 3.
ECOSPOR IV Investigators

SUPPLEMENT 4.
Data Sharing Statement