CLINICAL STUDY PROTOCOL

SER-109

(Eubacterial Spores, Purified Suspension, Encapsulated)

ECOSPOR IV: AN OPEN-LABEL EXTENSION OF STUDY SERES-012 EVALUATING SER-109 IN ADULT SUBJECTS WITH RECURRENT CLOSTRIDIUM DIFFICILE INFECTION (RCDI)

SPONSOR:

Seres Therapeutics, Inc.
200 Sidney Street, Suite 410
Cambridge, MA 02139
Telephone: +1-617-945-9626

TITLE:

ECOSPOR IV: An Open-Label Extension of Study SERES-012 Evaluating SER-109 in Adult Subjects with Recurrent *Clostridium difficile* Infection (RCDI)

CLINICAL PHASE: 2

IND NUMBER: 16262

VERSION: 1.0

VERSION DATE: March 06, 2017

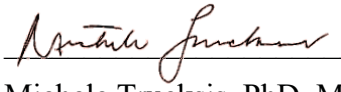
CLINICAL RESEARCH ORGANIZATION (CRO):

ICON
South County Business Park
Leopardstown,
Dublin 18,
Ireland
Telephone: +353 (1) 291 2000

Declaration of Sponsor or Responsible Medical Officer

Title: ECOSPOR IV: An Open-Label Extension of Study SERES-012 Evaluating SER-109 in Adult Subjects with Recurrent *Clostridium difficile* Infection (RCDI)

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, 1989, and the guidelines on Good Clinical Practice.



03/06/2017

Michele Trucksis, PhD, MD

Executive Vice President, Chief Medical Officer

Seres Therapeutics, Inc.

INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for SER-109. I have read the SERES-012 Protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

STUDY CONTACTS

Sponsor Responsible Medical Officer

Michele Trucksis, PhD, MD

Executive Vice President, Chief Medical Officer

Seres Therapeutics, Inc.

200 Sidney Street

Cambridge, MA 02139

Email: mtrucksis@serestherapeutics.com

CRO Medical Monitor

Anthony Japour MD

Medical Director

ICON Clinical Research, PLC.

2100 Pennbrook Parkway

North Wales, PA

Telephone: +1 215 616 6439

Mobile: +1 267 429 6601

Email: Anthony.Japour@iconplc.com

1. PROTOCOL SYNOPSIS

SPONSOR NAME: Seres Therapeutics, Inc.

ACTIVE INGREDIENT: SER-109 (Eubacterial Spores, Purified Suspension, Encapsulated)

PROTOCOL TITLE: ECOSPOR IV: An Open-Label Extension of Study SERES-012 Evaluating SER-109 in Adult Subjects with Recurrent *Clostridium difficile* Infection (RCDI)

STUDY CENTERS: Approximately 100 study centers in the North America

PLANNED STUDY PERIOD:	**CLINICAL PHASE:** 2
Estimated date first patient enrolled: 2Q2017 Estimated date last patient completed: 3Q2019	

DEFINITIONS:

For this study, CDI recurrence during the study is defined by the following criteria:

- Positive *Clostridium difficile* test on a stool sample determined by a toxin assay
- ≥ 3 unformed bowel movements per day over 2 consecutive days and the requirement that patients must continue to have diarrhea until antibiotic treatment is initiated
- Assessment by the investigator (based on clinical assessment) that the patient's condition warrants antibiotic treatment

OBJECTIVES:

Primary Efficacy Objective

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined by a toxin assay, up to 8 weeks after initiation of treatment

Secondary Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a PCR algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109

- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, diagnosis-related group (DRG)-adjusted hospital costs up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

STUDY DESIGN:

ECOSPOR IV is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a treatment regimen SER-109 in adult subjects 18 years of age or older with recurrent *Clostridium difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012.

Subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a treatment regimen of SER-109 or placebo in Study SERES-012, and who have responded to 7 to 14 days of standard-of-care (SOC) antibiotic treatment for CDI (i.e. vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID]) will be eligible to enroll and receive a treatment regimen of SER-109 in Study SERES-013. A treatment regimen of SER-109 is administered orally as 3×10^7 spore colony forming units (SCFUs) in 4 capsules once daily for 3 consecutive days. Approximately 142 eligible subjects with recurrent CDI disease from Study SERES-012 are expected to enroll.

Screening will begin at the Early Termination (ET) Visit of Study SERES-012. Eligible subjects will provide informed consent and undergo all baseline evaluations at the Screening Visit. Subjects will receive 7 to 14 days of oral vancomycin 125 mg QID, or oral fidaxomicin 200 mg BID, starting from Day -17 to Day -13. On Day -1, within 3 days of completion of SOC antibiotic treatment for their CDI, subjects will undergo a bowel cleanse by consuming 10 oz. (~300 mL) of oral magnesium citrate followed by overnight fasting. Subjects with impaired kidney function who are unable to take magnesium citrate will take 250 mL of GoLytely (polyethylene glycol electrolyte solution). Subjects will come to the clinic after an overnight fast on the morning of Day 1 to receive a single dose of oral SER-109 (3×10^7 SCFUs) in 4 capsules and have all safety evaluations performed. On Day 1, subjects will be dispensed a 2-day supply of SER-109 (3×10^7 SCFUs) in 4 capsules with instructions for home-administration of a single daily dose in the morning before breakfast on Day 2 and Day 3. Subjects will be contacted by phone on Day 2 and Day 3 to confirm they have taken study drug before breakfast and to inquire about their general health. If subjects have not taken study drug when contacted, they will be reminded to do so as soon as possible. From Day 1 to Week 8, all subjects will be contacted by phone by study site personnel weekly, with the exception of a home or in-clinic visit at Week 2 and an in-clinic visit at Week 8, and queried for adverse events (AEs) and diarrheal symptoms. After Week 8, all subjects will be contacted by phone by study site personnel every 4 weeks (i.e., Weeks 12, 16, 20, and 24) and queried for serious adverse events [SAEs] and adverse events of special interest [AESIs]). Health-related quality of life and health outcomes will be assessed throughout the study via the CDI-specific, Cdiff32 Health Related Quality of Life (HRQoL) and EuroQol 5 Dimension 5 Level (EQ-5D-5L) questionnaires.

To document episodes of diarrhea, subjects will complete a diarrhea log (see Investigator Site File) when they experience 1 or more daily episodes of unformed stools. If diarrheal symptoms recur (≥ 3 unformed stools per day over 2 consecutive days) between scheduled visits, subjects will be instructed to contact the investigator and return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI (Recurrence Visit).

Subjects who have a confirmed CDI recurrence should continue to be followed for safety assessments through Week 24. Favorable clinical outcome in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of the SER-109 treatment regimen. CDI recurrence is defined as ≥3 unformed stools per day over 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* toxin assay on a stool sample and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. To inform subject care, a *C. difficile* stool test may be performed locally at the study site. Stool samples collected for suspected CDI recurrence will also be processed and shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual). Data from the *C. difficile* toxin test (either EIA or CCNA), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

The schedule of assessments and procedures is provided in Table 1.

PLANNED NUMBER OF SUBJECTS:

Approximately 142 adults who experienced recurrent *C. difficile* by Week 8 in Study SERES-012.

PRIMARY DIAGNOSIS: Recurrent CDI

INCLUSION CRITERIA:

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study-related procedures:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012.
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject must be able to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES-012 must have met the protocol definition of:
   a. ≥3 unformed stools per day for 2 consecutive days
   b. A positive *C. difficile* stool toxin assay
   c. The requirement of CDI SOC antibiotic therapy (defined as 7 to 14 days of treatment with vancomycin 125 mg QID and/or fidaxomicin 200 mg BID).
   d. An adequate clinical response following SOC antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
   e. The requirement that the subject can be dosed with study drug within 4 days of SOC antibiotic completion.
4. If female, subject is non-lactating, and is either:
   a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
   b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

EXCLUSION CRITERIA:

A subject will not be enrolled if the subject meets any of the following criteria:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.

2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. History of irritable bowel syndrome
4. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding).
5. Currently receiving steroids, ≥20 mg/day of prednisone or equivalent, for >2 weeks, or maintenance levels of immunosuppression therapy (e.g. cyclosporine, tacrolimus).
6. Taking antibacterial therapy other than SOC antibiotics for the most recent episode of CDI within 14 days prior to randomization (a single-day antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.
7. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery.
8. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past 12 months.
9. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to start of study.
10. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term opiate use is permitted (e.g., for a dental extraction).
11. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
12. Known stool studies positive for ova and/or parasites within the 30 days before enrollment.
13. Poor concurrent medical risks with clinically significant co-morbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
14. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
15. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
16. Any history of immunoglobulin (IgG) replacement therapy
17. Any history of fecal microbiota transplantation (FMT).
18. Previously enrolled in this study or any Seres Therapeutics, Inc. sponsored study other than SERES-012.
19. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
20. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
21. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives.
22. Life expectancy is 24 weeks or less.

INVESTIGATIONAL PRODUCT, DOSE, AND MODE OF ADMINISTRATION:

Study drug is for investigational use only. Study drug dispensed at the study site will be stored at the study site or pharmacy. Subjects will be provided instructions for proper storage of study drug dispensed for home-administration at home. Instructions for storage are provided in the Investigator Site File. SER-109 will be supplied in size 00 capsules.

The dose, route, and schedule of study drug administration are presented in the following table:

Treatment	Dose	Dosage Form and Amount	Route	Number of Subjects
SER-109	3×10^7 spore colony forming units (SCFUs)	4 capsules once daily for 3 consecutive days	Oral	~ 142

STATISTICAL METHODS:

Analysis Populations:

Intent-to-Treat Population

The Intent-to-Treat- (ITT) Population will consist of all enrolled subjects.

Modified Intent-to-Treat Population

The Modified ITT (mITT) Population will consist of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.

Safety Population

The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

Study Endpoints:

Primary Efficacy Endpoint

- Recurrence of CDI as determined by a toxin assay up to 8 weeks after initiation of treatment

Secondary Efficacy Endpoints

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay
- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

Exploratory Efficacy Endpoints

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after treatment initiation (for subjects hospitalized)
- Diagnosis-related group-adjusted hospital costs (for subjects hospitalized) after initiation of treatment
- Changes from Baseline in Health-Related Quality of Life (HRQoL) and health outcomes as assessed by the EQ-5D-5L from Day 1 through Weeks 8 and 24, and assessed by the Cdiff32 HRQoL from Day 1 to Week 1 and Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

Safety Endpoints

- Incidence of AEs
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

Analysis of Primary Efficacy Endpoint:

The primary efficacy outcome is the recurrence of CDI determined by a toxin assay through Week 8 after receipt of a SER-109 treatment regimen in the ITT Population. Subjects will be categorized as having favorable (no CDI recurrence) or unfavorable outcomes (had CDI recurrence). Rules for imputing CDI recurrence status for subjects with missing data will be provided in the Statistical Analysis Plan (SAP).

The number and percentage of subjects defined as having favorable and unfavorable outcomes will be reported with exact 95% confidence intervals (CIs) for subjects who were randomized to SER-109 and placebo in SERES-012 separately, as well as both groups combined. The CIs will be derived using the Clopper-Pearson exact method.

Table 1: Schedule of Assessments and Procedures

	SCREENING PERIOD			**EFFICACY PERIOD**							**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC*[b]**	***TC*[c]**	***Clinic***	***TC*[d]**	***TC*[TCd]**	***TC***	***Clinic or home***	***TC Weekly***	***Clinic***	***TC***	***TC – Study Completion***	***Clinic***	***Clinic***
Day/Week	**Screening** *Note: Assessments performed at the ET visit in SERES-012 do not need to be repeated*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 3-7 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
Informed Consent	X													
Eligibility criteria review	X		X	X										
Confirm clinical response to SOC antibiotic			X	X										
IxRS registration	X			X										
Medical History	X													
Physical Exam	X			X										
Focused History and Physical										X			X	X
Weight	X			X						X			X	X
Vital signs[e]	X			X[f]						X			X	X
Chemistry and hematology	X			X[g]						X			X	X
Serum for FBMR				X[g]				X					X	X
Routine urine dipstick				X[g]										
Urine pregnancy test (WOCBP)	X			X[g]						X			X	X
Stool: Study Entry: central lab: *C. Diff* toxin assay	X													

	SCREENING PERIOD			**EFFICACY PERIOD**							**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC*[b]**	***TC*[c]**	***Clinic***	***TC*[d]**	***TC*[TCd]**	***TC***	***Clinic or home***	***TC Weekly***	***Clinic***	***TC***	***TC – Study Completion***	***Clinic***	***Clinic***
Day/Week	**Screening** *Note: Assessments performed at the ET visit in SERES-012 do not need to be repeated*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 3-7 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
Stool: On Study Recurrence/ET: central lab: *C. Diff* testing													X	X
Stool: microbiome testing			X[i]				X[i]	X[h]		X[i]		X[i]	X[i]	X[i]
Stool: metabolomics testing			X[i]				X[i]	X[h]		X[i]			X	X
Provide stool collection kits	X			X						X				
Stop SOC Abx		X												
Administer bowel cleanse			X											
Confirm subject fasted for ≥8 h prior to study drug dosing				X										
Confirm subject administered bowel cleanse on Day -1				X										
Study drug dosing				X	X	X								
Confirmation of study drug administration					X	X								
Study drug accountability										X				
Prior/ concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE assessment		X	X	X	X	X	X	X	X	X	X	X	X	X

	SCREENING PERIOD			**EFFICACY PERIOD**							**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC*[b]**	***TC*[c]**	***Clinic***	***TC*[d]**	***TC*[TCd]**	***TC***	***Clinic or home***	***TC Weekly***	***Clinic***	***TC***	***TC – Study Completion***	***Clinic***	***Clinic***
Day/Week	**Screening** *Note: Assessments performed at the ET visit in SERES-012 do not need to be repeated*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 3-7 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
Evaluation of diarrheal episodes	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cdiff32 HRQoL Survey				X			X			X			X[j]	X[j]
EuroQol 5 Dimensions 5 Level (EQ-5D-5L)				X						X		X	X	X

Abbreviations: Abx=antibiotics; AE=adverse event; *C.diff=clostridium difficile*; ET=early termination; FBMR=future biomedical research; HRQoL=Health-Related Quality of Life; IxRS=interactive voice and web response system; SOC=standard of care; TC=telephone call; WOCBP=women of childbearing potential

[a] Subjects with a confirmed CDI recurrence after Week 8 should continue to be followed for safety assessments through Week 24.

[b] Phone call to remind subjects to stop taking antibiotics and to collect a stool sample on Day -1 before magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel cleanse.

[c] Phone call to confirm termination of antibiotics on day of last scheduled antibiotic dose and review instructions for Day -1 activities including collection of a stool sample before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel cleanse. Stool sample collected at Day -1 will be returned to the clinic on Day 1.

[TCd] Subjects should be called to confirm they have taken study drug before breakfast and to inquire about their general health

[e] Blood pressure, pulse, respiratory rate, and body temperature assessed in the supine position

[f] To be assessed immediately before and approximately 30 minutes after study drug dosing

[g] To be assessed prior to study drug dosing

[h] Stool samples may be collected in the clinic or at home. If the subject elects to have an in-clinic visit, the sample may be collected at home prior to the visit and brought to the study site. If the subject is unable to bring the stool sample to the study site, arrangements may be made to pick up the sample at the subject's home and bring it to the study site or may ship directly to the central laboratory (i.e., home visit by nurse or courier).

[i] The stool sample collected on Day -1 should be collected prior to the bowel cleanse and may be brought to the study site for the Day 1 visit. The stool samples collected on Week 8 (in clinic visit) may be collected at home prior to the visit and brought to the study site. If the subject is unable to bring the sample to the study site at any other visit, a courier may be arranged to pick up the sample at the subject's home and bring it to the study site.

[j] Administer if prior to Week 8 visit

2.0 TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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3. LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS OF TERMS

AE	Adverse Event
AESI	Adverse Event of Special Interest
BMI	Body Mass Index
CCNA	Cell Cytotoxicity Neutralization Assay
CDI	*Clostridium difficile* infection
Cdiff32 HRQoL	*Clostridium difficile* (CDiff32) Health-Related Quality of Life Survey
CFR	Code of Federal Regulations
CI	Confidence interval
CLIA	Clinical Laboratory Improvement Amendment
CMH	Cochran-Mantel-Haenszel
DRG	Diagnosis-related group
DSMC	Data Safety Monitoring Committee
eCRF	electronic Case Report Form
EAIR	exposure-adjusted incidence rates
EIA	Enzyme immunoassay
ET	Early Termination
EQ-5D-5L	EuroQol 5 Dimensions 5 Level
FDA	Food and Drug Administration
FMT	Fecal microbiota transplantation
GCP	Good Clinical Practice
GDH	Glutamate dehydrogenase
GI	Gastrointestinal
HRQoL	Health-Related Quality of Life
IBS	Irritable Bowel Syndrome
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ISC	Independent Statistical Center

ITT	Intent-to-Treat
IxRS	Interactive voice and web response system
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified Intent-to-Treat
OTU	Operational taxonomic unit
PCR	Polymerase Chain Reaction
PP	Per-Protocol
RCDI	Recurrent *Clostridium difficile* infection
RR	Relative risk
SAE	Serious adverse event
SAER	Serious adverse event report
SAP	Statistical Analysis Plan
SCFU	Spore Colony Forming Units
SOC	Standard of care
SporQs	Spore Equivalents, a dosing unit measured by dipicolinic acid content
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
WOCBP	Women of childbearing potential

4. INTRODUCTION

Clostridium difficile is a spore-forming Gram-positive anaerobe present throughout the environment and, in low amounts, can be a component of the gut flora of a healthy individual. *Clostridium difficile* infection (CDI) usually develops in patients with a history of antibiotic use that depletes the normal gut flora, enabling *C. difficile* to colonize and proliferate within the colon, elaborating virulent toxins A and B. These toxins invade epithelial cells disrupting their cytoskeleton, resulting in damage to the epithelial barrier and promoting mucosal inflammation. The clinical manifestations of CDI vary broadly, ranging from nuisance diarrhea lasting a few days, to more pronounced disease with severe colonic inflammation that can develop into pseudomembraneous colitis with associated systemic toxicity requiring lifesaving colectomy.

With ever-increasing use of antibiotics, particularly in the aging populations in hospitals and in nursing homes, the incidence of *C. difficile*-associated disease has been increasing such that *C. difficile* is the leading cause of nosocomial infection in the United States (US). The Centers for Disease Control and Prevention estimate that *C. difficile* causes diarrhea linked to approximately 29,000 American deaths each year (Lessa et al, 2015). In Canada, there are approximately 37,900 CDI episodes each year (2012); 7980 (21%) of these are relapses (Levy, 2015). In the European Union (EU), the number of reported cases of CDI has also increased in recent years, and is estimated to affect 172,000 patients per year.

Clostridium difficile spores can survive for months in hospitals and long-term care facilities where they can cause repeated CDI episodes. Virtually all antibiotics have been implicated in association with CDI. The mechanistic link to antibiotic use is based on the finding that a healthy microbial ecology resists pathogen colonization by competing for nutrients and resources in the gut (Theriot et al, 2014; Weingarden et al, 2014). Antibiotic use disrupts the microbiota and liberates nutrients that enable colonization by *C. difficile* (Ng et al, 2013).

The incidence of recurrent CDI has paralleled the increased incidence of primary infection. CDI recurs in approximately 25% of patients after antibiotic treatment for first-time disease (Bakken et al, 2011; Depestel and Aronoff, 2013; Surawicz et al, 2013). After the first recurrent episode, patients are at an even higher risk for subsequent CDI, estimated to be > 60% after the second or subsequent episode (Higa and Kelly, 2013). There are few proven, approved therapeutic options for significantly reducing CDI recurrence in patients with recurrent CDI. Some patients are treated with antibiotics indefinitely to avoid persistent diarrhea and other sequelae of CDI.

SER-109 is an Ecobiotic® drug being developed for the treatment of adults with recurrent CDI. SER-109 is an ecology of bacteria in spore form, enriched from stool donations obtained from healthy, screened donors. The bacterial spores are enriched by thorough killing of the vegetative microorganisms, then fractionating the resulting spore population away from inactive components and formulating and encapsulating the spores for oral delivery. SER-109 is administered to subjects after completion of a course of antibiotic therapy for recurrent CDI.

SER-109 has been shown to prevent CDI and to treat *C. difficile* relapse in nonclinical studies in mice and hamsters (see Investigator's Brochure for more information). Clinical experience with SER-109 includes three studies: 1) a completed open-label, two-part study in 30 subjects with a history of 3 or more occurrences of CDI (SERES-001); 2) a completed double-blind, placebo-controlled, parallel-group study in adults with recurrent CDI (SERES-004) and 3) an expanded access for intermediate-size patient populations and open-label extension of study SERES-004

(SERES-005). To date, a total of 142 subjects have received 1 or 2 doses of SER-109 in the completed and ongoing clinical studies with SER-109. The available safety data collected to date suggests that SER-109 is well-tolerated with an acceptable safety profile, although it is associated with a slight increase in gastrointestinal adverse effects, particularly diarrhea, compared to placebo (25% vs 14%). There have been 2 deaths (one in Study SERES-004 and one in the ongoing, open-label Study SERES-005), both of which were deemed by the investigators not related to SER-109. There have been no concerning trends in laboratory values, vital sign values, or physical examination findings in the completed SERES-001 or SERES-004 studies or in the SERES-005 preliminary safety datasets. A summary of clinical efficacy and safety is presented below.

1.1.1. Summary of Clinical Efficacy and Safety

Study SERES-001 was a 2-part study exploring the safety and efficacy of SER-109 in adult subjects (22 to 88 years of age) with recurrent CDI. The primary efficacy measure was response to SER-109 treatment up to 8 weeks after initiation of therapy. Response was defined as the absence of CDI during the efficacy evaluation period (Day 1 to Week 8). Fifteen subjects in Part 1 of the study received oral SER-109 (a mean dose of $1.7x10^9$ spore equivalents [SporQs], a dosing unit measured by dipicolinic acid content) administered over 2 days. Fifteen subjects in Part 2 of the study received oral SER-109 (mean dose of $1x10^8$ SporQs) administered over 1 day.

In the open label study, SERES-001, SER-109 resulted in per-protocol efficacy of 86.7% (26/30 subjects) and an 8-week clinical cure rate of 96.7% (29/30 subjects). One subject had a recurrence at Day 5 and declined re-treatment. Three subjects reported diarrhea with a concomitant positive test result for *C. difficile* between 5 and 9 days after receiving SER-109. All 3 subjects were negative for *C. difficile* carriage and clinically CDI-free at 8 weeks, and were judged to be clinically cured without treatment with a course of antibiotics. One subject had a recurrence at 26 days after dosing, was re-treated per protocol, and was CDI-free 8 weeks after their second dose.

Most subjects (27/30) experienced ≥ 1 AE in Study SERES-001, all of which were treatment-emergent AEs (TEAEs). Fourteen TEAEs were considered related to study drug and all were mild or moderate. The most common system organ class (SOC) was GI disorders, and the most common preferred term was diarrhea. Four subjects experienced a total of 7 serious AEs (SAEs), none of which were considered by the investigators to be drug-related.

SERES-004 was a randomized, double-blind, placebo-controlled Phase 2 study conducted in the U.S. Eighty-nine (89) subjects were randomized 2:1 to receive either SER-109 or placebo, respectively, following antibiotic treatment for recurrent CDI, and stratified by age (< 65 years; ≥ 65 years). The primary objective was to demonstrate the superiority of SER-109 versus placebo based on the proportion of subjects experiencing a CDI recurrence up to 8 weeks after treatment. The primary safety objective was to evaluate the safety of SER-109 in adults with recurrent CDI up to 12 weeks after treatment as determined by clinical and laboratory safety assessments.

The study did not meet the primary objective of reducing the relative risk of CDI recurrence up to 8 weeks following dosing. Overall, recurrence of *C. difficile* positive diarrhea requiring antibiotic treatment during the 8 weeks post-treatment occurred in 42 (47.2%) subjects (16 [53.3%] subjects randomized to receive placebo vs. 26 [44.1%] subjects randomized to receive SER-109. The relative risk of recurrence in subjects receiving placebo vs. SER-109, adjusted for age stratum, was 1.22, with a corresponding 95% CI of (0.79, 1.88). Of the 43 subjects stratified to the <65 years of

age strata, recurrence was observed in 12/28 (42.9%) subjects randomized to receive SER-109 and 4/15 (26.7%) subjects randomized to receive placebo. Of the 46 subjects stratified to the ≥65 years of age strata, recurrence was observed in 14/31 (45.2%) subjects randomized to receive SER-109 and 12/15 (80.0%) subjects randomized to receive placebo. Overall, of the 42 subjects who met the primary endpoint of CDI recurrence by Week 8, 35 subjects discontinued the study due to their CDI recurrence prior to Week 8. Of the 35 subjects who discontinued the study due to a CDI recurrence prior to Week 8, 34 enrolled in the open-label extension study SERES-005.

In Study SERES-004, a total of 66 of the 89 subjects randomized (74.2%), 46 of the 60 (76.7%) subjects who received SER-109 and 20 of the 29 (69.0%) subjects who received placebo, experienced at least 1 TEAE. Fifteen of the 89 (16.9%) subjects, 11 of the 60 (18.3%) subjects who received SER-109 and 4 of the 29 (13.8%) subjects who received placebo, experienced at least 1 TEAE that was considered by the investigators to be drug-related. Like Study SERES-001, the most commonly reported SOC was GI disorders (55% in the SER-109 group and 44.8% in the Placebo group). The most commonly reported (incidence ≥5%) preferred terms in the GI SOC reported in subjects who received SER-109 were diarrhea, abdominal pain, flatulence, nausea, and constipation. The majority of TEAEs were mild or moderate in severity. Six of the 60 (10%) subjects who received SER-109 experienced an event that has been reported as severe. Twelve of the 89 (10.1%) subjects enrolled (9 subjects who received SER-109 [15%] and 3 subjects who received placebo [10.3%] have experienced a total of 43 treatment-emergent SAEs, none of which were considered to be drug-related by the investigator. One subject had an SAE (metastatic non-small cell lung cancer) that was fatal and led to study withdrawal.

SERES-005 began as an open-label extension study of SERES-004 conducted in the U.S, offered to subjects who received an investigational product in SERES-004, but recurred prior to 8-weeks post-treatment. In April 2016, the study was amended to include expanded access to an intermediate-size patient population of adults, 18 years of age or older with recurrent CDI, for whom there is no comparable or alternative therapy. Seventy-three patients, 34 who enrolled from Study SERES-004 and 39 who enrolled under an expanded access met the eligibility criteria for the study. SERES-005 subjects who enrolled from study SERES-004 have completed the study; the subjects enrolled under expanded access are continuing in the study. The primary efficacy objective is to evaluate CDI recurrence rates in adults up to 8 weeks post-treatment with SER-109. The primary safety objective is to evaluate the safety and tolerability of SER-109 in adults with recurrent CDI. Among the 34 subjects who enrolled from Study SERES-004 and who have completed the study, recurrence of CDI up to 8 weeks post-treatment with SER-109 was observed in 11 (32.4%) subjects.

Preliminary results from the ongoing SERES-005 study indicate that, overall, 3 out of the 44 (6.8%) subjects, for whom safety data is available, experienced at least 1 treatment-emergent SAE. None of these SAEs are considered to be related to study treatment. One subject enrolled under the extension study experienced a total of 8 treatment-emergent SAEs. Five of these treatment-emergent SAEs resulted in death. Cerebrovascular accident, congestive cardiac failure, myocardial infarction, aspiration pneumonia, and type 2 diabetes mellitus were reported as the cause of death. The most commonly reported SOCs have been GI disorders in the extension study population and Infections and Infestations in the expanded access population. The most commonly reported (incidence ≥5%) preferred terms overall have been diarrhea, abdominal pain, nasopharyngitis, constipation, flatulence, nausea, and urinary tract infection. The majority of TEAEs have been mild or moderate in severity.

Thus, clinical experience to date suggests that SER-109 is well-tolerated with an acceptable safety profile. Overall, there have been no drug-related treatment-emergent SAEs and the majority of related TEAEs have been mild or moderate in severity and most commonly associated with the gastrointestinal tract. Additional information regarding clinical experience with SER-109 can be found in the Investigator's Brochure.

Although safety data with SER-109 has been relatively consistent across studies, efficacy data in the placebo-controlled Study SERES-004 was inconsistent with results from the open-label Study SERES-001. Hypotheses to explain why the primary endpoint of reducing the relative risk of CDI recurrence at up to 8-weeks was not achieved in Study SERES-004 include that the diagnostic test for entry may not have differentiated subjects with active CDI disease from those with *C. difficile* carriage. This would have led to enrolling subjects who may have been experiencing post-CDI irritable bowel syndrome (IBS)-like symptoms but, were only colonized by *C. difficile* and not an active infection, the diagnostic test for recurrences which primarily used PCR overestimated recurrences, and although analysis of the microbiome identified that SER-109 in SERES-004 was biologically active, the dose administered in Phase 2 may need to be increased for optimal efficacy (see rationale below).

1.2. Rationale

There are few proven, approved therapeutic options for significantly reducing CDI recurrence in patients with recurrent CDI. The aim of this study is to further evaluate the safety, tolerability, and efficacy of a treatment regimen SER-109 in adult subjects 18 years of age or older with recurrent *Clostridium difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012.

1.2.1. Rationale for Dose and Treatment Regimen

In this study, subjects will receive a dose of SER-109 ($3x10^7$ spore colony forming units [SCFUs]) per day for 3 consecutive days. The dose and treatment regimen was chosen based on several factors.

In Study SERES-001, subjects received doses of SER-109 ranging from $3x10^7$ to $2x10^{10}$ SporQs ($2x10^5$ to $5x10^9$ SCFU) given over one or two days. Analysis of changes in the subject microbiome demonstrates that spore forming species richness in the subjects' GI tract at 1 week was positively correlated with SER-109 dose. Importantly, of subjects who recurred in SERES-004, about 50% of recurrences in both placebo and SER-109 arms happened by Day 10 and 75% by Day 20, starting as early as Day 3. In SERES-004, the engraftment of SER-109 spore-forming bacteria in treated subjects' gastrointestinal tracts was less robust and less rapid as compared to that observed in SERES-001, although it was significantly greater than the changes in placebo-treated subjects. Engraftment improved at later time points, but due to early recurrences, the SER-109-induced microbiome change in SERES-004 may have been too late from a therapeutic perspective. In addition, it was generally observed that commensal spore-forming species richness at 1 week post dosing is correlated with better clinical outcome. In aggregate, these observations are central to the design of the proposed regimen that provides a higher daily dose ($3x10^7$ SCFU) repeated daily over 3 days following the completion of antibiotics as compared to dosing in SERES-004. Due to the fact that recurrence happens early, the slower SER-109-induced microbiome changes in SERES-004 suggest that the $1x10^8$ dose target (SporQ) was likely below

the required amount to achieve a therapeutic response. To account for variations in antibiotic washout, and to provide dosing prior to the earliest observed recurrences, three (3) doses will be administered on Days 1 - 3 following antibiotic cessation. We have chosen a three-fold higher dose level based on the SCFU metric, $3x10^7$ SCFU, as an amount commensurate with engraftment richness to the degree correlating with protection against recurrence.

1.2.2. Rationale for Endpoints and *C. difficile* Diagnostic Criteria

The introduction of molecular tests, which are more sensitive and detect microbial DNA instead of toxin, has led to greater detection of *Clostridium difficile* but detect *C. difficile* bacteria regardless of toxin production. This phenomenon has called into question whether a positive PCR result reflects clinical disease or represents *C. difficile* colonization (Polange et al, 2015).

Thus, in SERES-004, the diagnostic test for entry may not have differentiated subjects with active CDI disease from those with *C. difficile* carriage. This would lead to enrolling subjects who may be experiencing a post-CDI irritable bowel syndrome (IBS) if colonized by *C. difficile.* IBS following CDI is reported to occur in up to 25% of CDI patients (Wadhwa et al, 2016). This would have decreased the power of the study to differentiate the treatment arms as those subjects without a true diagnosis of RCDI are less likely to recur.

Since PCR diagnostics in SERES-004 may have led to misclassification of subjects with diarrhea and *C. difficile* colonization as recurrence, the primary efficacy endpoint in this study is the recurrence of CDI in subjects who receive SER-109 or placebo using a *C. difficile* toxin positive diagnostic (not toxin gene-based) up to 8 weeks after initiation of treatment. Unlike the PCR diagnostic for *C. difficile*, the toxin based tests, such as enzyme immunoassay (EIA) for toxin A and B or the cell cytotoxicity neutralization assay (CCNA) detects the presence of *C. difficile* toxin in fecal samples. Thus, recurrence is defined as ≥ 3 unformed stools per day for 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay, and assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment.

2. STUDY OVERVIEW

ECOSPOR IV is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a treatment regimen SER-109 in adult subjects 18 years of age or older with recurrent *Clostridium difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012.

This study will be conducted at approximately 100 study centers in the North America. Subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a treatment regimen of SER 109 or placebo in Study SERES-012, and who have responded to 7 to 14 days of standard-of-care (SOC) antibiotic treatment for CDI (i.e. vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID]) will be eligible to enroll and receive a treatment regimen of SER-109 in Study SERES-013. A treatment regimen of SER-109 is administered orally as 3×10^7 spore colony forming units (SCFUs) in 4 capsules once daily for 3 consecutive days. Approximately 142 eligible subjects with recurrent CDI disease from Study SERES-012 are expected to enroll.

The study duration is approximately 27 weeks, including a ~3-week Screening Period, an 8-week Efficacy Period, and a 16-week Follow-up Period from initiation of treatment on Day 1.

Favorable clinical outcome in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of treatment of study drug, with CDI recurrence defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

2.1. Trial Conduct

Screening will begin at the Early Termination (ET) Visit of Study SERES-012. Eligible subjects will provide informed consent and undergo all baseline evaluations at the Screening Visit. Assessments performed at the ET Visit of Study SERES-012 do not need to be repeated. Subjects will receive 7 to 14 days of oral vancomycin 125 mg QID, or oral fidaxomicin 200 mg BID, starting from Day -17 to Day -13. To be eligible for study drug dosing, all subjects must have an adequate clinical response following antibiotic therapy to treat their CDI, defined as <3 unformed stools in 24 hours for 2 or more consecutive days up to study drug initiation on Day 1. On Day -1, within 3 days of completion of SOC antibiotic treatment for their CDI, subjects will undergo a bowel cleanse by consuming 10 oz. (~300 mL) of oral magnesium citrate followed by overnight fasting. Subjects with impaired kidney function who are unable to take magnesium citrate will take 250 mL of GoLytely (polyethylene glycol electrolyte solution). Subjects will come to the clinic after an overnight fast on Day 1 to receive a dose of oral SER-109 (3×10^7 SCFUs) in 4 capsules and have all safety evaluations performed. On Day 1, subjects will be dispensed a 2-day supply of SER-109 (3×10^7 SCFUs) in 4 capsules with instructions for home-administration of a single daily dose in the morning before breakfast on Day 2 and Day 3. Subjects will be contacted by phone on Day 2 and Day 3 to confirm they have taken study drug before breakfast and to inquire about their general health. If subjects have not taken study drug when contacted, they will be reminded to do

so as soon as possible. From Day 1 to Week 8, all subjects will be contacted by phone by study site personnel weekly, with the exception of a home or in-clinic visit at Week 2 and an in-clinic visit at Week 8, and queried for adverse events (AEs) and diarrheal symptoms. After Week 8, all subjects will be contacted by phone by study site personnel every 4 weeks (i.e., Weeks 12, 16, 20, and 24) and queried for serious adverse events [SAEs] and adverse events of special interest [AESIs]). Health-related quality of life and health outcomes will be assessed throughout the study via the CDI-specific, Cdiff32 Health Related Quality of Life (HRQoL) and EuroQol 5 Dimension 5 Level (EQ-5D-5L) questionnaires.

All AEs, SAEs/AESIs, and concomitant medications will be collected from initiation of study drug administration up to Week 8. From Week 8 up to Week 24, all SAEs/AESIs and SAE/AESI-related data, and any antibiotic medication and its corresponding indication will be collected.

To document episodes of diarrhea, subjects will complete a diarrhea log (see Investigator Site File) when they experience 1 or more daily episodes of unformed stools. If diarrheal symptoms recur (≥ 3 unformed stools per day over 2 consecutive days) between scheduled visits, subjects will be instructed to contact the investigator and return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI (Recurrence Visit).

Subjects who have a confirmed CDI recurrence should continue to be followed for safety assessments through Week 24. Favorable clinical outcome in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of the SER-109 treatment regimen. CDI recurrence is defined as ≥3 unformed stools per day over 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* toxin assay on a stool sample and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. To inform subject care, a *C. difficile* stool test may be performed locally at the study site. Stool samples collected for suspected CDI recurrence will also be processed and shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual). Data from the *C. difficile* toxin test (either EIA or CCNA), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

The schedule of assessments and procedures is provided in Table 1.

3. STUDY OBJECTIVES

3.1. Primary Efficacy Objective

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined by a toxin assay, up to 8 weeks after initiation of treatment

3.2. Secondary Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a PCR algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

3.3. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

3.4. Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109

- To determine, for subjects who are hospitalized, diagnosis-related group (DRG)-adjusted hospital costs (where available) up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

4. STUDY ENROLLMENT AND WITHDRAWAL

4.1. Inclusion Criteria

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study-related procedures:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012.
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject must be able to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES-012 must have met the protocol definition of:
   a. ≥3 unformed stools per day for 2 consecutive days
   b. A positive *C. difficile* stool toxin assay
   c. The requirement of CDI SOC antibiotic therapy (defined as 7 to 14 days of treatment with vancomycin 125 mg QID and/or fidaxomicin 200 mg BID).
   d. An adequate clinical response following SOC antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1
   e. The requirement that the subject can be dosed with study drug within 4 days of SOC antibiotic completion.
4. If female, subject is non-lactating, and is either:
   a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
   b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

4.2. Exclusion Criteria

A subject will not be enrolled if the subject meets any of the following criteria:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.
2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. History of irritable bowel syndrome
4. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding).
5. Currently receiving steroids, ≥20 mg/day of prednisone or equivalent, for >2 weeks, or maintenance levels of immunosuppression therapy (e.g. cyclosporine, tacrolimus).
6. Taking antibacterial therapy other than SOC antibiotics for the most recent episode of CDI within 14 days prior to randomization (a single-day antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.
7. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery.
8. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past 12 months.
9. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to start of study.
10. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term opiate use is permitted (e.g., for a dental extraction).
11. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
12. Known stool studies positive for ova and/or parasites within the 30 days before enrollment.
13. Poor concurrent medical risks with clinically significant co-morbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
14. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
15. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
16. Any history of immunoglobulin (IgG) replacement therapy.
17. Any history of fecal microbiota transplantation (FMT).

18. Previously enrolled in this study or any Seres Therapeutics, Inc. sponsored study other than SERES-012.
19. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
20. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
21. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives.
22. Life expectancy is 24 weeks or less.

4.3. Subject Monitoring and Withdrawal

4.3.1. Reasons for Withdrawal

Subjects should continue to be followed for safety assessments up to 24 weeks after treatment, even after a CDI recurrence. However, a subject may withdraw from the study at any time for any reason, without any consequence. In addition, a subject may be withdrawn from the study for reasons including the following:

- AE (typically an SAE)
- Subject choice (withdrawal of consent; investigator will attempt to ascertain reason)
- Protocol violation/non-compliance

4.3.2. Handling of Withdrawals and Discontinuations of Treatment

The primary reason for withdrawal from the study will be recorded in an electronic case report form (eCRF). Subjects who voluntarily withdraw, or who are withdrawn from the study will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section 7.3.3. Although subjects are free to withdraw at any time, subjects will be encouraged to remain in the study for follow-up safety evaluation.

Those subjects who withdraw from the study will be referred to a physician for follow-up care.

4.3.3. Lost to Follow-up

If a subject fails to appear for a follow up assessment, all attempts to contact the subject and information received during contact attempts must be documented in the subject's medical record. In any circumstance, every effort should be made to contact the subject and document subject outcome, (i.e., 3 documented contact attempts via phone calls, e-mail, etc., on separate occasions will be made to locate or contact the subject, and/or to determine health status).

4.3.4. Termination of Study

Although the sponsor has every intention of completing the study, the sponsor may terminate the study at any time for clinical or administrative reasons.

5. INVESTIGATIONAL PRODUCT

5.1. SER-109

SER-109 is an ecology of bacterial spores enriched from stool donations obtained from healthy, screened donors. SER-109 is formulated as an oral capsule for administration to patients following cessation of antibiotic therapy.

5.1.1. Donor Screening

Donors undergo a general health examination including gastrointestinal (GI) medical history, familial GI medical history, blood chemistry, hematology with complete blood count, urinalysis, and blood and fecal viral and bacterial pathogen testing before donating stool. The donor must successfully complete the physical screening and laboratory tests after the donation period before the material can be released for manufacturing. A description of donor screening procedures is provided in the Investigator's Brochure.

5.1.2. Manufacturing and Storage

SER-109 is manufactured using current Good Manufacturing Practice (GMP). Stool raw material is sourced from donors who are screened for health history, physical status, and a panel of pathogen tests; materials from a single donor are pooled to make a manufacturing lot. The manufacturing process inactivates non-spore forms of live bacteria and fungi, and potential parasites and viruses, and substantially reduces the amount of undigested food and inactivated non-spore components via successive separation steps. The purified material is then concentrated to enable oral capsule formulation, stored frozen, and quality control tested until formulation.

5.2. SER-109 Kit Storage and Handling

The investigational product (SER-109) will be provided as a per subject kit to include 3 bottles containing four size 00 capsules (3×10^7 SCFUs) in an opaque, 40 mL high density polyethylene container sealed with foil.

SER-109 is odorless and tasteless as prescribed. If chewed or if capsule integrity is compromised, SER-109 has a sweet taste.

Instructions for shipment, storage, accountability, reconciliation, and destruction of study drug are provided in the Investigator Site File.

5.3. Compliance

Subjects will be instructed to return all unused medication and all used packaging materials to the clinic at the Week 8 visit. Subject compliance to study drug will be checked by the investigator or their designee(s) and documented in the CRFs (e.g., tablet count). Subjects will be instructed to take all study drug doses in the morning after an overnight fast (nothing by mouth except for small amounts of water) of $\geq$ 8 hours. Subjects will be asked to remain fasting for up to 60 minutes following dosing.

5.4. METHOD OF ASSIGNING PATIENTS TO STUDY TREATMENT

This is an open-label study. All subjects who qualify for dosing will receive single daily doses of SER-109 (3×10^7 SCFUs) in 4 capsules administered over 3 consecutive days.

The interactive voice and web response system (IxRS) will assign appropriate bottles of SER-109 that will be available at the site for all subjects on their Day 1 study visit. Subjects who discontinue this study or who have previously received SER-109 in this study will not be permitted to re-enter. Similarly, SER-109 dispensed to a subject may not be re-used, even if the bottle(s) are returned unopened.

5.5. MAINTAINING THE RANDOMZATION CODES AND BREAKING THE STUDY BLIND

Not applicable. This study is not randomized or blinded.

5.6. Concomitant Medications

5.6.1. Prohibited Concomitant Medications

The following therapies are prohibited for the duration of the study:

- Probiotics
- Steroids (≥ 20 mg/day of prednisone or equivalent)
- Immunosuppressant therapy (e.g. cyclosporine, tacrolimus).
- Loperamide
- Diphenoxylate/atropine
- Cholestyramine
- Opiate treatment unless on a stable dose. Note: Short term opiate use is permitted (e.g., for a dental extraction).
- Chemotherapy, radiotherapy, or biologic treatment for active malignancy

5.7. Antibiotics for Suspected *Clostridium Difficile* Episodes Post-Randomization

Subjects suspected of having CDI will be asked to contact the investigator and return to the clinic for a *C. difficile* stool toxin test and evaluation for recurrence of CDI (Recurrence Visit) (see Section **7.3.3**).

Subjects must fulfill the following criteria for antibiotic use:

1. ≥ 3 unformed stools per day over 2 consecutive days
   - Diarrhea as defined here should continue up until the day antibiotics to treat CDI are initiated.
2. A positive *C. difficile* test on a stool sample determine by a toxin assay

- A *C. difficile* stool test may be performed at the local laboratory to inform subject care; the central laboratory result will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator.

3. Assessment by the investigator that the clinical condition of the subject warrants treatment

6. STUDY PROCEDURES

The schedule of assessments and procedures is presented in Table 1.

6.1. Duration of Participation

The duration of study participation is up to approximately 27 weeks, consisting of a Screening Period lasting up to ~3 weeks, an 8-week Efficacy Period, and a 16-week Follow-Up Period from initiation of study drug on Day 1.

6.2. Medical History

At the time of Screening, subjects' medical history will be updated with particular attention to the most recent CDI history. The antibiotic regimen, including dose and duration after the most recent CDI recurrence, will be documented. Subjects must demonstrate an adequate clinical response to the antibiotic regimen to treat the most recent CDI recurrence defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before study drug dosing.

6.3. Physical Examination

A physical examination will be conducted by a physician at the timepoints indicated in Table 1. A focused history and physical will be conducted at Week 8 and at the Early Termination Visit or any Recurrence Visit to the study site, if applicable.

6.4. Body Weight

Body weight will be obtained at all in-clinic visits according to the schedule in Table 1.

6.5. Vital Signs

Vital sign assessments including systolic and diastolic blood pressure, pulse, respiratory rate, and oral body temperature measurements will be obtained at the visits indicated in Table 1. Vital sign assessments on Day 1 should be obtained immediately before and approximately 30 minutes after dosing.

6.6. Laboratory Assessments

All hematology and blood chemistry laboratory tests will be performed by the central laboratory. The laboratory facilities for analysis of clinical laboratory samples obtained under this protocol will have adequate licensure and accreditation. Urine pregnancy tests will be performed at the sites. Details of sample handling, specific tests performed, and methodology will be provided in the Laboratory Manual.

6.6.1. Hematology and blood chemistry

Blood samples for hematology and blood chemistry will be obtained according to the schedule in Table 1. Blood samples for hematology and blood chemistry obtained on Day 1 (pre-dose) will be used to determine baseline data.

The central laboratory will flag subjects if they have all of the following abnormal laboratory results:

- Alanine aminotransferase (ALT) ≥ 3 x upper limit of normal (ULN)
- Aspartate aminotransferase (AST) ≥ 3 x ULN
- Total bilirubin > 2 x ULN
- Alkaline phosphatase < 2 x ULN

These subjects meet the conditions of a Hy's Law case, and should be reported in the same manner as an SAE (see Section Section 8.1).

6.6.2. Urinalysis

Urine dipstick testing will be performed at the study site according to the schedule in Table 1. If results for nitrates or leukocytes are positive, the urine sample may be sent to the central laboratory for analysis at the discretion of the investigator.

6.6.3. Pregnancy Testing

Women of childbearing potential (WOCBP) will have urine pregnancy tests according to the schedule in Table 1.

6.7. Stool Sample Collection and Analysis

Subjects will be asked to collect stool at home or in the clinic according to the schedule in Table 1. The sample collected on Day -1 may be brought to the study site for the Day 1 Visit. If the subject is unable to bring the stool sample to the study site for any visit, arrangements may be made to pick up the sample at the subject's home and bring it to the study site or may ship directly to the central laboratory (i.e., home visit by nurse or courier). Samples brought to the study site will be processed and then shipped to a central laboratory according to procedures defined in the Laboratory Manual. Stool collection kits will be provided to the subjects by the study sites.

Microbiome and metabolomics testing may be performed on some or all stool samples collected on Day -1 (prior to administering the bowel cleanse), at Week 1, Week 2, Week 8, Week 24 (for microbiome testing only), and at the Early Termination Visit or any Recurrence Visit to the study site, if applicable.

If recurrent CDI is suspected, to inform subject care, a *C. difficile* test can be performed by a CLIA-certified local laboratory using an FDA-approved test in order to inform subject treatment. Stool samples collected for suspected CDI recurrence must also be processed and shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual for details of *C. difficile* testing performed at the central laboratory). Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be

communicated to the investigator and the decision to treat with antibiotics will be based upon their assessment.

6.8. Biological Specimen Collection for Future Biomedical Research

The sponsor may conduct future biomedical research on specimens (including serum and stool) routinely and specifically collected during this clinical study for potential commercial use by Seres Therapeutics, Inc. and specimens may be stored for up to 10 years.

6.9. Monitoring of Diarrheal Symptoms and General Health

Subjects will be instructed to complete a diarrhea log (see Investigator Site File) when they experience 1 or more daily episodes. At all scheduled telephone calls and study site visits, subjects will be queried regarding general well-being; AEs; diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea; and concomitant medications according to a standardized questionnaire. Any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day lasting ≥ 2 consecutive days) will be asked to come in for an in-clinic visit, where possible, for a *C. difficile* stool toxin test and evaluation for recurrence of CDI (see Section 7.3.3).

6.10. Health Outcome Assessment

Information such as mortality from any cause, hospitalizations, and hospital length of stay (in days), including days in the intensive care unit, and diagnosis-related group (DRG)-adjusted hospital costs (where available), will be collected as part of the health outcomes assessment throughout this study.

6.11. Quality of Life Assessment

The EQ-5D-5L is a standardized measure of health status. The CDiff32 HRQoL is a newly developed and validated health-related quality of life questionnaire specific to patients with CDI (Garey et al, 2016). Subjects will complete the EQ-5D-5L and Cdiff32 HRQoL at the time points indicated in the Schedule of Assessments (Table 1).

6.12. Clinical Response Evaluation

Recurrence of CDI will be determined by the investigator based on the following definition:

- A CDI episode is defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* stool test on a stool sample determined by a toxin assay and a decision by the investigator, based on clinical assessment, that antibiotic treatment is needed.

If subjects experience diarrhea symptoms (≥ 3 unformed stools per day for 2 consecutive days) or suspect a CDI episode, they should contact the investigator immediately (including on weekends) to arrange a Recurrence Visit for clinical evaluation and a *C. difficile* stool toxin test. The subject should not initiate antibiotic treatment for suspected CDI until instructed to do so by the investigator.

7. STUDY SCHEDULE

The Schedule of Assessments and Procedures is presented in Table 1. Study days are relative to the oral administration of the first dose of study drug on Day 1. Assessments will be performed and noted in each subject's chart or record.

7.1. Screening Period

7.1.1. Clinic Visit (Day -17 to Day -5)

Screening for this study begins at the Early Termination Visit of Study SERES-012. Assessments performed at the ET Visit in Study SERES-012 do not need to be repeated.

- After a full explanation of the study protocol, have each subject sign an informed consent form (ICF) before performing any study-related activity (including Screening activities).
- Ensure that the antibiotic regimen for the current CDI episode is consistent with the protocol (i.e., 7 to 14 days of oral vancomycin 125 mg QID, or oral fidaxomicin 200 mg BID)
- Assess each patient to ensure all inclusion criteria are met and no exclusion criteria are met.
- Update medical history. Ensure documentation of most recent CDI episode to include dates, test results, duration, and antibiotic treatment received.
- Perform a physical examination including vital sign measurements (blood pressure, pulse, respiratory rate, and body temperature), and weight.
- Collect blood and urine samples and ship to the central laboratory for evaluation of:
  - Blood chemistry
  - Hematology
  - Urine dipstick performed at the study site; if positive for nitrates and/or leukocytes, sample may be sent to central laboratory for analysis at the investigator's discretion
  - Urine pregnancy test, if applicable

    Note: Laboratory values obtained within 7 days of Screening and from the central laboratory of Study SERES-012 (e.g., SERES-012 visit due to CDI recurrence) do not need to be repeated at the Screening Visit.
- Obtain information regarding prior medication use within the 8 weeks before anticipated enrollment as well as concomitant medications. Comprehensive prior medication and concomitant medicine information from SERES-012 study can be used for this purpose.
- Ensure documentation of recent antibiotic or immunosuppressive medication use that may affect eligibility in the study.
- Assess AEs.

- Register subject in IWRS.
- Instruct subjects that, should they enroll in the study, they will need to meet the following requirements:
  - Probiotic use is prohibited during the study.
  - On Day -2, subject should take their last dose of antibiotic treatment for their CDI.
  - On Day -1, before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel preparation, they should collect a stool sample.
  - On the evening of Day -1, they must take 1 bottle (10 oz/~300 mL) of magnesium citrate (or, for patients with impaired kidney function, 250 mL of GoLytely [polyethylene glycol electrolyte solution]) followed by a fast (abstinence from food for ≥ 8 hours before receipt of SER-109) until at least 1 hour after receipt of SER-109 the next day.
  - On Day 1, patients must take 4 oral capsules of SER-109 and continue their fast for 1 hour after dosing.
  - Subjects will receive a 2-day supply of study drug with instructions for home-administration of single daily doses in the morning before breakfast on Day 2 and Day 3.
  - Stool samples will be collected by the subject at home and brought to the clinic at Screening, Day-1, Week 1, Week 2, Week 8, Week 24, and at the Early Termination Visit or any Recurrence Visit (if applicable). If the subject is unable to bring the stool sample to the study site for any visit, arrangements may be made to pick up the sample at the subject's home and bring it to the study site or may ship directly to the central laboratory (i.e., home visit by nurse or courier). Samples brought to the study site will be processed and then shipped to a central laboratory according to procedures defined in the Laboratory Manual. Stool collection kits will be provided for these at home collections.
  - Subjects are to contact the investigator immediately (including on weekends) if they experience diarrheal symptoms or suspect a CDI episode to arrange a Recurrence Visit (see Section 7.3.3) for clinical evaluation and a *C. difficile* stool toxin test. Advise subjects that antibiotic treatment should be initiated only after a positive *C. difficile* test, and clinical assessment by the investigator.

7.1.2. Pre-treatment Preparation Phone Call Visit (Day -4 to -2)

Contact subject by phone to:

- Perform diarrhea assessment to ensure that subject's diarrhea has been controlled (< 3 unformed stools per day for 2 consecutive days).
- Assess AEs.
- Review concomitant medications.
- Remind subject to not take antibiotics beyond Day -2.

- Remind subject to collect a stool sample before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel cleanse on Day -1.

7.1.3. Pre-treatment Preparation Phone Call Visit (Day -1)

Contact subject by phone to:

- Ensure all inclusion criteria continue to be met and no exclusion criteria are met, including that subject's CDI has responded to antibiotics without diarrhea over the previous 2 days (< 3 unformed stools per day).
- Ensure subject has discontinued antibiotics to control CDI symptoms, and has had their last dose of antibiotic on any day from Day- 4 to Day -2.
- Remind subject to collect a stool sample before beginning the magnesium citrate or GoLytely bowel cleanse.
- Ensure subject consumes a 10 oz (~300 mL) bottle of magnesium citrate (or, for subjects with impaired renal function, 250 mL of GoLytely and is prepared to fast overnight (no food or drink other than small amounts of water for ≥8 hours) before anticipated receipt of study drug.
- Assess AEs.
- Review concomitant medications.
- Remind subject to bring their Day -1 stool sample collected at home to the study site to be processed for shipment to the central laboratory.

7.2. Efficacy Period

7.2.1. Clinic Visit (Day 1)

7.2.1.1. Before Administering Study Drug (Pre-dose)

- Assess subject to ensure all inclusion criteria are met and no exclusion criteria are met, including that subject's CDI has responded to antibiotics without diarrhea for the previous 2 days (< 3 unformed stools per day).
- Review concomitant medications and update information regarding prior medication use. Confirm that subject took their last dose of standard of care antibiotic treatment for their CDI on any day from Day -4 to Day -2.
- Ensure subject consumed a 10 oz (~300 mL) bottle of magnesium citrate or 250 mL of GoLytely on Day -1.
- Ensure subject is undergoing a fast (no food or drink other than small amounts of water) for ≥ 8 hours before anticipated receipt of study drug).
- Obtain stool sample from subject's Day -1 at home collection and process, store, and ship per Laboratory Manual.
- Perform a physical examination, including vital sign measurements (i.e., blood pressure, pulse, respiratory rate, and body temperature) and weight.

- Collect blood and urine samples for the following laboratory tests:
  - Blood chemistry
  - Hematology
  - Serum for future biomedical research
  - Urine dipstick performed at the study site; if positive for nitrates and/or leukocytes, the sample may be sent to central laboratory for analysis at the discretion of the investigator
  - Urine pregnancy test, if applicable
- Assess AEs.
- Instruct subject to complete the EQ-5D-5L and CDiff32 questionnaires.
- Access the IxRS to obtain the bottle number of SER-109

7.2.1.2. Administering Study Drug

On Day 1, administer 4 study drug capsules orally with at least 8 oz of water (capsules are to be swallowed, not chewed).

7.2.1.3. After Administering Study Drug (Post-dose)

- Observe subject in the clinic for ≥ 60 minutes.
- Assess vital sign measurements (i.e., blood pressure, pulse, respiratory rate, and oral body temperature) approximately 30 minutes after dosing.
- Assess AEs.
- Provide subject with stool collection kits.
- Provide specific instructions and a reminder card on reporting and follow-up of symptoms including diarrhea and abdominal discomfort, collection of samples, reporting of any concerns, and, in particular, notification of the investigator of the occurrence of diarrhea.
- Ensure subject continues to fast for a total of 1 hour after dosing (post-dose).
- Dispense a 2-day supply of study drug to subjects with instructions for proper storage and home-administration on Day 2 and Day 3.
- Release subject from the clinic upon authorization by the investigator.
- Review instructions with subject for recording episodes of diarrhea (See Investigator Site Manual)
- Investigators should manage subjects' expectations, they may have diarrhea early-on after receiving drug in the study.

7.2.2. Phone Call Visit (Day 2)

Contact subject by phone to:

- Confirm administration of 2nd dose (4 capsules) of study drug before breakfast. If subject has not yet taken study drug, remind subject to take study drug as soon as possible.
- Inquire about general health
- Perform diarrhea assessment:
  - Remind subjects they may have diarrhea early-on after receiving drug in the study.
  - Remind subjects to complete the diarrhea log (see Investigator Site File)
- Assess AEs
- Review concomitant medications

7.2.3. Clinic, Home, or Phone Call Visit (Day 3)

Contact subject by phone to:

- Confirm administration of 3rd dose (4 capsules) of study drug before breakfast. If subject has not yet taken study drug, remind subject to take study drug as soon as possible.
- Inquire about general health
- Perform diarrhea assessment:
  - Remind subjects they may have diarrhea early-on after receiving drug in the study.
  - Remind subjects to complete the diarrhea log (see Investigator Site File)
- Assess AEs
- Review concomitant medications
- Remind subject to bring their Week 1 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site.

7.2.4. Phone Call Visit (Week 1)

Contact subject by phone at Week 1 (± 2 days).

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 7.3.3).
- Assess AEs.
- Review concomitant medications.
- Instruct subject to complete the CDiff32 questionnaire.

- Remind subject to bring their Week 1 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site.

7.2.5. Clinic or Home Visit (Week 2)

Arrange for a home visit or an in-clinic visit at Week 2 (± 2 days) to:

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 7.3.3).
- Assess AEs.
- Review concomitant medications.
- Collect a serum sample and a stool sample.

7.2.6. Phone Call Visits (Weeks 3-7)

Contact subject by phone at Weeks 3-7 (± 2 days).

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, arrange a Recurrence Visit (see Section 7.3.3).
  - Advise subject to continue diarrhea log, and collect stool sample for the Recurrence Visit.
  - Advise subject to not initiate antibiotic treatment for CDI until advised to do so by the study investigator.
- Assess AEs.
- Review concomitant medications.
- Remind subject to bring their Week 8 stool sample collected at home to the study site to be processed for shipment to the central laboratory.

7.2.7. End of Efficacy Period Clinic Visit (Week 8)

Subjects will be seen in the clinic at the study site at Week 8 (± 2 days).

- Obtain stool sample from subject's Week 8 at home collection and process, store, and ship per Laboratory Manual.
- Perform diarrhea assessment:

  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 7.3.3).
- Assess AEs.
- Review concomitant medications.
- Perform a focused history and physical exam.
- Collect blood and urine samples for the following laboratory tests:
  - Blood chemistry
  - Hematology
  - Urine pregnancy test, if applicable
- Instruct subject to complete the EQ-5D-5L and CDiff32 questionnaires.
- Provide subject with stool collection kits as necessary.

7.3. Follow-up Period

7.3.1. Phone Call Visits (Every 4 Weeks)

Contact subject by phone at Weeks 12, 16 and 20 (± 3 days) to:

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, arrange a Recurrence Visit (see Section 7.3.3)
  - Advise subject to continue diarrhea log, and collect stool sample for the Recurrence Visit
  - Advise subject not to initiate antibiotic treatment for CDI until advised to do so by the study investigator.
- Assess AEs.
- Review concomitant medications.
- Remind subject to bring their Week 24 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site.

7.3.2. Phone Call Visit - Study Completion (Week 24)

Contact subject by phone at Week 24 (± 3 days) to:

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 7.3.3).
- Assess AEs.
- Review concomitant medications.
- Instruct subject to complete the EQ-5D-5L questionnaire
- Remind subject to bring their Week 24 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site.

7.3.3. Recurrence and Early Termination (ET) Visits

Any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day over 2 consecutive days) will be asked to contact the investigator and return to the clinic for a *C. difficile* stool test and evaluation for recurrence of CDI. Additionally, all subjects will be seen in the clinic if the subject withdraws early from the study, whenever possible.

Perform the following assessments and procedures:

- Obtain stool sample from subject's at home collection and process, store, and ship sample per Laboratory Manual.
- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI that has not already been reported, obtain information regarding the day, frequency, and quality of bowel movements during diarrhea episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. To inform subject care, a *C. difficile* test on unformed stool may be performed locally at the study site (see Laboratory Manual); ship stool to the central laboratory for *C. difficile* stool testing (see Laboratory Manual).
  - If the *C. difficile* test on a stool sample determined by a toxin assay is positive and the investigator determines that antibiotic treatment is appropriate per protocol guidelines (see Section 5.7), prescribe standard of care antibiotic regimen to control CDI.
  - Advise subject to continue diarrhea log up until the day of initiation of antibiotic treatment for their CDI.
- Assess AEs.
- Review concomitant medications.

- Perform a focused history and physical exam.
- Collect blood and urine samples for the following laboratory tests:
  - Blood chemistry
  - Hematology
  - Serum for future biomedical research
  - Urine pregnancy test, if applicable
- Instruct subject to complete the EQ-5D-5L and CDiff32 questionnaires (*Note: the CDiff32 questionnaire should only be completed for an ET or Recurrence Visit prior to Week 8).

8. ASSESSMENT OF SAFETY AND ADVERSE EVENT REPORTING

All AEs, SAEs/AESIs, and concomitant medications will be collected from the time of initiation of study drug up to Week 8. From Week 8 up to Week 24, all SAEs/AESIs and SAE/AESI-related data, and any antibiotic medication and its corresponding indication will be collected.

The investigator is responsible for:

- Informing the sponsor in the event that a subject or a subject's partner becomes pregnant during the study. A "Pregnancy Report Form" will be generated and the pregnancy will be captured in the safety database and will be followed through to the outcome.
- Instructing subjects in the self-reporting of selected AEs including diarrhea and abdominal discomfort.
- Evaluating subject safety including assessment of AEs for seriousness, severity, and causality.
- Informing the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) of AEs as required and SAEs as per IRB/IEC guidelines.

For the purpose of this study, an AE is defined as any untoward medical occurrence in a subject who was administered study drug, regardless of its causal relationship to the study drug. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not it is considered related to the study drug.

An SAE is any AE regardless of causality that:

- Results in death.
- Is life threatening. Life threatening means that the subject was at immediate risk of death from the adverse event as it occurred. This does not include an event that, hypothetically had it occurred in a more severe form, it might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization; hospital admissions and/or surgical operations scheduled to occur during the study period, but

planned before study entry are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not worsen in any unexpected manner during the study (e.g., surgery performed earlier than planned).

- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a subject's ability to conduct normal life functions.
- Is associated with a congenital anomaly/birth defect.
- Is an important medical event. An important medical event is an event that may not result in death, be life threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition of an SAE. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization, and the development of drug dependency or drug abuse.

An adverse event of special interest (AESI) (serious or non-serious) is one of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor is appropriate.

In this protocol, an invasive infection (e.g., bacteremia, abscess, meningitis) is designated as an AESI, and as such, will be reported and followed in the same manner as an SAE during the course of the study.

All AEs, including SAEs and AESIs, will be graded for severity by using the following grading system:

- **Mild**: Events require minimal or no treatment and do not interfere with the subject's daily activities
- **Moderate**: Events result in a low level of inconvenience or concern and may require treatment; moderate events may cause some interference with functioning
- **Severe**: Events interrupt a subject's usual daily activity and may require systemic drug therapy or other treatment; severe events are usually incapacitating

Changes in the severity of an AE will be documented, and documentation will include assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent will be documented based on the severity, onset, and duration of each episode.

An abnormal laboratory test finding that meets any of the criteria below will be considered an AE:

- Is associated with accompanying symptoms
- Requires additional diagnostic testing or medical/surgical intervention
- Leads to a concomitant drug treatment or any change in a concomitant medication or therapy
- Is considered an AE by the investigator

Laboratory results that fall outside the reference range and do not meet one of the criteria above will not be reported as AEs. Repeating a test because of an abnormal result, in the absence of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error will not be reported as an AE.

For all AEs, including SAEs, the investigator will report on the relationship of the AE to the study drug by using the following definitions:

- **Unrelated**: There is little or no chance that the study drug caused the AE; other conditions, including concurrent illnesses, progression or expression of the disease state, or a reaction to a concomitant medication best explain the event
- **Related** or **Possibly Related**: The association of the AE with the study drug is unknown; however, the AE is not clearly due to another condition, or a reasonable temporal association exists between the AE and treatment administration and, based on the investigator's clinical experience, the association of the AE with the study drug seems likely

Adverse events, including local and systemic reactions not considered medically serious, will be recorded. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to study drug, date of resolution of the event, seriousness, and outcome. Additionally, serious criteria will be collected for all SAEs.

Any medical condition that is present at the time that the subject is screened will be considered as a baseline condition and not be reported as an AE. However, if it worsens at any time during the study, it should be recorded as an AE.

With regards to events of diarrhea, diarrhea that meets the protocol definition of CDI recurrence (≥ 3 unformed stools per day over 2 or more consecutive days, a positive *C. difficile* test on a stool sample determined by a toxin assay, and assessment by the investigator that treatment is required) should NOT be entered as an AE. Events of diarrhea that are not associated with CDI recurrence (eg, due to food poisoning or flu), should be reported as an AE (eg, Diarrhea [Not CDI related]).

When CDI recurrence is deemed serious due to hospitalization, CDI recurrence should be included as an SAE term and recorded as the reason for hospitalization in the Hospitalization CRF page.

8.1. Serious Adverse Event Reporting

The sponsor has requirements for expedited reporting of SAEs meeting specific criteria to worldwide regulatory authorities. Therefore, the sponsor (or sponsor's designee) must be notified immediately regarding any SAE that occurs after administration of the study drug.

All SAEs must be reported to the medical monitor within 24 hours of knowledge of the event at the study site. Refer to the Investigator Site File for detailed instructions.

The study site will transmit an SAE report (SAER) to the sponsor or sponsor's designee by facsimile or email. The study site will be provided with SAER forms wherein the following information is requested:

- Subject identification, investigator name, and study site number
- SAE information: event term, onset date, severity, and causal relationship to study drug

- The outcomes attributable to the event (i.e. serious criteria) (e.g., death, life threatening, inpatient hospitalization, prolongation of existing hospitalization, a congenital anomaly, a persistent or significant disability or incapacity, or other important medical event)
- A summary of relevant test results, pertinent laboratory data, and any other relevant medical history
- The date of study drug administration
- Whether or not the study drug was discontinued
- Supplemental information, which may include the following hospital records: laboratory results, radiology reports, progress notes, admission and emergency room notes, holding and observation notes, discharge summaries, autopsy reports, and death certificates

In addition, relevant eCRF pages should be appended to communicate relevant study drug and subject outcome information.

The SAER should be faxed or emailed within 24 hours with as much of the above information as available at the time. The following minimum information is required for an initial SAE report: subject identification, reporting source (i.e., Site Name and Site Number), and an event or outcome. Supplemental information may be transmitted by using a follow-up report and should not delay the initial report. The sponsor may contact the study site to solicit additional information or follow-up on the event.

The investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications or procedures necessary for treatment of the SAE must be recorded in the appropriate pages of the subject's eCRF.

9. STATISTICAL METHODS

9.1. STUDY ENDPOINTS

9.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the recurrence of CDI as determined by a toxin assay up to 8 weeks after initiation of treatment. A recurrence is defined as ≥ 3 unformed stools per day for 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay, and assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment.

9.1.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints are the following:

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay

- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

9.1.3. Exploratory Efficacy Endpoints

Exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 24 weeks after treatment initiation (for subjects hospitalized)
- Diagnosis-related group-adjusted hospital costs (for subjects hospitalized when available) after initiation of treatment
- Changes from Baseline in Health-Related Quality of Life (HRQoL) and health outcomes as assessed by the EQ-5D-5L from Day 1 through Weeks 8 and 24, and assessed by the Cdiff32 HRQoL from Day 1 to Week 1 and Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

9.1.4. Safety Endpoints

Safety endpoints are the following:

- Incidence of AEs
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

9.2. Analysis Populations

Three analysis populations will be defined:

- Intent to Treat (ITT) Analysis Population - The ITT Population will consist of all enrolled subjects.

- Modified Intent-to-Treat (mITT) Analysis Population - The mITT Population will be composed of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.
- Safety Population - The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

9.3. Determination of Sample Size

Approximately 142 subjects are anticipated to enroll from SERES-012. This estimate assumes that 30% of subjects in the SER-109 group and 59% of subjects in the placebo group will have a CDI recurrence in the SERES-012 study and rollover into this study.

9.4. General Statistical Considerations

Descriptive statistics, including the numbers and percentages for dichotomous or categorical variables, and the numbers, means, standard deviations, medians, minimums, and maximums for continuous variables will be provided. Listings of individual subject data will be produced.

All summary tables will be presented based on the following groups: 1) subjects randomized to SER-109 in SERES-012, 2) subjects randomized to placebo in SERES-012, and 3) overall (both groups combined).

A comprehensive statistical analysis plan (SAP) will be submitted to regulatory authorities.

9.5. Subject Population and Baseline Characteristics

Enrollment, protocol deviations, and discontinuations from the study will be summarized by group for the ITT Population. Demographics (e.g., age, race, ethnicity, sex), baseline characteristics (e.g., weight,), medical history, and other baseline characteristics will be summarized by group for the Safety, ITT and mITT Populations.

9.6. Study Drug Exposure

SER-109 exposure will be summarized as the total number of capsules taken with counts and percentages of subjects by group. The summary will be presented for the Safety, ITT, and mITT Populations.

9.7. Efficacy Analysis

9.7.1. Primary Efficacy Analysis

The primary efficacy endpoint is the recurrence of CDI up to Week 8 in the ITT Population. Subjects will be categorized as having favorable (no CDI recurrence) or unfavorable outcomes (had CDI recurrence). The number and percentage of subjects defined as having favorable and unfavorable outcomes will be reported with exact 95% confidence intervals (CIs) for each group. The CIs will be derived using the Clopper-Pearson exact method (Clopper and Pearson, 1934). Subjects who are lost to follow-up, terminated the trial early, or died without a recorded recurrence

of CDI before Week 8 will be defined as having an unfavorable outcome for the primary analysis. Subjects who miss any contact with the site (phone calls or the Week 2 visit) before Week 8 but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent contact will be defined as having a favorable outcome for the primary analysis. Additional rules for imputing CDI recurrence status for subjects with at least one component of the CDI recurrence endpoint criteria missing will be provided in the SAP.

9.7.2. Sensitivity Analysis for the Primary Efficacy Endpoint

Sensitivity analyses of the primary efficacy endpoint as described in Section 9.7.1 will also be conducted as follows:

- All subjects who are lost to follow-up, terminated the study prematurely, or died without having a CDI recurrence by Week 8, will be considered to have a favorable outcome. Subjects who missed any contact with the site before Week 8 (phone calls or the Week 2 visit) but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent contact will continue to be defined as having a favorable outcome for this analysis, which will be conducted in the ITT Population.

The primary efficacy outcome will also be analyzed for the mITT Population.

9.7.3. Secondary Efficacy Analysis

Time to recurrence of CDI will be summarized by group for the ITT and mITT Populations using the median, 25th and 75th percentiles from a Kaplan-Meier analysis. The 95% CI for the median will also be provided. Subjects who complete the follow-up period and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminated the study prematurely before experiencing a CDI recurrence will be censored in the analysis on the date of last contact. Subjects who die before having a CDI recurrence will be censored on the date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Subjects who were not dosed will have their time to recurrence measured from their enrollment date.

9.8. Exploratory Analysis

Planned analyses of the exploratory efficacy endpoints will be detailed in the SAP.

9.9. Safety Analysis

All safety analyses will be conducted in the Safety Population. All safety summary tables will be presented for the following groups: 1) randomized to SER-109 in SERES-012, 2) randomized to placebo in SERES-012, and 3) overall (both groups combined).

Adverse events will be coded by using the Medical Dictionary for Regulatory Activities (MedDRA). Summary tables of TEAEs will be provided. A TEAE is any AE that newly appeared, increased in frequency, or worsened in severity after initiation of SER-109. A listing of all AEs, including those occurring before the start of study drug, will be provided. The percentage of patients with TEAEs will be tabulated by system organ class and preferred term. The incidence of TEAEs based on the number of days the patients were on study drug (per patient on therapy day)

will also be presented by system organ class and preferred term, by severity, and by relationship to treatment. Tables of any TEAE leading to SER-109 discontinuation and SAEs will also be provided.

An additional analysis will determine the incidence of TEAEs based on the number of days the patients were followed before receiving antibiotics for treatment of recurrent CDI. The incidence of TEAEs per patient for days before receiving antibiotics will be tabulated by system organ class and preferred term, and by severity and relationship to treatment.

Descriptive statistics of the laboratory parameters and vital sign measurements will be presented for all study visits at which they were collected. The change from Baseline to each post-baseline visit and to the overall worst post-baseline value will also be summarized. Laboratory parameters will be defined, as within or outside normal limits, and shift tables from baseline to each postbaseline visit will be provided.

9.10. Handling of Missing Data

Every effort will be made to collect all data at specified times, according to the schedule of study events.

For the primary endpoint, subjects who are lost-to-follow-up, terminated from the study prematurely, or died without a CDI recurrence before 8 weeks after treatment are defined as having an unfavorable outcome for the primary analysis. Subjects who miss any contact with the site before Week 8 (phone calls or Week 2 visit) but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent telephone contact or by Week 8 will be defined as having a favorable outcome for the primary analysis. If the Week 8 visit is missed, a subject will be considered as having an unfavorable outcome for the primary analysis if he reports 2 or more consecutive days with ≥ 3 unformed stools at the next unmissed telephone contact or visit. If any of the 3 components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then an unfavorable outcome for the primary analysis is imputed. However, if some of the 3 components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a favorable outcome for the primary analysis is imputed. The imputed recurrence status for subjects who have at least one component of the CDI recurrence endpoint criteria missing will be detailed in the SAP.

Missing data for the time to CDI recurrence analyses will be handled with censoring by the Kaplan-Meier method. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminate the trial prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before experiencing a CDI recurrence will be censored on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Sensitivity analyses of the time to recurrence endpoint will be provided in the SAP.

No other imputations for missing data will be made (except as detailed in the SAP for missing dates and times).

10. ADMINISTRATIVE REQUIREMENTS

10.1. Good Clinical Practice

This study will be conducted in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) and the appropriate regulatory requirements. The investigator will be thoroughly familiar with the appropriate use of the investigational product. Essential clinical documents will be maintained to demonstrate the validity of the study and integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

- The principal investigator has the overall responsibility for the conduct and administration of the study at the study site and for contacts with the sponsor, the IRB/IEC, and local authorities.
- The principal investigator is responsible for ensuring the privacy, health, and welfare of the subjects during and after the clinical study.
- All investigators are responsible for performing the study in accordance with the protocol and the above guidelines and regulations, and for collecting, documenting, and reporting the data accurately.
- All investigators must be familiar with the background and requirements of the study and with the properties of the investigational product as described in the current version of the Investigator's Brochure.
- The principal investigator is responsible for distributing study information and documentation to all appropriate staff members before and during the course of the study as updated information becomes available.

10.2. Trial Governance and Oversight

This study was developed in collaboration with a Clinical Advisory Committee, which comprises both sponsor employed and independent scientific experts who provide input on study design, interpretation of study results, and subsequent peer reviewed scientific publications.

10.3. Ethical Considerations

This study will be conducted in accordance with ethical principles in the Belmont Report, and in compliance with local IRB/IEC requirements and institutional guidelines.

The investigator must obtain IRB/IEC approval of the protocol, ICF, and other required study documentation before starting the study. It is the responsibility of the investigator to ensure that all aspects of IRB/IEC review are conducted in accordance with current governmental regulations.

A progress report must be submitted to the IRB/IEC at the required intervals and not less than annually. At the completion or termination of the study, the investigator must submit a closeout letter to the IRB/IEC.

10.4. Subject Information and Informed Consent

Before any testing under this protocol, including screening tests and assessments, written informed consent with the IRB/IEC approved ICF must be obtained from the subject in accordance with local practice and regulations.

The background of the proposed study, procedures, and benefits and risks of the study must be explained to the subject. The subject must be given sufficient time to consider whether to participate in the study.

A copy of the ICF, signed and dated by the subject, must be given to the subject. Each ICF should contain an authorization allowing the investigator to use and disclose subject health information (i.e., subject identifiable health information) in compliance with local law.

10.5. Subject Confidentiality

Subject confidentiality is held strictly in trust by the investigator and medical and laboratory staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. The investigator will grant a regulatory authority access to the subject's original medical records for verification of data gathered, and to audit the data collection process. The subjects' and donors' confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Subjects will not be identified by name in any study reports, and these reports will be used for research purposes only.

10.6. Protocol Compliance

The investigator will conduct the study in compliance with the IRB/IEC approved protocol without any changes or deviations. Modifications to the protocol will require approval from the sponsor and written IRB/IEC approval before implementation, except when the modification is needed to eliminate an immediate hazard to the subject. Any change, intentional or otherwise, must be reported immediately to the sponsor and to the relevant IRB/IEC and/or regulatory authority as required by guidelines or regulation. Study sites that fail to comply may be terminated.

10.7. Future Use of Stored Specimens

The sponsor may, where permitted by local regulations, conduct future biomedical research on specimens (including serum, and stool) routinely and specifically collected during this clinical study for potential commercial use by Seres Therapeutics, Inc., and specimens may be stored for up to 10 years.

10.8. Study Monitoring

Regular monitoring is defined in ICH Guidance for Industry E6 Good Clinical Practice: Consolidated Guidance, Section 1.38, as "The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures, GCP, and the applicable regulatory requirement(s)." The purpose of monitoring is to verify that:

- The rights and well-being of the human subjects are protected.

- The reported study data are accurate, complete, and verifiable from source documents.
- The conduct of the study is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirements.

It will be the responsibility of the investigator to ensure that the essential documents are available at the investigator or institutional site. Any or all of these documents may be pertinent to, and should be available for, monitoring by the sponsor or inspection by the regulatory authorities as defined in the monitoring plan.

The sponsor or an authorized sponsor representative will conduct regular study site monitoring visits to review and validate study data as defined in the monitoring plan by reviewing subjects' medical records and eCRFs in accordance with written standard operating procedures, ICH guidelines, GCP, and applicable regulations and guidelines. The investigator will allow representatives of the sponsor or regulatory authorities to inspect facilities and records relevant to this study.

10.9. Case Report Forms and Study Records

Data will be collected for this study by using an eCRF. The investigator and study site staff will receive training and support on the use of the eCRF. All eCRF data are to be completed by the study coordinator or other designated study site personnel. All data entry, modification, or deletion will be recorded automatically in the electronic audit trail. All data changes will be clearly indicated with a means to locate prior values. A unique user identification and password will be assigned to all personnel approved to enter or change data to prevent unauthorized access to the data.

All electronic data entered by the study site (including the electronic audit trail) will be maintained or made available at the study site in compliance with Title 21 Part 11 of the Code of Federal Regulations (CFR) and other applicable retention regulations. The computerized system is able to generate accurate and complete copies of records in paper or electronic form for inspection and review by applicable regulatory authorities, the IRB/IEC/Research Ethics Board, and auditors or other designees authorized by the sponsor.

In addition to capturing the user identification as part of the audit trail for all data entry, the eCRF allows for application of electronic signatures. The investigator or designated sub-investigator, after review of the data in the eCRF, will confirm the validity of each subject's data by electronic signature. This electronic signature will be certified as outlined in 21 CFR Part 11.

The sponsor will retain the original eCRF data and audit trail. An electronic or certified paper copy of all completed eCRF data, including query resolution correspondence, will be provided to the investigator at the end of the study.

10.10. Study Completion

The sponsor requires the following data and materials to be submitted before a study can be considered complete or terminated:

- Laboratory findings, clinical data, and all special test results from the time of informed consent through the End of Study Visit at Week 24

- Electronic CRFs properly completed by appropriate study personnel and signed and dated by the investigator
- Complete study drug accountability records
- Copies of IRB/IEC approval and notification of the original protocol and of any protocol amendments, if appropriate
- A summary of the study prepared by the investigator (an IRB/IEC summary letter is acceptable)

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CLINICAL STUDY PROTOCOL

SER-109

(Eubacterial Spores, Purified Suspension, Encapsulated)

SERES-013: ECOSPOR IV: AN OPEN-LABEL EXTENSION OF STUDY SERES-012 AND OPEN-LABEL PROGRAM FOR EVALUATING SER-109 IN ADULT SUBJECTS WITH RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION (RCDI)

SPONSOR:

Seres Therapeutics, Inc.
200 Sidney Street, Suite 410
Cambridge, MA 02139
Telephone: +1-617-945-9626

TITLE:

ECOSPOR IV: An Open-Label Extension of Study SERES-012 and Open-Label Program for Evaluating SER-109 in Adult Subjects with Recurrent *Clostridioides difficile* Infection (RCDI)

CLINICAL PHASE: 3

IND NUMBER: 16262

Original Protocol: March 06, 2017

Amendment 1 date: 6 June 2017

Amendment 2 date: 08 September 2017

Amendment 3 date: 9 January 2018

Amendment 4 date: 4 January 2019

Amendment 5 date: 9 May 2019

Amendment 6 date: 18 May 2020

Amendment 7 date: 31 July 2020

Amendment 8 date: 16 February 2021

CLINICAL RESEARCH ORGANIZATION (CRO):

ICON
South County Business Park
Leopardstown,
Dublin 18,
Ireland
Telephone: +353 (1) 291 2000

Declaration of Sponsor or Responsible Medical Officer

Title: ECOSPOR IV: An Open-Label Extension of Study SERES-012 and Open-Label Program for Evaluating SER-109 in Adult Subjects with Recurrent *Clostridioides difficile* Infection (RCDI)

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki, 1989, and the guidelines on Good Clinical Practice.

Signature: Elaine Wang MD. *Electronically signed by: Elaine Wang Reason: I am a document author Date: Feb 16, 2021 09:18 EST*

Email: ewang@serestherapeutics.com

Feb 16, 2021

Date

Elaine Wang, MD

Senior Medical Director

Seres Therapeutics, Inc.

INVESTIGATOR'S AGREEMENT

I have read the SERES-013 Protocol Amendment 8, dated 16 February 2021, and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____

Printed Name of Investigator

_____

Signature of Investigator

_____

Date

STUDY CONTACTS

Sponsor Responsible Medical Officer

Elaine Wang, MD

Senior Medical Director

Seres Therapeutics, Inc.

200 Sidney Street

Cambridge, MA 02139

Email: ewang@serestherapeutics.com

CRO Medical Monitor

e-mail address: STUDY-MA-DL-3647-0010@iconplc.com

Address: 601 Gateway Blvd Suite 1250

San Francisco, CA 94080

Telephone: 415-371-2124

1. PROTOCOL SYNOPSIS

SPONSOR NAME: Seres Therapeutics, Inc.

ACTIVE INGREDIENT: SER-109 (Eubacterial Spores, Purified Suspension, Encapsulated)

PROTOCOL TITLE: ECOSPOR IV: An Open-Label Extension of Study SERES-012 and Open-Label Program for Evaluating SER-109 in Adult Subjects with Recurrent *Clostridioides difficile* Infection (RCDI)

STUDY CENTERS: Approximately 140 study centers in North America

PLANNED STUDY PERIOD:	**CLINICAL PHASE:** 3
Estimated date first patient enrolled: 4Q2017 Estimated date last patient completed: 2Q2022	

DEFINITIONS:

For this study, CDI recurrence during the study is defined by the following criteria:

- Positive *Clostridioides difficile* test on a stool sample determined by a toxin assay
- ≥ 3 unformed bowel movements per day over 2 consecutive days and the requirement that patients must continue to have diarrhea until antibiotic treatment is initiated
- Assessment by the investigator (based on clinical assessment) that the patient's condition warrants antibiotic treatment

OBJECTIVES:

Subjects who are participating in the open-label extension of Study SERES-012 (Cohort 1) have the following objectives.

Primary Efficacy Objective

- To evaluate SER-109 in the reduction of CDI recurrence rate and increase in sustained clinical response rate determined by a toxin assay, up to 8 weeks after initiation of treatment

Secondary Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a PCR algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109

- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

After the last subject rolls over from SERES-012 (Cohort 1), SERES-013 will commence enrollment of Cohort 2, the Open-Label population. The objectives for Cohort 2 are the following:

Primary safety objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

Efficacy objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates and increase in sustained clinical response rates, determined by a toxin assay, up to 8 and 12 weeks after initiation of treatment

No Secondary efficacy objectives

Exploratory objectives

- To evaluate changes in the composition of the gut microbiome from Baseline to 1 week after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline to 1 week after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109

- To assess the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) Visual Analog Scale at Screening and at Week 8
- To assess a Bowel Cleanse Patient Satisfaction Survey Measure at Week 8 on using a Bowel Cleanse Patient Satisfaction Survey Measure on a bowel cleanse administered prior to SER-109

STUDY DESIGN:

ECOSPOR IV; Cohort 1 is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a SER-109 treatment regimen in adult subjects 18 years of age or older with recurrent *Clostridioides. difficile* infection (RCDI), who received a SER-109 or placebo treatment regimen in Study SERES-012.

Approximately 30 eligible subjects with recurrent CDI disease from Study SERES-012 are expected to enroll in Cohort 1.

Subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a SER109 or placebo treatment regimen in Study SERES-012 and who have responded to CDI SOC antibiotic therapy defined as 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg BID] will be eligible to enroll in Study SERES-013, and will receive a SER-109 treatment regimen. A SER-109 treatment regimen is 3×10^7 spore colony forming units (SCFU) administered orally in 4 capsules once daily for 3 consecutive days.

Subjects being considered for SERES-013 will start screening for 013 at the Recurrence Visit of Study SERES-012. Subjects with a confirmed recurrence of CDI will receive 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg BID]. On Day -1, within 3 days of completion of SOC antibiotic treatment for their CDI, subjects will undergo a bowel cleanse followed by overnight fasting prior to scheduled randomization on Day 1.

Laboratory values or procedures obtained during the screening window and from the central laboratory of Study SERES-012 (e.g. Recurrence Visit) do not need to be repeated at the Screening Visit for SERES-013. The SERES-012 Recurrence Visit may be used for the SERES-013 Screening Visit. However, if the SERES-012 Recurrence Visit occurred outside the SERES-013 screening window (Day -24 to Day -2), the required screening procedures for SERES-013 must be repeated within the screening window.

Subjects will come to the clinic after an overnight fast on the morning of Day 1 to receive a single dose of oral SER-109 (3×10^7 SCFU) in 4 capsules and have all safety evaluations performed. On Day 1, subjects will be dispensed a 2-day supply of SER-109 (3×10^7 SCFU) in 4 capsules with instructions for home-administration of a single daily dose in the morning before breakfast on Day 2 and Day 3. Subjects will be contacted by phone on Day 2 and Day 3 to confirm they have taken study drug before breakfast and to inquire about their general health. If subjects have not taken study drug when contacted, they will be reminded to do so as soon as possible. From Day 1 to Week 8, all subjects will be contacted by phone by study site personnel weekly, with the exception of a home or in-clinic visit at Week 2 and an in-clinic visit at Week 8, and queried for adverse events (AEs) and diarrheal symptoms. After Week 8, all subjects will be contacted by phone by study site personnel every 4 weeks (i.e., Weeks 12, 16, 20, and 24) and queried for serious adverse events [SAEs] and adverse events of special interest [AESIs]). Health-related quality of life and health outcomes will be assessed throughout the study via the CDI-specific, Cdiff32 Health Related Quality of Life (HRQoL) and EuroQol 5 Dimension 5 Level (EQ-5D-5L) questionnaires.

To document episodes of diarrhea, subjects will complete a diarrhea log (see Investigator Site File) to include days with diarrhea as well as no diarrhea. If diarrheal symptoms recur (≥ 3 unformed stools per day over 2 consecutive days) between scheduled visits, subjects will be instructed to contact the investigator and return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI (Recurrence Visit).

Subjects who have a confirmed CDI recurrence should continue to be followed for safety assessments through Week 24. Favorable clinical outcome (or Sustained clinical response) in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of the SER-109 treatment regimen. CDI recurrence is defined as ≥3 unformed stools per day over 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* toxin assay on a stool sample and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. To inform subject

care, a *C. difficile* stool test may be performed locally at the study site. Stool samples collected for suspected CDI recurrence will also be processed and shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual). The subject should not initiate antibiotics for suspected CDI prior to providing a stool sample for the central laboratory stool testing. Data from the *C. difficile* toxin test (either EIA or CCNA), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

The schedule of assessments and procedures is provided in Table 1.

The Open-Label cohort (Cohort 2) will commence after the last subject from SERES-012 rolls over into SERES-013. The primary objective in Cohort 2 is to describe safety and tolerability of SER-109 in subjects 18 years of age or older with at least a first recurrence of CDI. Approximately -200 subjects are expected to be enrolled in Cohort 2

In Cohort 2, subjects with symptoms suggestive of RCDI may submit a stool sample for CDI toxin or PCR testing at a Clinical Laboratory Improvement Amendment (CLIA)- certified local laboratory or the central laboratory with a prescreening or screening consent. Stool samples submitted to the central lab for pre-screening and screening will be tested using a PCR . test. All subjects who are confirmed to have toxin or PCR positive CDI at prescreening will undergo all Screening Visit assessments after providing signed informed consent. Subjects with one or more recurrences of CDI (including the current episode) who have responded to CDI antibiotic therapy defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days of fidaxomicin [200 mg] will be potentially eligible to enroll in Study SERES-013 to receive a SER-109 treatment regimen. A SER-109 treatment regimen is 3×10^7 spore colony forming units (SCFU) administered orally in 4 capsules once daily for 3 consecutive days. During the screening window (Day -24 to Day -2), after informed consent is obtained, subjects will be assessed for eligibility and undergo physical examination. Vital signs and laboratory specimens will be obtained. At screening, subjects will complete a EuroQol 5 Dimension 5 Level (EQ-5D-5L) Visual Analog Scale.
On Day -1, within 3 days of completion of antibiotic treatment for their CDI, subjects will undergo a bowel cleanse with magnesium citrate, or GoLytely if they have renal impairment, prior to scheduled allocation on Day 1.

Subjects will come to the clinic on the morning of Day 1 to receive a single dose of oral SER-109 (3×10^7 SCFU) in 4 capsules. On Day 1, subjects will be dispensed a 2-day supply of SER-109 (3×10^7 SCFU) in 4 capsules with instructions for home-administration of a single daily dose in the morning on Day 2 and Day 3. Subjects will be contacted by phone on Day 2 and Day 3 to confirm they have taken study drug and to inquire about their general health. If subjects have not taken study drug when contacted, they will be reminded to do so as soon as possible. From Day 1 to Week 8, all subjects will be contacted by phone by study site personnel weekly, with the exception of a home or in-clinic visit at Week 8,and queried for adverse events (AEs) and diarrheal symptoms. At the Week 8 visit, subjects will complete the EuroQol 5 Dimension 5 Level (EQ-5D-5L) Visual Analog Scale and the Bowel Cleanse Patient Satisfaction Survey Measure. After Week 8, all subjects will be contacted by phone by study site personnel every 4 weeks (i.e., Weeks 12, 16, 20, and 24) and queried for serious adverse events [SAEs] and adverse events of special interest [AESIs]) and diarrheal symptoms. If diarrheal symptoms recur ($\geq$ 3 unformed stools per day over 2 consecutive days), subjects will be instructed to contact the investigator and have an in-home or clinic visit for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI (Recurrence Visit).

Subjects who have a confirmed CDI recurrence should continue to be followed for safety assessments through Week 24. Favorable clinical outcome (or Sustained clinical response) in this study will be determined by the absence of CDI recurrence up to 8 and 12 weeks after initiation of the SER-109 treatment regimen. CDI recurrence is defined as $\geq$3 unformed stools per day over 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* toxin assay on a stool sample and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. Stool samples collected for suspected CDI recurrence will be processed and shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual). The subject should not initiate antibiotics for suspected CDI prior to providing a stool sample for the central laboratory stool testing. Data from the *C. difficile* toxin test (either EIA or CCNA), performed at the central laboratory, will be used for the primary efficacy endpoint. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

The schedule of assessments and procedures for Cohort 2 is provided in Table 2.

PLANNED NUMBER OF SUBJECTS:

Approximately 30 adults who experienced recurrent *C. difficile* by Week 8 in Study SERES-012 will make up Cohort 1. Approximately 200 subjects will be enrolled in Cohort 2 for a total of 230 subjects across Cohorts 1 and 2.

PRIMARY DIAGNOSIS: Recurrent CDI

INCLUSION CRITERIA:

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study related- procedures:

For Cohort 1:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES012 must have met the protocol definition of:
   a. ≥3 unformed stools per day for 2 consecutive days
   b. A positive *C. difficile* stool toxin assay
   c. The requirement of CDI SOC antibiotic therapy defined as 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg BID].
   d. An adequate clinical response following SOC antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
4. If female, subject is nonlactating, and is either:
   a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
   b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

For Cohort 2

1. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
2. ≥ 2 episodes of CDI inclusive of the current episode, with estimated total number of prior episodes
3. The CDI recurrence must have met the protocol definition of:
   a. ≥3 unformed stools per day for 2 consecutive days
   b. A positive *C. difficile* stool toxin assay or PCR test (either local or central laboratory)
   c. The requirement of CDI antibiotic therapy defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg]. It is acceptable if subject was started on metronidazole, switched to vancomycin or fidaxomicin and is treated for a minimum of 10 days of vancomycin or fidaxomicin with a total treatment duration of up to a maximum of 42 days for switch to vancomycin or 25 days for switch to fidaxomicin.
   d. An adequate clinical response following antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
   e. The requirement that the subject can be dosed with study drug within 4 days of antibiotic completion
4. Male or Female Subject ≥ 18 years of age

5. If female, subject is nonlactating, and is either:
   a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
   b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
6. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.
7. If currently taking probiotics, must be willing to stop at time of consent, for the duration of the study.

EXCLUSION CRITERIA:

A subject will not be enrolled if the subject meets any of the following criteria:

For Cohort 1:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.
2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding). Note: nursing homes, rehabilitation, assisted living centers and acute care hospitals are acceptable.
4. Absolute neutrophil count of <500 cells/mm^3
5. Taking antibacterial therapy other than antibiotics for the most recent episode of CDI during the screening period (a single-day antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.
6. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery. (bariatric surgery which does not disrupt the gastrointestinal lumen, i.e., restrictive procedures such as banding, are permitted).
7. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past3 months.
8. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to start of study.
9. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term (One day) opiate use is permitted (e.g., for a dental extraction).
10. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
11. Known stool studies positive for ova and/or parasites within the 30 days before enrollment.
12. Poor concurrent medical risks with clinically significant co-morbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
13. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
14. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
15. Any history of immunoglobulin (IgG) replacement therapy within the past 3 months.
16. Any history of fecal microbiota transplantation (FMT) in the past 3 months.
17. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
18. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
19. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives.
20. Life expectancy is 24 weeks or less.

For Cohort 2 (all Cohort 1 exclusion criteria plus number 21 below apply)

21. Previously enrolled in a Seres Therapeutics clinical study. An exception is made for subjects who screened in SERES-012 who did not receive SER-109 and did not previously roll-over to SERES-013.

INVESTIGATIONAL PRODUCT, DOSE, AND MODE OF ADMINISTRATION:

Study drug is for investigational use only. Study drug dispensed at the study site will be stored at the study site or pharmacy. Subjects will be provided instructions for proper storage of study drug dispensed for home-administration at home. Instructions for storage are provided in the Investigator Site File. SER-109 will be supplied in size 00 capsules.

The dose, route, and schedule of study drug administration are presented in the following table:

Treatment	Dose	Dosage Form and Amount	Route	Number of Subjects
SER-109	3×10^7 spore colony forming units (SCFU)	4 capsules once daily for 3 consecutive days	Oral	~ 30 subjects Cohort 1 ~200 subjects Cohort 2 Total of ~230 subjects

STATISTICAL METHODS:

Analysis Populations:

Intent-to-Treat Population

The Intent-to-Treat (ITT) Population will consist of all enrolled subjects.

Modified Intent-to-Treat Population

The Modified ITT (mITT) Population will consist of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.

Safety Population

The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

Study Endpoints: For Cohort 1,

The terminology for primary, secondary, and exploratory efficacy endpoints are retained for Cohort 1, but have been changed for Cohort 2.

Primary Efficacy Endpoint

- Recurrence of CDI and sustained clinical response as determined by a toxin assay up to 8 weeks after initiation of treatment

Secondary Efficacy Endpoints

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay
- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

Exploratory Efficacy Endpoints

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after treatment initiation (for subjects hospitalized)
- Changes from Baseline in Health-Related Quality of Life (HRQoL) and health outcomes as assessed by the EQ-5D-5L up to Week 24, and assessed by the Cdiff32 HRQoL from up to Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

Safety Endpoints

- Incidence of AEs
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

Study Endpoints for Cohort 2

Primary Safety Endpoints

- Incidence of AEs
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

Efficacy Endpoint

- Recurrence of CDI and sustained clinical response as determined by a toxin assay up to 8 and 12 weeks after initiation of treatment
- There are no secondary endpoints

Exploratory Endpoints

- Change in the composition of the gut microbiome from Baseline up to 1 week after initiation of treatment in a subgroup of subjects
- Change in the fecal metabolome from Baseline up to 1 week after initiation of treatment in a subgroup subjects
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment

- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after treatment initiation (for subjects hospitalized)
- Change in EQ-5D-5L visual analog score from Screening to Week 8 visit after initiation of treatment
- Assess Bowel Cleanse Patient Satisfaction Survey Measure at Week 8

Analysis of Primary Efficacy Endpoint:

In Cohort 1, the primary efficacy outcome is the recurrence of CDI determined by a toxin assay through Week 8 after receipt of a SER-109 treatment regimen in the ITT Population. Subjects will be categorized as having favorable (sustained clinical response) (no CDI recurrence) or unfavorable outcomes (had CDI recurrence). Rules for imputing CDI recurrence status for subjects with missing data will be provided in the Statistical Analysis Plan (SAP).

The number and percentage of subjects defined as having favorable and unfavorable outcomes will be reported with exact 95% confidence intervals (CIs) for subjects who were randomized to SER-109 and placebo in SERES-012 separately, as well as both groups combined. The CIs will be derived using the Clopper-Pearson exact method.

In Cohort 2, the efficacy outcome is the recurrence of CDI determined by a toxin assay through Week 8 and 12 after receipt of a SER-109 treatment regimen in the ITT Population. Subjects will be categorized as having favorable (sustained clinical response) (no CDI recurrence) or unfavorable outcomes (had CDI recurrence). Rules for imputing CDI recurrence status for subjects with missing data will be provided in the Statistical Analysis Plan (SAP).

The number and percentage of subjects defined as having favorable and unfavorable outcomes will be reported with exact 95% confidence intervals (CIs) derived using the Clopper-Pearson exact method.

Table 1: Schedule of Assessments and Procedures for Cohort 1

	SCREENING PERIOD			EFFICACY PERIOD							FOLLOW UP PERIOD		Recurrence Visit(s)[a]	ET Visit
	Clinic	*TC*[b]	*TC*	*Clinic*	*TC*[c]	*TC*	*TC*	*Clinic or home*[j]	*TC Weekly*	*Clinic*[j,k]	*TC*	*TC – Study Completion*	*Clinic*[j,k]	*Clinic*[j]
Day/Week	**Screening (-24 to -2)** *Note: Assessments performed at Recurrence Visit in SERES-012 do not need to be repeated if performed within the SERES-013 screening window*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 37 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
Informed Consent	X													
Eligibility criteria review	X		X	X										
Confirm clinical response to SOC antibiotic			X	X										
IxRS registration	X			X										
Medical History	X													
Physical Exam	X									X			X	X
Focused History and Physical				X						X			X	X
Weight	X			X						X			X	X
Vital signs[d]	X			X[e]						X			X	X
Chemistry and hematology	X			X[f]						X			X	X

	SCREENING PERIOD			**EFFICACY PERIOD**							**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC*[b]**	***TC***	***Clinic***	***TC*[c]**	***TC***	***TC***	***Clinic or home*[j]**	***TC Weekly***	***Clinic*[j,k]**	***TC***	***TC – Study Completion***	***Clinic*[j,k]**	***Clinic*[j]**
Day/Week	**Screening (-24 to -2)** *Note: Assessments performed at Recurrence Visit in SERES-012 do not need to be repeated if performed within the SERES-013 screening window*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 37 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
Serum for FBMR				X[f]				X					X	X
Routine urine dipstick	X			X[f]										
Urine pregnancy test (WOCBP)	X			X[f]						X			X	X
Stool: Study Entry: central lab: *C. Diff* toxin assay	X													
Stool: On Study Recurrence/ET: central lab: *C. Diff* testing													X	X
Stool: microbiome testing			X[h]				X[h]	X[g]		X[h]		X[h]	X[h]	X[h]
Stool: metabolomics testing			X[h]				X[h]	X[g]		X[h]			X	X
Provide stool collection kits	X			X						X				
Stop SOC Abx		X												
Administer bowel cleanse			X											

	SCREENING PERIOD			**EFFICACY PERIOD**							**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC[b]***	***TC***	***Clinic***	***TC[c]***	***TC***	***TC***	***Clinic or home[j]***	***TC Weekly***	***Clinic[j,k]***	***TC***	***TC – Study Completion***	***Clinic[j,k]***	***Clinic[j]***
Day/Week	**Screening (-24 to -2)** *Note: Assessments performed at Recurrence Visit in SERES-012 do not need to be repeated if performed within the SERES-013 screening window*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 37 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
Confirm subject fasted for ≥8 h prior to study drug dosing				X										
Confirm subject administered bowel cleanse on Day -1				X										
Study drug dosing				X	X	X								
Confirmation of study drug administration					X	X								
Study drug accountability										X				
Prior/ concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE assessment		X	X	X	X	X	X	X	X	X	X	X	X	X
Evaluation of diarrheal episodes	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cdiff32 HRQoL Survey				X			X			X			X[i]	X[i]

	SCREENING PERIOD			EFFICACY PERIOD							FOLLOW UP PERIOD		Recurrence Visit(s)[a]	ET Visit
	Clinic	*TC*[b]	*TC*	*Clinic*	*TC*[c]	*TC*	*TC*	*Clinic or home*[j]	*TC Weekly*	*Clinic*[j,k]	*TC*	*TC – Study Completion*	*Clinic*[j,k]	*Clinic*[j]
Day/Week	**Screening (-24 to -2)** *Note: Assessments performed at Recurrence Visit in SERES-012 do not need to be repeated if performed within the SERES-013 screening window*	**-4 to -2** *Complete SOC Abx*	**Day -1** *(Within 3d of completing SOC Abx)*	**Day 1**	**Day 2**	**Day 3**	**Week 1 (±2 d)**	**Week 2 (±2 d)**	**Week 37 (±2 d)**	**Week 8 (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures														
EuroQol 5 Dimensions 5 Level (EQ-5D-5L)				X						X		X	X	X

Abbreviations: Abx=antibiotics; AE=adverse event; *C.diff=Clostridioides difficile*; ET=early termination; FBMR=future biomedical research; HRQoL=Health-Related Quality of Life; IxRS=interactive voice and web response system; SOC=standard of care; TC=telephone call; WOCBP=women of childbearing potential

[a] Subjects with a confirmed CDI recurrence after Week 8 should continue to be followed for safety assessments through Week 24.

[b] Phone call: Confirm termination of antibiotics on day of last scheduled antibiotic dose and review instructions for Day -1 activities including collection of a stool sample before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel cleanse. All subject should be reminded to collect a stool sample at Day -1. It will be returned to the clinic on Day 1.

[c] Subjects should be called to confirm they have taken study drug in the morning and to inquire about their general health

[d] Blood pressure, pulse, respiratory rate, and body temperature

[e] To be assessed immediately before and approximately 30 minutes after study drug dosing

[f] To be assessed prior to study drug dosing

[g] Stool samples may be collected in the clinic or at home. If the subject elects to have an in-clinic visit, the sample may be collected at home prior to the visit and brought to the study site. If the subject is unable to bring the stool sample to the study site, arrangements may be made to pick up the sample at the subject's home and bring it to the study site or may ship directly to the central laboratory (i.e., home visit by nurse or courier).

[h] The stool sample collected on Day -1 should be collected prior to the bowel cleanse and may be brought to the study site for the Day 1 visit. The stool samples collected on Week 8 (in clinic visit) may be collected at home prior to the visit and brought to the study site. If the subject is unable to bring the sample to the study site at any other visit, a courier may be arranged to pick up the sample at the subject's home and bring it to the study site.

[i] Administer if prior to Week 8 visit.

[j.] As necessary for safety of subject, study visits including Week 2, Week 8, Unscheduled, and Early Termination, may be conducted remotely at subject's home, with qualified nurse or site personnel who will be appropriately documented on the site's delegation log to perform these Remote Study Visits and associated procedures (Modification due to COVID-19 pandemic). If a nurse or site personnel cannot perform the visits, a telephone call or a video conference (Zoom, Skype, FaceTime, etc.) should be conducted in place of the Week 2, Week 8, Unscheduled, and Recurrence / Early Termination visits and must be documented in the source. Any required procedures not performed during a remote visit at the subject's home will be documented as a protocol deviation by site staff.

[k] When visits are conducted over the phone, transcription of the EQ-5D-5L may be performed over the phone by site staff. The discussion with the subject must be documented in source files. (Modification due to COVID-19 pandemic).

Table 2: Schedule of Assessments and Procedures for Cohort 2

	SCREENING PERIOD			**EFFICACY PERIOD**				**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC[b]***	***TC***	***Clinic***	***TC***	***TC[c] Weekly***	***Clinic or Home***	***TC***	***TC – Study Completion***	***Clinic or Home[j]***	***Clinic or Home[g,j]***
Day/Week	**Screening (-24 to -2)**	**-4 to -2** *Complete Abx*	**Day -1** *(Within 3d of completing Abx)*	**Day 1**	**Days 2 and 3[c]**	**Weeks 1 to 7**	**Week 8[j] (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures											
Informed Consent	X[k]										
Eligibility criteria review	X		X	X							
Confirm clinical response to antibiotic			X	X							
IxRS registration	X			X							
Medical History	X										
Physical Exam	X										
Focused History and Physical				X			X			X	X
Weight	X										
Vital signs[d]	X			X[e]			X			X	X
Chemistry and hematology	X						X				X
Urine pregnancy test (WOCBP)	X			X[f]			X			X	X
Stool: Study Entry: central or local lab: *C. diff* toxin or PCR test	X										
Stool: On Study Recurrence/ET: central lab: *C. Diff* testing										X	X

	SCREENING PERIOD			**EFFICACY PERIOD**				**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC[b]***	***TC***	***Clinic***	***TC***	***TC[c] Weekly***	***Clinic or Home***	***TC***	***TC – Study Completion***	***Clinic or Home[j]***	***Clinic or Home[g,j]***
Day/Week	**Screening (-24 to -2)**	**-4 to -2** *Complete Abx*	**Day -1** *(Within 3d of completing Abx)*	**Day 1**	**Days 2 and 3[c]**	**Weeks 1 to 7**	**Week 8[j] (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures											
Provide stool collection kits	X			X			X				
Stop Abx	X	X									
Administer bowel cleanse			X								
Confirm subject administered bowel cleanse on Day -1				X							
Study drug dosing				X	X						
Confirmation of study drug administration											
Study drug accountability							X				
Prior/ concomitant medications	X	X	X	X	X	X	X	X	X	X	X
AE assessment		X	X	X	X	X	X	X	X	X	X
Evaluation of diarrhea episodes	X	X	X	X	X	X	X	X	X	X	X
Telephone Contact[h]		X	X			X		X	X		
Stool for microbiome and metabolomics testing and future biomedical research			X[i]	X[i]		X					

	SCREENING PERIOD			**EFFICACY PERIOD**				**FOLLOW UP PERIOD**		**Recurrence Visit(s)[a]**	**ET Visit**
	Clinic	***TC[b]***	***TC***	***Clinic***	***TC***	***TC[c] Weekly***	***Clinic or Home***	***TC***	***TC – Study Completion***	***Clinic or Home[j]***	***Clinic or Home[g,j]***
Day/Week	**Screening (-24 to -2)**	**-4 to -2** *Complete Abx*	**Day -1** *(Within 3d of completing Abx)*	**Day 1**	**Days 2 and 3[c]**	**Weeks 1 to 7**	**Week 8[j] (±2 d)**	**Weeks 12, 16, 20 (± 3 d)**	**Week 24 (±3 d)**		
Assessments and Procedures											
Bowel Cleanse Patient Satisfaction Survey Measure							X				
EQ-5D-5L Visual Analog Scale	X						X				

Abbreviations: Abx=antibiotics; AE=adverse event; *C.diff=Clostridioides difficile*; ET=early termination; FBMR=future biomedical research; HRQoL=Health-Related Quality of Life; IxRS=interactive voice and web response system; ; TC=telephone call; WOCBP=women of childbearing potential

[a] Subjects with a confirmed CDI recurrence after Week 8 should continue to be followed for safety assessments through Week 24.

[b] Phone call: Confirm termination of antibiotics on day of last scheduled antibiotic dose and review instructions for Day -1 activities including collection of a stool sample before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel cleanse. All subject should be reminded to collect a stool sample collected at Day -1 will be returned to the clinic on Day 1

[c] Subjects should be called on Day 2 and Day 3 to confirm they have taken study drug and to inquire about their general health

[d] Blood pressure, pulse, respiratory rate, and body temperature

[e] To be assessed immediately before and approximately 30 minutes after study drug dosing

[f] To be assessed prior to study drug dosing

[g] If termination visit precedes 8 weeks, safety laboratory tests and EQ-5D-5L Visual Analog Scale and Bowel Cleanse Patient Satisfaction Survey Measure will be obtained at this visit

[h] For all visits performed remotely by a telephone contact, a standardized script will be used to ask about the subject's general health, AEs, concomitant medications and to perform a diarrhea assessment

[i] Stool sample will be collected for all subjects at Day -1 and brought to the clinic at the Day 1 visit. In a subgroup of subjects who consent, a stool specimen obtained at Week 1 may be couriered or brought in to the study site

[j] If a nurse or site personnel cannot perform the visits, a telephone call or a video conference (Zoom, Skype, FaceTime, etc.) should be conducted in place of the Week 8, Unscheduled, and Recurrence / Early Termination visits and must be documented in the source. Any required procedures not performed during a remote visit at the subject's home will be documented as a protocol deviation by site staff.

[k] Subjects may sign a pre-screening consent to obtain stool specimen for toxin or PCR assay at Local or Central Laboratory. Prescreening may be repeated but requires a separate consent and a new subject number. Note that Central Laboratory will only perform PCR test on qualifying CDI

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3. LIST OF ACRONYMS, ABBREVIATIONS, AND DEFINITIONS OF TERMS

AE	Adverse Event
Abx	Antibiotics
AESI	Adverse Event of Special Interest
BMI	Body Mass Index
CCNA	Cell Cytotoxicity Neutralization Assay
CDI	*Clostridioides difficile* infection
C. difficile or C. diff	*Clostridioides difficile*
Cdiff32 HRQoL	*Clostridium difficile* (CDiff32) Health-Related Quality of Life Survey
CFR	Code of Federal Regulations
CI	Confidence interval
CLIA	Clinical Laboratory Improvement Amendment
CMH	Cochran-Mantel-Haenszel
DSMC	Data Safety Monitoring Committee
eCRF	electronic Case Report Form
EAIR	exposure-adjusted incidence rates
EIA	Enzyme immunoassay
ET	Early Termination
EQ-5D-5L	EuroQol 5 Dimensions 5 Level
FDA	Food and Drug Administration
FMT	Fecal microbiota transplantation
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GDH	Glutamate dehydrogenase
GI	Gastrointestinal
HRQoL	Health-Related Quality of Life
IBS	Irritable Bowel Syndrome
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee

IRB	Institutional Review Board
ISC	Independent Statistical Center
ITT	Intent-to-Treat
IxRS	Interactive voice and web response system
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified Intent-to-Treat
NAAT	Nucleic acid amplification testing
OTU	Operational taxonomic unit
PCR	Polymerase Chain Reaction
PP	Per-Protocol
RCDI	Recurrent *Clostridioides difficile* infection
RR	Relative risk
SAE	Serious adverse event
SAER	Serious adverse event report
SAP	Statistical Analysis Plan
SCFU	Spore Colony Forming Units
SOC	Standard of care
SporQs	Spore Equivalents, a dosing unit measured by dipicolinic acid content
TEAE	Treatment-emergent adverse event
ULN	Upper limit of normal
WOCBP	Women of childbearing potential

4. INTRODUCTION

Clostridioides difficile is a sporeforming Gram-positive anaerobe present throughout the environment and, in low amounts, can be a component of the gut flora of a healthy individual. *Clostridioides difficile* infection (CDI) usually develops in patients with a history of antibiotic use that depletes the normal gut flora, enabling *C. difficile* to colonize and proliferate within the colon, elaborating virulent toxins A and B. These toxins invade epithelial cells disrupting their cytoskeleton, resulting in damage to the epithelial barrier and promoting mucosal inflammation. The clinical manifestations of CDI vary broadly, ranging from nuisance diarrhea lasting a few days, to more pronounced disease with severe colonic inflammation that can develop into pseudomembranous colitis with associated systemic toxicity requiring lifesaving colectomy.

With ever increasing use of antibiotics, particularly in the aging populations in hospitals and in nursing homes, the incidence of *C. difficile* -associated disease has been increasing such that *C. difficile* is the leading cause of nosocomial infection in the United States (US). The Centers for Disease Control and Prevention estimate that *C. difficile* causes diarrhea linked to approximately 29,000 American deaths each year (Lessa et al, 2015). In Canada, there are approximately 37,900 CDI episodes each year (2012); 7980 (21%) of these are relapses (Levy, 2015). In the European Union (EU), the number of reported cases of CDI has also increased in recent years, and is estimated to affect 172,000 patients per year.

Clostridioides difficile spores can survive for months in hospitals and long term care facilities where they can cause repeated CDI episodes. Virtually all antibiotics have been implicated in association with CDI. The mechanistic link to antibiotic use is based on the finding that a healthy microbial ecology resists pathogen colonization by competing for nutrients and resources in the gut (Theriot et al, 2014; Weingarden- et al, 2014). Antibiotic use disrupts the microbiota and liberates nutrients that enable colonization by *C. difficile* (Ng et al, 2013).

The incidence of recurrent CDI has paralleled the increased incidence of primary infection. CDI recurs in approximately 25% of patients after antibiotic treatment for first time disease (Bakken et al, 2011; Depestel and Aronoff, 2013; Surawicz- et al, 2013). After the first recurrent episode, patients are at an even higher risk for subsequent CDI, estimated to be > 60% after the second or subsequent episode (Higa and Kelly, 2013). There are few proven, approved therapeutic options for significantly reducing CDI recurrence in patients with recurrent CDI. Some patients are treated with antibiotics indefinitely to avoid persistent diarrhea and other sequelae of CDI.

SER-109 is an Ecobiotic® drug being developed for the treatment of adults with recurrent CDI. SER-109 is an ecology of bacteria in spore form, enriched from stool donations obtained from healthy, screened donors. The bacterial spores are enriched by thorough killing of the vegetative microorganisms, then fractionating the resulting spore population away from inactive components and formulating and encapsulating the spores for oral delivery. SER-109 is administered to subjects after completion of a course of antibiotic therapy for recurrent CDI.

SER-109 has been shown to prevent CDI and to treat *C. difficile* relapse in nonclinical studies in mice and hamsters (see Investigator's Brochure for more information). Clinical experience with SER-109 includes four studies: 1) a completed open-label, two-part study in 30 subjects with a history of 3 or more occurrences of CDI (SERES-001); 2) a completed double-blind, placebo-controlled, parallel-group study in adults with recurrent CDI (SERES-004); 3) a completed expanded access for intermediate-size patient populations and open-label extension of study

SERES-004 (SERES-005); and 4) a completed randomized controlled, blinded study of 3 daily doses in subjects with RCDI (SERES- 012). There have been 8 deaths (one in Study SERES-004, four in SERES-005 and three in SERES-012), all of which were deemed by the investigators not related to SER-109. There have been no concerning trends in laboratory values, vital sign values, or physical examination findings in the completed SERES-001, SERES-004, SERES-005, or SERES-012 safety datasets. A summary of clinical efficacy and safety is presented below.

4.1. Summary of Clinical Efficacy and Safety

Study SERES-001 was a 2-part study exploring the safety and efficacy of SER-109 in adult subjects (22 to 88 years of age) with recurrent CDI. The primary efficacy measure was response to SER-109 treatment up to 8 weeks after initiation of therapy. Response was defined as the absence of CDI during the efficacy evaluation period (Day 1 to Week 8). Fifteen subjects in Part 1 of the study received oral SER-109 (a mean dose of $1.7x10^9$ spore equivalents [SporQs], a dosing unit measured by dipicolinic acid content) administered over 2 days. Fifteen subjects in Part 2 of the study received oral SER-109 (mean dose of $1x10^8$ SporQs) administered over 1 day.

In the open label study, SERES-001, SER-109 resulted in per protocol efficacy of 86.7% (26/30 subjects) and an 8-week clinical cure rate of 96.7% (29/30 subjects). One subject had a recurrence at Day 5 and declined re-treatment. Three subjects reported diarrhea with a concomitant positive test result for *C. difficile* between 5 and 9 days after receiving SER-109. All 3 subjects were negative for *C. difficile* carriage and clinically CDI free at 8-weeks and were judged to be clinically cured without treatment with a course of antibiotics. One subject had a recurrence at 26 days after dosing, was re-treated per protocol, and was CDI free 8-weeks after their second dose.

Most subjects (27/30) experienced ≥ 1 AE in Study SERES-001, all of which were -treatment emergent- AEs (TEAEs). Fourteen TEAEs were considered related to study drug and all were mild or moderate. The most common system organ class (SOC) was GI disorders, and the most common preferred term was diarrhea. Four subjects experienced a total of 7 serious AEs (SAEs), none of which were considered by the investigators to be drug-related.

SERES-004 was a randomized, double-blind, placebo-controlled Phase 2 study conducted in the U.S. Eighty-nine (89) subjects were randomized 2:1 to receive either SER-109 or placebo, respectively, following antibiotic treatment for recurrent CDI, and stratified by age (< 65 years; ≥ 65 years). The primary objective was to demonstrate the superiority of SER-109 versus placebo based on the proportion of subjects experiencing a CDI recurrence up to 8 weeks after treatment. The primary safety objective was to evaluate the safety of SER-109 in adults with recurrent CDI up to 12 weeks after treatment as determined by clinical and laboratory safety assessments.

The study did not meet the primary objective of reducing the relative risk of CDI recurrence up to 8 weeks following dosing. Overall, recurrence of *C. difficile* positive diarrhea requiring antibiotic treatment during the 8 weeks post-treatment occurred in 42 (47.2%) subjects (16 [53.3%] subjects randomized to receive placebo vs. 26 [44.1%] subjects randomized to receive SER-109. The relative risk of recurrence in subjects receiving placebo vs. SER-109, adjusted for age stratum, was 1.22, with a corresponding 95% CI of (0.79, 1.88). Of the 43 subjects stratified to the <65 years of age strata, recurrence was observed in 12/28 (42.9%) subjects randomized to receive SER-109 and 4/15 (26.7%) subjects randomized to receive placebo. Of the 46 subjects stratified to the ≥65 years of age strata, recurrence was observed in 14/31 (45.2%) subjects randomized to receive SER-109

and 12/15 (80.0%) subjects randomized to receive placebo. Overall, of the 42 subjects who met the primary endpoint of CDI recurrence by Week 8, 35 subjects discontinued the study due to their CDI recurrence prior to Week 8. Of the 35 subjects who discontinued the study due to a CDI recurrence prior to Week 8, 34 enrolled in the open-label extension study SERES-005.

In Study SERES-004, a total of 66 of the 89 subjects randomized (74.2%), 46 of the 60 (76.7%) subjects who received SER-109 and 20 of the 29 (69.0%) subjects who received placebo, experienced at least 1 TEAE. Fifteen of the 89 (16.9%) subjects, 11 of the 60 (18.3%) subjects who received SER-109 and 4 of the 29 (13.8%) subjects who received placebo, experienced at least 1 TEAE that was considered by the investigators to be drug-related. Like Study SERES-001, the most commonly reported SOC was GI disorders (55% in the SER-109 group and 44.8% in the Placebo group). The most commonly reported (incidence ≥5%) preferred terms in the GI SOC reported in subjects who received SER-109 were diarrhea, abdominal pain, flatulence, nausea, and constipation. The majority of TEAEs were mild or moderate in severity. Six of the 60 (10%) subjects who received SER-109 experienced an event that has been reported as severe. Twelve of the 89 (10.1%) subjects enrolled (9 subjects who received SER-109 [15%] and 3 subjects who received placebo [10.3%] have experienced a total of 43 treatment-emergent SAEs, none of which were considered to be drug-related by the investigator. One subject had an SAE (metastatic non-small cell lung cancer) that was fatal and led to study withdrawal.

SERES-005 began as an open-label extension study of SERES-004 conducted in the U.S, offered to subjects who received an investigational product in SERES-004, but recurred prior to 8-weeks post-treatment. In April 2016, the study was amended to include expanded access to an intermediate-size patient population of adults, 18 years of age or older with recurrent CDI, for whom there is no comparable or alternative therapy. Seventy-two subjects, 34 who enrolled from Study SERES-004 and 38 who enrolled under expanded access met the eligibility criteria for the study. The primary efficacy objective was to evaluate CDI recurrence rates in adults up to 8 weeks post-treatment with SER-109. The primary safety objective was to evaluate the safety and tolerability of SER-109 in adults with recurrent CDI.

Overall, CDI recurrence was observed within 8 weeks post-treatment in 38.9% (28/72) of subjects, including 52.4% (11/21) in the SERES-004 SER-109 group, 15.4% (2/13) in the SERES-004 Placebo group, and 39.5% (15/38) in the Expanded Access group. Among the 58 subjects treated with vancomycin followed by SER-109, CDI recurrence within 8 weeks post-treatment was observed in 41.4% (24/58). Among the 13 subjects treated with fidaxomicin followed by SER-109, CDI recurrence was observed within 8 weeks post-treatment in 30.8% (4/13) of subjects. Among subjects ≥ 65 years old, CDI occurred in 16/44 subjects (36.4%) and in those < 65 years old, CDI occurred 12/28 subjects (42.9%). Hospitalizations overall occurred in 9 (12.5%), 10 (13.9%), and 12 (16.7%) subjects by 8, 12, and 24 weeks, respectively. All four (5.6%) hospitalizations for CDI occurred within the first 8 weeks.

Overall, 55 (76.4%) subjects had 241 TEAEs. Seven (7) subjects had 11 study-drug related or possibly related AEs. Eleven subjects experienced 21 SAEs. There was a tendency for more subjects with SAEs in those ≥ 65 years old than those < 65 years old, 20.5% vs. 7.1%, respectively. None were drug-related or possibly related. Eight subjects, all ≥ 65 years old had 25 severe TEAEs. Four subjects, all ≥ 65 years old had TEAEs leading to study withdrawal. The most frequently affected system organ class (SOC) was gastrointestinal (GI) accounting for 52.8% of events with preferred terms diarrhea, abdominal pain, constipation, nausea, flatulence, vomiting followed by

Infections and infestations (36.1%) with preferred terms urinary tract infection, nasopharyngitis, and cellulitis. Overall, most events were of mild or moderate intensity: 33.3% of subjects had TEAEs that were mild in intensity, 31.9% subjects had at least TEAE of moderate intensity. Related events mainly affected the GI SOC.

Eleven subjects had 21 SAEs leading to three of four deaths and study discontinuation. One subject had 8 SAEs: diastolic congestive heart failure, myocardial infarction, left face zoster shingles, severe sepsis and septic shock, cerebrovascular accident, pneumonia and aspiration pneumonia. Myocardial infarction, cerebrovascular accident and aspiration pneumonia were fatal. One subject had inflammatory diarrhea and sepsis that was fatal. One subject had dehydration and cerebrovascular accident that was fatal. One subject had *C. difficile* colitis that was fatal. All SAEs were not considered related to study drug.

Of the other SAEs, 3 were severe including diarrhea related to *C. difficile*, recurrent syncope, and CDI recurrence, and 4 were moderate in intensity including lumbar compression fracture, diarrhea, hyperkalemia and exacerbation of end-stage renal disease, and pancolitis. These SAEs and fatality rates are consistent with those expected in the study population with multiple comorbidities.

SERES-012 was a stratified, randomized, double-blind placebo-controlled Phase 3 study, that enrolled subjects with recurrent CDI defined as 2 or more recurrences of CDI in the previous 12 months. Subjects were stratified by age (<65 years; ≥ 65 years) and antibiotic treatment of the qualifying CDI (vancomycin; fidaxomicin), and were randomized in a 1:1 ratio to SER-109 administered as 4 capsules (3×10^7 SCFU) for 3 consecutive days or identical appearing placebo after administration of a bowel cleanse on completion of antibiotics. The primary objective was to demonstrate the superiority of SER-109 vs. placebo in reducing recurrences by eight weeks, as determined by symptoms (≥ 3 unformed bowel movements for 2 days) and by using toxin detection by EIA, reflexing to CCNA. Investigator assessment that the subject required treatment must also have been met.

This is a summary from the interim analysis dataset. The majority of the 182 subjects were female (n = 109; 59.9%). Most of them were white (n = 170; 93.4%). The mean age was 65.5 years and 103 (56.6%) subjects were ≥ 65 years of age. There were more females in the SER-109 group 62/90 (68.9%) vs. 47/92 (51.1%) and there were fewer subjects with a second recurrence in the SER-109 group (56.7%) vs. placebo group (64.1%). Other demographic and baseline characteristics were similar between the two groups.

The study met the primary endpoint of reducing the relative risk of CDI recurrence by toxin assay up to 8 weeks following dosing in the ITT population. Overall, recurrence of CDI up to 8 weeks post-treatment was observed in 48 of 182 (26.4%) subjects of all subjects enrolled. Ten (10) of the 90 (11.1 %) subjects randomized to receive SER-109, and 38 of the 92 (41.3%) subjects randomized to receive placebo experienced a CDI recurrence prior to Week 8. The relative risk of CDI recurrence in the SER-109 versus placebo arm up to Week 8 was 0.27 (95% CI: 0.15 to 0.51) and the absolute difference in recurrence rates was 30.2%. Sustained clinical response was observed in 80 of 90 (88.9%) SER-109 subjects and 54 of 92 (58.7%) placebo subjects.

With regard to age < 65 years, recurrence was observed by Week 8 in 2/40 (5.0%) subjects randomized to receive SER-109 and 13/40 (32.5%) subjects randomized to receive placebo (relative risk of CDI recurrence in the SER-109 versus placebo arm up to Week 8 was 0.15 [95% CI: 0.04 to 0.64]). For age ≥ 65 years, recurrence was observed by Week 8 in 8/50 (16.0%) subjects

randomized to receive SER-109 and 25/52 (48.1%) subjects randomized to receive placebo (relative risk of CDI recurrence in the SER-109 versus placebo arm up to Week 8 was 0.33 [95% CI: 0.17 to 0.67]). With regard to the prior antibiotic vancomycin regimen, recurrence was observed by Week 8 in 9/65 (13.8%) subjects randomized to receive SER-109 and 27/68 (39.7%) subjects randomized to receive placebo (relative risk of CDI recurrence in the SER-109 versus placebo arm up to Week 8 was 0.35 [95% CI: 0.18 to 0.68]). For the prior antibiotic fidaxomicin regimen, recurrence was observed by Week 8 in 1/25 (4.0%) subjects randomized to receive SER-109 and 11/24 (45.8%) subjects randomized to receive placebo (relative risk of CDI recurrence in the SER-109 versus placebo arm up to Week 8 was 0.09 [95% CI: 0.01 to 0.63]).

Overall, 168 of the 182 subjects (92.3%) experienced at least 1 TEAE. Eighty-four (84) of the 90 (93.3%) subjects who received SER-109 experienced a total of 529 TEAEs, and 84 of the 92 (91.3%) subjects who received placebo experienced a total of 598 TEAEs. Overall, 94 of the 182 (51.6%) subjects experienced a total of 452 TEAEs that were considered by the Investigator to be related or possibly related to study drug – 51.1% in the SER-019 group and 52.2% in the placebo group. Fifteen (15) of the 90 (16.7%) subjects who received SER-109 experienced a total of 26 treatment-emergent SAEs and 19 of the 92 (20.7%) subjects who received placebo experienced a total of 32 treatment-emergent SAEs. Four (4) of the 90 (4.4%) subjects who received SER-109 experienced a total of 4 treatment-emergent adverse events of special interest (for example, blood stream infection, meningitis, abscess) (AESI) and 3 of the 92 (3.3%) subjects who received placebo experienced a total of 3 treatment-emergent AESIs. None of these SAEs or AESIs were considered related to investigational product by the Investigator. Three deaths were outcomes of SAEs, all in the SER-109 group, consisting of worsening glioblastoma, atrial fibrillation and sepsis, and subdural hematoma after a fall.

The most commonly reported (incidence ≥ 5%) preferred terms in both treatment groups were flatulence, abdominal distension, abdominal pain, constipation, diarrhea, nausea, fatigue, chills, and decreased appetite. In addition, the commonly reported (incidence ≥ 5%) preferred terms were urinary tract infection in subjects who received SER-109; and vomiting and *C. difficile* colitis in subjects who received placebo. A larger number of some GI AEs in the placebo group may be because of the higher rate of recurrences in that group, Overall, the safety profile was similar between SER-109 and placebo recipients.

Thus, clinical experience to date suggests that SER-109 is well-tolerated with an acceptable safety profile. Overall, there have been no drug-related treatment-emergent SAEs and the majority of related TEAEs have been mild or moderate in severity and most commonly associated with the gastrointestinal tract. Additional information regarding clinical experience with SER-109 can be found in the Investigator's Brochure.

Although safety data with SER-109 has been relatively consistent across studies, efficacy data in the placebo-controlled Study SERES-004 was inconsistent with results from the open-label Study SERES-001. There are a number of factors that may explain why the primary endpoint of reducing the relative risk of CDI recurrence at up to 8-weeks was not achieved in Study SERES-004. First, the diagnostic test for entry may not have differentiated subjects with active CDI disease from those with *C. difficile* carriage. This would have led to enrolling subjects who may have been experiencing post-CDI irritable bowel syndrome (IBS)-like symptoms but, were only colonized by *C. difficile* and did not have an active infection. These subjects may not have been distributed equally between the treatment arms. Additionally, the diagnostic test for recurrences which

primarily used polymerase chain reaction testing (PCR), also known as nucleic acid amplification testing (NAAT), which could have overestimated recurrences during the trial. Finally, although analysis of the microbiome identified that SER-109 in SERES-004 was biologically active, the dose administered in Phase 2 may not have been optimal for efficacy (see rationale below). Both changing the dose and definition of recurrence in SERES-012 likely led to a more robust and well-controlled trial, with statistically significant and clinically meaningful improvement observed.

4.2. Rationale

There are few proven, approved therapeutic options for significantly reducing CDI recurrence in patients with recurrent CDI.

This study is being conducted primarily to gather safety and tolerability data as well as efficacy from subjects exposed to SER-109 at the dose used in SERES-012. In addition to subjects rolling over from SERES-012 (Cohort 1), subjects with first recurrence or more will be eligible for Cohort 2. There are no data to suggest differences in safety profile in subjects with at least one recurrence versus those with at least two recurrences. In a Phase 3b/4 study that described safety in groups with 0, 1, or 2 recurrences, there were no differences when comparing an extended pulsed course of fidaxomicin with vancomycin (Cornely 2019).

	Extended FID			Vancomycin		
Number randomized	181			181		
Number preceding CDI episodes	0	1	2	0	1	2
N in group	141	26	10	140	29	10
N with AEs (%)	94 (67)	19 (73)	8 (80)	101 (72)	18 (62)	9 (90)
N with SAEs (%)	58 (41)	7 (27)	3 (30)	61 (44)	11 (38)	5 (50)
N with deaths (%)	25 (18)	1 (4)	2 (20)	32 (23)	3 (10)	1 (10)

The majority of subjects enrolled in the trial were having their first occurrence of CDI. Those with first and second recurrence make up a small proportion of subjects. However, there does not appear to be an increased AE, SAE, or death rate going from first occurrence to second and third occurrences. These data support inclusion of first recurrence (second occurrence) subjects in the larger safety population. Enrollment of patients with first CDI recurrence will allow participating centers to present the protocol to a higher number of subjects, who previously did not qualify.

The reason to require toxin testing in the pivotal efficacy study was to ensure exclusion of carriers which could potentially be imbalanced across treatment arms. However, this significantly limited availability of treatment to a population in whom toxin testing was available. Inclusion criteria have been broadened to allow evaluation of safety and tolerability in subjects who may receive SER-109 where toxin testing using EIA is not available, but who meet the diagnostic criteria based on clinical indicators and a positive PCR test for *C. difficile*. In the IDSA treatment guidelines, diagnosis of *C. difficile* infection using PCR alone is acceptable in patients who meet clinical diagnostic criteria (McDonald 2018). Subjects who have been treated with longer duration and

dose of vancomycin or duration of fidaxomicin will also be enrolled given changes in standard-of-care durations of antibiotics towards prolonged and tapering courses (McDonald 2018, Cornely 2019). Broadening eligibility to subjects with *C. difficile* infection who do not have access to toxin EIA testing or who have received longer courses of antibiotics prior to study participation better reflect 'real world' management of CDI patients. Subjects previously screened in SERES-012 who did not receive SER-109 and had not previously rolled over to SERES-013 will also be eligible.

4.2.1. Rationale for Dose and Treatment Regimen

In this study, subjects will receive a dose of SER109 ($3x10^7$ spore colony forming units [SCFU]) per day for 3 consecutive days. The dose and treatment regimen was the one used in SERES-012.

In Study SERES-001, subjects received doses of SER-109 ranging from $3x10^7$ to $2x10^{10}$ SporQs ($2x10^5$ to $5x10^9$ SCFU) given over one or two days. Analysis of changes in the subject microbiome demonstrates that spore forming species richness in the subjects' GI tract at 1 week was positively correlated with SER-109 dose. Importantly, of subjects who recurred in SERES-004, about 50% of recurrences in both placebo and SER109 arms happened by Day 10 and 75% by Day 20, starting as early as Day 3. In SERES-004, the engraftment of SER-109 spore-forming bacteria in treated subjects' gastrointestinal tracts was less robust and less rapid as compared to that observed in SERES-001, although it was significantly greater than the changes in placebo-treated subjects. Engraftment improved at later time points, but due to early recurrences, the SER-109-induced microbiome change in SERES-004 may have been too late from a therapeutic perspective. In addition, it was generally observed that commensal spore-forming species richness at 1-week post dosing is correlated with better clinical outcome. In aggregate, these observations are central to the design of the proposed regimen that provides a higher daily dose ($3x10^7$ SCFU) repeated daily over 3 days following the completion of antibiotics as compared to dosing in SERES-004. Due to the fact that recurrence happens early, the slower SER-109-induced microbiome changes in SERES-004 suggest that the $1x10^8$ dose target (SporQ) was likely below the required amount to achieve a therapeutic response. To account for variations in antibiotic washout, and to provide dosing prior to the earliest observed recurrences, three (3) doses will be administered on Days 1-3 following antibiotic cessation. We have chosen a three-fold higher dose level based on the SCFU metric, $3x10^7$ SCFU, as an amount commensurate with engraftment richness to the degree correlating with protection against recurrence.

4.2.2. Rationale for Endpoints and *C. difficile* Diagnostic Criteria

The introduction of molecular tests, which are more sensitive and detect microbial DNA to the C. *difficile* toxin gene instead of toxin, has led to greater detection of *Clostridioides difficile* but detect *C. difficile* bacteria regardless of toxin production. This phenomenon has called into question whether a positive PCR or NAAT result reflects clinical disease or represents *C. difficile* colonization (Polange et al, 2015).

Thus, in SERES-004, the diagnostic test for entry may not have differentiated subjects with active CDI disease from those with *C. difficile* carriage. This would lead to enrolling subjects who may be experiencing a post-CDI irritable bowel syndrome (IBS) if colonized by *C. difficile*. IBS following CDI is reported to occur in up to 25% of CDI patients (Wadhwa et al, 2016). This would

have decreased the power of the study to differentiate the treatment arms as those subjects without a true diagnosis of RCDI are less likely to recur.

Since PCR diagnostics in SERES-004 may have led to misclassification of subjects with diarrhea and *C. difficile* colonization as recurrence, the primary efficacy endpoint in this study is the recurrence of CDI in subjects who receive SER-109 or placebo using a *C. difficile* toxin positive diagnostic (not toxin gene-based) up to 8 weeks after initiation of treatment. Unlike the PCR diagnostic for *C. difficile*, the toxin-based tests, such as enzyme immunoassay (EIA) for toxin A and B or the cell cytotoxicity neutralization assay (CCNA) detects the presence of *C. difficile* toxin in fecal samples. Thus, recurrence is defined as ≥ 3 unformed stools per day for 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay, and assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment.

5. STUDY OVERVIEW

ECOSPOR IV – Cohort 1 is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a treatment regimen SER-109 in adult subjects 18 years of age or older with recurrent *Clostridioides difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012. In Cohort 2 of ECOSPOR IV, the primary study objective is to examine safety and tolerability in a cohort of subjects receiving SER-109 at the dose used in SERES-012.

This study will be conducted at approximately 140 study centers in the North America. For Cohort 1, subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a treatment regimen of SER 109 or placebo in Study SERES-012, and who have responded to 10 to 21 days of standard-of-care (SOC) antibiotic treatment for CDI (i.e. vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID]) will be eligible to enroll and receive a treatment regimen of SER-109 in Study SERES-013. A treatment regimen of SER-109 is administered orally as 3×10^7 spore colony forming units (SCFU) in 4 capsules once daily for 3 consecutive days. Approximately 30 eligible subjects with recurrent CDI disease from Study SERES-012 are expected to enroll in Cohort 1. Approximately 200 subjects will be enrolled through the Open-Label program in Cohort 2For Cohort 2, subjects with one or more recurrences of CDI (including the current episode) who have responded to CDI antibiotic therapy defined as 10 to 42 days of treatment with vancomycin [125 mg QID] or 10 – 25 days of fidaxomicin [200 mg BID] will be potentially eligible to enroll in Study SERES-013 to receive a SER-109 treatment regimen.

The study duration is approximately 27 weeks, including a ~3-week Screening Period, an 8-week Efficacy Period, and a 16-week Follow-up Period from initiation of treatment on Day 1. Subjects treated with prolonged or tapering doses of antibiotics after prescreening confirmation of CDI will be screened during the 3-week screening period (Day -24 to Day -2)

In Cohort 1, favorable clinical outcome, or sustained clinical response, will be determined by the absence of CDI recurrence up to 8 weeks after initiation of treatment of study drug, with CDI recurrence defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay and a decision by the investigator (based on clinical assessment) that antibiotic treatment is needed. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

In Cohort 2, favorable clinical outcome, or sustained clinical response, will be determined by the absence of CDI recurrence up to 8 or 12 weeks after initiation of treatment of study drug, with CDI recurrence defined in the same manner as for Cohort 1.

5.1. Trial Conduct

For Cohort 1, screening will begin at the Recurrence Visit of Study SERES-012. Eligible subjects, or their legally authorized representative, will provide informed consent and undergo all baseline evaluations at the Screening Visit. Assessments performed at the Recurrence Visit of Study SERES-012 do not need to be repeated, if performed within the SERES-013 screening window (Day -24 to Day -2) and from the central laboratory of study SERES-012.

For Cohort 2, subjects with symptoms suggestive of RCDI may submit a stool sample for CDI toxin or PCR testing at a Clinical Laboratory Improvement Amendment (CLIA)- certified local laboratory or the central laboratory with a prescreening or screening consent. The central laboratory will only be performing PCR testing on qualifying CDI. All subjects who are confirmed to be toxin or PCR positive at prescreening will undergo all Screening Visit assessments after providing signed informed consent. Subjects who were previously test negative who have a new episode may repeat pre-screening or screening using a new consent and study number.

Cohort 1 subjects will receive 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg BID]. On Day 1, within 3 days of completion of SOC antibiotic treatment for their CDI, subjects will undergo a bowel cleanse by consuming 10 oz. (~300 mL) of oral magnesium citrate followed by overnight fasting. Subjects with impaired kidney function who are unable to take magnesium citrate will take 250 mL of GoLytely (polyethylene glycol electrolyte solution).

Subjects will come to the clinic after an overnight fast on Day 1 to receive a dose of oral SER-109 (3×10^7 SCFU) in 4 capsules and have all safety evaluations performed. On Day 1, subjects will be dispensed a 2-day supply of SER-109 (3×10^7 SCFU) in 4 capsules with instructions for at-home administration of a single daily dose in the morning before breakfast on Day 2 and Day 3. Subjects will be contacted by phone on Day 2 and Day 3 to confirm they have taken study drug in the morning before breakfast and to inquire about their general health. If subjects have not taken study drug when contacted, they will be reminded to do so as soon as possible. From Day 1 to Week 8, all subjects will be contacted by phone by study site personnel weekly, with the exception of a home or in-clinic visit at Week 2 and an in-clinic visit at Week 8, and queried for adverse events (AEs) and diarrheal symptoms. After Week 8, all subjects will be contacted by phone by study site personnel every 4 weeks (i.e., Weeks 12, 16, 20, and 24) and queried for serious adverse events [SAEs] and adverse events of special interest [AESIs]). Health-related quality of life and health outcomes will be assessed throughout the study via the CDI-specific, Cdiff32 Health Related Quality of Life (HRQoL) and EuroQol 5 Dimension 5 Level (EQ-5D-5L) questionnaires.

All AEs, SAEs/AESIs, and concomitant medications will be collected from initiation of study drug administration up to Week 8. From Week 8 up to Week 24, all SAEs/AESIs and SAE/AESI-related data, and any antibiotic medication and its corresponding indication will be collected.

To document episodes of diarrhea, subjects will complete a daily diarrhea log (see Investigator Site File) to include days with diarrhea as well as no diarrhea. If diarrheal symptoms recur (≥ 3 unformed stools per day over 2 consecutive days) between scheduled visits, subjects will be instructed to contact the investigator and return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI (Recurrence Visit).

Subjects who have a confirmed CDI recurrence should continue to be followed for safety assessments through Week 24. Favorable clinical outcome in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of the SER-109 treatment regimen. Sustained clinical response is favorable clinical outcome. CDI recurrence is defined as ≥3 unformed stools per day over 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* toxin assay on a stool sample and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. To inform subject care, a *C. difficile* stool test may be performed locally at the study site. Stool samples collected for suspected CDI recurrence will also be processed and

shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual). The subject should not initiate antibiotics for the suspected CDI prior to providing a stool sample for the central laboratory stool testing. Data from the *C. difficile* toxin test (either EIA or CCNA), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

The schedule of assessments and procedures is provided in Table 1.

Cohort 2 subjects will receive 10 to 42days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg]. Subjects will undergo screening at Day -24 to Day -2 of the study. On the screening visit, the EuroQol 5 Dimension 5 Level (EQ-5D-5L) Visual Analog Scale will be completed by the patient. On Day -1, within 3 days of completion of antibiotic treatment for their CDI, subjects will undergo a bowel cleanse by consuming 10 oz. (~300 mL) of oral magnesium citrate followed by overnight fasting. Subjects with impaired kidney function who are unable to take magnesium citrate will take 250 mL of GoLytely (polyethylene glycol electrolyte solution).

Subjects will come to the clinic on Day 1 to receive a dose of oral SER-109 (3×10^7 SCFU) in 4 capsules. On Day 1, subjects will be dispensed a 2-day supply of SER-109 (3×10^7 SCFU) in 4 capsules with instructions for at-home administration of a single daily dose in the morning on Day 2 and Day 3. Subjects will be contacted by phone on Day 2 and Day 3 to confirm they have taken study drug and to inquire about their general health. If subjects have not taken study drug when contacted, they will be reminded to do so as soon as possible. From Day 1 to Week 8, all subjects will be contacted by phone by study site personnel weekly, with the exception of a home or in-clinic visit at Week 8, and queried for adverse events (AEs) and diarrheal symptoms. On Week 8, the EuroQol 5 Dimension 5 Level (EQ-5D-5L) Visual Analog Scale and Bowel Cleanse Patient Satisfaction Survey Measure will be completed by the patient. After Week 8, all subjects will be contacted by phone by study site personnel every 4 weeks (i.e., Weeks 12, 16, 20, and 24) and queried for serious adverse events [SAEs] and adverse events of special interest [AESIs]) and diarrheal symptoms.

All AEs, SAEs/AESIs, and concomitant medications will be collected from initiation of study drug administration up to Week 8. From Week 8 up to Week 24, all SAEs/AESIs and SAE/AESI-related data, and any antibiotic medication and its corresponding indication will be collected.

If diarrheal symptoms recur (≥ 3 unformed stools per day over 2 consecutive days) between scheduled visits, subjects will be instructed to contact the investigator and return to the clinic or have a home visit for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI (Recurrence Visit).

Subjects who have a confirmed CDI recurrence should continue to be followed for safety assessments through Week 24. Favorable clinical outcome, or sustained clinical response, will be determined by the absence of CDI recurrence up to 8 or 12 weeks after initiation of the SER109 treatment regimen. CDI recurrence is defined as ≥3 unformed stools per day over 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* toxin assay on a stool sample and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. Stool samples collected for suspected CDI recurrence will be processed and shipped to the central laboratory for *C. difficile* stool testing (see Laboratory Manual). The subject should not initiate antibiotics for the suspected

CDI prior to providing a stool sample for the central laboratory stool testing. Data from the *C. difficile* toxin test (either EIA or CCNA), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

The schedule of assessments and procedures is provided in Table 2.

6. STUDY OBJECTIVES

The study objectives remain the same for Cohort 1.

6.1. Primary Efficacy Objective

- To evaluate SER-109 in the reduction of CDI recurrence rates and increased sustained clinical response rate, determined by a toxin assay, up to 8 weeks after initiation of treatment

6.2. Secondary Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a PCR algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

6.3. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

6.4. Exploratory Efficacy Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109

- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

For Cohort 2, the primary objectives are safety and tolerability.

6.5. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

6.6. Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates and increase in sustained clinical response rates, determined by a toxin assay, up to 8 and 12 weeks after initiation of treatment

6.7. Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline to 1 week after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline to 1 week after initiation of treatment
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) Visual Analog Scale at Screening and at 8 weeks
- To assess patient satisfaction with a bowel cleanse administered prior to SER-109, a Bowel Cleanse Patient Satisfaction Survey Measure at 8 weeks

7. STUDY ENROLLMENT AND WITHDRAWAL

7.1. Inclusion Criteria

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study related- procedures:

For Cohort 1:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012.
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES-012 must have met the protocol definition of:
    a. ≥3 unformed stools per day for 2 consecutive days
    b. A positive *C. difficile* stool toxin assay
    c. The requirement of CDI SOC antibiotic therapy (defined as 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg] mg BID).
    d. An adequate clinical response following SOC antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
4. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

For Cohort 2:

1. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
2. ≥ 2 episodes of CDI, inclusive of the current episode, with.estimated total number of prior episodes
3. The CDI recurrence must have met the protocol definition of:
    a. ≥ 3 unformed stools per day for 2 consecutive days

b. A positive *C. difficile* stool toxin or PCR assay (either local or central laboratory).
c. The requirement of CDI antibiotic therapy (defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg]. It is acceptable if subject was started on metronidazole, switched to vancomycin or fidaxomicin and is treated for a minimum of 10 days of vancomycin or fidaxomicin with a total treatment duration of up to a maximum of - 42 days for vancomycin or 25 days for fidaxomicin.
d. An adequate clinical response following antibiotic therapy, defined as <3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
e. The requirement that the subject can be dosed with study drug within 4 days of antibiotic completion.

4. Male or female subject ≥ 18 years of age.
5. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as post-menopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
6. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.
7. If currently taking probiotics, must be willing to stop at time of consent, for the duration of the study.

7.2. Exclusion Criteria

Cohort 1

A subject will not be enrolled if the subject meets any of the following criteria:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.
2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding). Note: nursing homes, rehabilitation, assisted living centers and acute care hospitals are acceptable.
4. Absolute neutrophil count of <500 cells/mm^3
5. Taking antibacterial therapy other than antibiotics for the most recent episode of CDI during the screening period (a single day antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.

6. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery. (Bariatric surgery which does not disrupt the gastrointestinal lumen, i.e., restrictive procedures such as banding, are permitted).
7. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past 3 months.
8. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to enrollment.
9. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term (1 day) opiate use is permitted (e.g., for a dental extraction).
10. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
11. Known stool studies positive for ova and/or parasites within the 30 days before enrollment
12. Poor concurrent medical risks with clinically significant comorbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
13. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
14. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
15. Any history of immunoglobulin (IgG) replacement therapy within the past 3 months.
16. Any history of fecal microbiota transplantation (FMT) within the past 3 months.
17. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
18. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
19. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives
20. Life expectancy is 24 weeks or less

For Cohort 2 , all Cohort 1 exclusion criteria plus number 21, below, apply.

21. Previously enrolled in a Seres Therapeutics clinical study An exception is made for subjects who screened in SERES-012 who did not receive SER-109 and did not previously roll-over to SERES-013.

7.3. Subject Monitoring and Withdrawal

7.3.1. Reasons for Withdrawal

Subjects should continue to be followed for safety assessments up to 24 weeks after treatment, even after a CDI recurrence. However, a subject may withdraw from the study at any time for any reason, without any consequence. In addition, a subject may be withdrawn from the study for reasons including the following:

- AE (typically an SAE)
- Subject choice (withdrawal of consent by subject or their legally authorized representative; investigator will attempt to ascertain reason)
- Protocol violation/non-compliance

7.3.2. Handling of Withdrawals and Discontinuations of Treatment

The primary reason for withdrawal from the study will be recorded in an electronic case report form (eCRF). Subjects who voluntarily withdraw, or who are withdrawn from the study will be encouraged to complete the Early Termination Visit. The Early Termination Visit procedures are listed in Section **10.3.3**. Although subjects are free to withdraw at any time, subjects will be encouraged to remain in the study for follow-up safety evaluation.

Those subjects who withdraw from the study will be referred to a physician for follow-up care.

7.3.3. Lost to Follow-up

If a subject fails to appear for a follow up assessment, all attempts to contact the subject and information received during contact attempts must be documented in the subject's medical record. In any circumstance, every effort should be made to contact the subject and document subject outcome, (i.e., 3 documented contact attempts via phone calls, e-mail, etc., on separate occasions will be made to locate or contact the subject, and/or to determine health status).

7.3.4. Termination of Study

Although the sponsor has every intention of completing the study, the sponsor may terminate the study at any time for clinical or administrative reasons.

8. INVESTIGATIONAL PRODUCT

8.1. SER-109

SER-109 is an ecology of bacterial spores enriched from stool donations obtained from healthy, screened donors. SER-109 is formulated as an oral capsule for administration to patients following cessation of antibiotic therapy.

8.1.1. Donor Screening

Donors undergo a general health examination including gastrointestinal (GI) medical history, familial GI medical history, blood chemistry, hematology with complete blood count, urinalysis,

and blood and fecal viral and bacterial pathogen testing before donating stool. The donor must successfully complete the physical screening and laboratory tests after the donation period before the material can be released for manufacturing. A description of donor screening procedures is provided in the Investigator's Brochure.

8.1.2. Manufacturing and Storage

SER-109 is manufactured using current Good Manufacturing Practice (GMP). Stool raw material is sourced from donors who are screened for health history, physical status, and a panel of pathogen tests; materials from a single donor are pooled to make a manufacturing lot. The manufacturing process inactivates non-spore forms of live bacteria and fungi, and potential parasites and viruses, and substantially reduces the amount of undigested food and inactivated non-spore components via successive separation steps. The purified material is then concentrated to enable oral capsule formulation, stored frozen, and quality control tested until formulation.

8.2. SER-109 Kit Storage and Handling

The investigational product (SER-109) will be provided as a per subject kit to include 3 bottles containing four size 00 capsules (3×10^7 SCFU) in an opaque, 40 mL high density polyethylene container sealed with foil.

SER-109 is odorless and tasteless as prescribed. If chewed or if capsule integrity is compromised, SER-109 has a sweet taste.

Instructions for shipment, storage, accountability, reconciliation, and destruction of study drug are provided in the Pharmacy Manual.

8.3. Compliance

Subjects will be instructed to return all unused medication and all used packaging materials to the clinic at the Week 8 visit. Subject compliance to study drug will be checked by the investigator or their designee(s) and documented in the CRFs (e.g., tablet count). Subjects will be instructed to take all study drug doses in the morning after an overnight fast (nothing by mouth except for small amounts of water) of $\geq$ 8 hours. Subjects will be asked to remain fasting for up to 60 minutes following dosing. In Cohort 2, fasting is not required.

8.4. Method of Assigning Patients to Study Treatment

This is an open-label study. All subjects who qualify for dosing will receive single daily doses of SER-109 (3×10^7 SCFU) in 4 capsules administered over 3 consecutive days.

The interactive voice and web response system (IxRS) will assign appropriate bottles of SER-109 that will be available at the site for all subjects on their Day 1 study visit. Subjects who discontinue this study or who have previously received SER-109 in this study will not be permitted to re-enter. Similarly, SER-109 dispensed to a subject may not be re-used, even if the bottle(s) are returned unopened.

8.5. Maintaining the Randomization Codes and Breaking the Study Blind

Not applicable. This study is not randomized or blinded.

8.6. Concomitant Medications

8.6.1. Prohibited Concomitant Medications and Procedures

The following therapies are prohibited for the duration of the study unless there has been a confirmed recurrence.

- Probiotics
- Loperamide
- Diphenoxylate/atropine
- Cholestyramine
- Opiate treatment unless on a stable dose. Note: Short term (one day) opiate use is permitted (e.g., for a dental extraction).
- Oral metronidazole, oral fidaxomicin and oral vancomycin except for treatment of suspected or confirmed CDI recurrence.
- Fecal Microbiota Transplant (FMT) prior to recurrence in the study

8.7. Criteria for Confirmed *Clostridioides difficile* Recurrence Post-Randomization

Cohort 1 subjects suspected of having CDI will be asked to contact the investigator and return to the clinic for a *C. difficile* stool toxin test and evaluation for recurrence of CDI (Recurrence Visit) (see Section **10.3.3**). For both Cohort 1 and Cohort 2 subjects, during the COVID-19 pandemic home visits will be allowed in place of clinic visits. subjects suspected of having CDI will be asked to contact the investigator and return to the clinic or have a home visit for a *C. difficile* stool toxin test to be sent to the central laboratory and evaluation for recurrence of CDI (Recurrence Visit). If the subject is seen in a home visit, the investigator will determine treatment based on the clinical evaluation and stool test result.

The home visit will remain an option for a clinic visit during follow-up visits after enrollment for Cohort 2.

Subjects must fulfill the following criteria:

1. ≥ 3 unformed stools per day over 2 consecutive days
2. Diarrhea as defined here should continue up until the day antibiotics to treat CDI are initiated. A positive *C. difficile* test on a stool sample determined by a toxin assay
   - A *C. difficile* stool test will be performed at the central laboratory. This result will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator.
3. Assessment by the investigator that the clinical condition of the subject warrants treatment

9. STUDY PROCEDURES

For Cohort 1, the schedule of assessments and procedures is presented in Table 1. For Cohort 2, the schedule of assessments and procedures is presented in Table 2.

9.1. Duration of Participation

The duration of study participation is up to approximately 27 weeks, consisting of a Screening Period lasting up to ~3 weeks, an 8-week Efficacy Period, and a 16-week Follow-Up Period from initiation of study drug on Day 1.

9.2. Medical History

At the time of Screening, subjects' medical history will be updated with particular attention to the most recent CDI history. The antibiotic regimen, including dose and duration after the most recent CDI recurrence, will be documented. Subjects must demonstrate an adequate clinical response to the antibiotic regimen to treat the most recent CDI recurrence defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before study drug dosing.

9.3. Physical Examination

For cohort 1, a physical examination will be conducted by a physician at the timepoints indicated in Table 1. A focused history and physical will be conducted at Week 8 and at the Early Termination Visit or any Recurrence Visit to the study site, if applicable.

For Cohort 2 subjects, a physical examination will be performed by the principal investigator or delegated qualified individual at screening. A focused history and physical by delegated qualified individual will be performed at Day 1, Week 8, any recurrence visit, and the Early Termination Visit if it occurs before Week 8.

9.4. Body Weight

For Cohort 1, body weight will be obtained at all in-clinic visits according to the schedule in Table 1.

For Cohort 2, Body Weight will be obtained at the Screening Visit according to the schedule in Table 2.

9.5. Vital Signs

For Cohort 1, vital sign assessments including systolic and diastolic blood pressure, pulse, respiratory rate, and oral body temperature measurements will be obtained at the visits indicated in Table 1. Vital sign assessments on Day 1 should be obtained immediately before and approximately 30 minutes after dosing.

For Cohort 2, Vital Sign assessments including systolic and diastolic blood pressure, pulse, respiratory rate, and oral temperature measurements will be obtained at the clinic or home visits.

9.6. Laboratory Assessments

All hematology and blood chemistry laboratory tests will be performed by the central laboratory. The laboratory facilities for analysis of clinical laboratory samples obtained under this protocol will have adequate licensure and accreditation. Urine pregnancy tests will be performed at the sites. Details of sample handling, specific tests performed, and methodology will be provided in the Laboratory Manual.

9.6.1. Hematology and blood chemistry

For Cohort 1, blood samples for hematology and blood chemistry will be obtained according to the schedule in Table 1. Blood samples for hematology and blood chemistry obtained on Day 1 (pre-dose) will be used to determine baseline data.

The central laboratory will flag subjects if they have all of the following abnormal laboratory results:

- Alanine aminotransferase (ALT) ≥ 3 x upper limit of normal (ULN)
- Aspartate aminotransferase (AST) ≥ 3 x ULN
- Total bilirubin > 2 x ULN
- Alkaline phosphatase < 2 x ULN

These subjects meet the conditions of a Hy's Law case, and should be reported in the same manner as an SAE (see Section 11.1).

For Cohort 2, blood samples for hematology and blood chemistry will be obtained on the Screening Visit, which will be used to determine baseline data. A second and final sample will be obtained at the 8 week visit or the Early Termination Visit (if before 8 weeks).

Subjects who do not have an absolute neutrophil count (ANC) result from the Screening visit must have a local or central laboratory result to ensure study eligibility before the first dose of SER-109 is administered.

9.6.2. Urinalysis

For Cohort 1, urine dipstick testing will be performed at the study site according to the schedule in Table 1. If results for nitrates or leukocytes are positive, the urine sample may be sent to the central laboratory for analysis at the discretion of the investigator.

For Cohort 2, urine dipstick testing will no longer be performed. (Table 2)

9.6.3. Pregnancy Testing

For Cohort 1, women of childbearing potential (WOCBP) will have urine pregnancy tests according to the schedule in Table 1.

For Cohort 2, Women of childbearing potential (WOCBP) will have urine pregnancy tests at the Screening Visit and at the 8 week visit or the Early Termination Visit (if before 8 weeks) according to the schedule in Table 12.

9.7. Stool Sample Collection and Analysis

Cohort 1, subjects will be asked to collect stool at home or in the clinic according to the schedule in Table 1. The sample collected on Day -1 may be brought to the study site for the Day 1 Visit. If the subject is unable to bring the stool sample to the study site for any visit, arrangements may be made to pick up the sample at the subject's home and bring it to the study site or may ship directly to the central laboratory (i.e., home visit by nurse or courier). Samples brought to the study site will be processed and then shipped to a central laboratory according to procedures defined in the Laboratory Manual. Stool collection kits will be provided to the subjects by the study sites.

Microbiome and metabolomics testing may be performed on some or all stool samples collected on Day -1 (prior to administering the bowel cleanse), at Week 1, Week 2, Week 8, Week 24 (for microbiome testing only), and at the Early Termination Visit or any Recurrence Visit to the study site, if applicable.

If recurrent CDI is suspected, to inform subject care, a *C. difficile* test can be performed by a CLIA-certified local laboratory using an FDA-approved test in order to inform subject treatment. Stool samples collected for suspected CDI recurrence must also be processed and shipped to a central laboratory for *C. difficile* stool testing (see Laboratory Manual for details of *C. difficile* testing performed at the central laboratory). The subject should not initiate antibiotics for suspected CDI prior to providing a stool sample for the central laboratory stool testing. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon their assessment.

For Cohort 2, subjects will be asked to collect stool prior to the bowel cleanse on Day -1. The sample collected on Day -1 may be brought to the study site for the Day 1 Visit. A subgroup of subjects who consent will collect a stool specimen at Week 1 and will make arrangements to return it to the clinic or ship directly to the central laboratory. Microbiome and metabolomics testing may be performed on some or all stool samples collected on Day -1 (prior to administering the bowel cleanse), and at Week 1.

The subjects will be instructed to call the study site if diarrhea develops. If recurrent CDI is suspected, stool sample must be collected and shipped to central laboratory for *C. difficile* stool testing (see Laboratory Manual for details of *C. difficile* testing performed at the central laboratory). The subject should not initiate antibiotics for suspected CDI prior to providing a stool sample for the central laboratory stool testing. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), will be used for the primary endpoint analysis. The central laboratory result will be communicated to the investigator and the decision to treat with antibiotics will be based upon their assessment.

9.8. Biological Specimen Collection for Future Biomedical Research

The sponsor may conduct future biomedical research on specimens (including serum and stool) routinely and specifically collected during this clinical study for potential commercial use by Seres Therapeutics, Inc. and specimens may be stored for up to 10 years.

9.9. Monitoring of Diarrheal Symptoms and General Health

Cohort 1 subjects will be instructed to complete a daily diarrhea log (see Investigator Site File) every day whether or not they experience diarrhea. At all scheduled telephone calls and study site visits, subjects will be queried regarding general well-being; AEs; diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea; and concomitant medications according to a standardized questionnaire. Any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day lasting ≥ 2 consecutive days) will be asked to come in for an in-clinic visit, where possible, for a *C. difficile* stool toxin test and evaluation for recurrence of CDI (see Section 10.3.3).

Cohort 2: at all scheduled telephone calls and clinic or home visits, subjects will be queried regarding general health; AEs; diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea; and concomitant medications according to a standardized questionnaire. Any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day lasting ≥ 2 consecutive days) will be asked to arrange for an in-clinic or home visit for a *C. difficile* stool toxin test (to be sent to central laboratory) and evaluation for recurrence of CDI.

9.10. Health Outcome Assessment

Information such as mortality from any cause, hospitalizations, and hospital length of stay (in days), including days in the intensive care unit, will be collected as part of the health outcomes assessment throughout this study.

9.11. Quality of Life Assessment

The EQ-5D-5L is a standardized measure of health status. The CDiff32 HRQoL is a newly developed and validated health-related quality of life questionnaire specific to patients with CDI (Garey et al, 2016). For Cohort 1 only, administer the EQ-5D-5L and Cdiff32 HRQoL at the time points indicated in the Schedule of Assessments (Table 1).

For Cohort 2, the EQ-5D-5L visual analog scale will be completed using a paper questionnaire at Screening and Week 8. In addition, subjects will be asked to complete a Bowel Cleanse Patient Satisfaction Survey Measure at Week 8 on the bowel cleanse procedure administered prior to SER-109. This survey is modified from one that has previously been validated in subjects undergoing colonoscopy (Hatoun 2016). This questionnaire has been minimally modified as shown below.

	Original for Colonoscopy Prep	**SER-109**	**Responses**
1	How easy or difficult was it to consume the study drug?	How easy or difficult was it to consume the bowel cleanse?	Very easy Easy Tolerable Difficult Very difficult
2	Were you able to consume the entire preparation as instructed?	Were you able to consume the entire bowel cleanse as instructed?	Yes No

3	Please describe your overall experience of the study preparation:	Please describe your overall experience of the bowel cleanse:	Excellent Goodc Fair Poor Bad
4	The taste of this study preparation was:	The taste of this bowel cleanse was:	Excellent Good Tolerable Poor Bad
5	Would you ask your doctor for this preparation again if you needed another colonoscopy in the future?	Would you take this bowel cleanse again if you needed to take the study drug, SER-109, in the future?	Yes No
6	Would you refuse the same preparation again if it were to be prescribed to you in the future?	Would you refuse the bowel cleanse again if it were to be prescribed to you in the future?	Yes No

9.12. Clinical Response Evaluation

Recurrence of CDI will be determined by the investigator based on the following definition:

- A CDI episode is defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* stool test on a stool sample determined by a toxin assay and a decision by the investigator, based on clinical assessment, that antibiotic treatment is needed.

If subjects experience diarrhea symptoms (≥ 3 unformed stools per day for 2 consecutive days) or suspect a CDI episode, they should contact the investigator immediately (including on weekends) to arrange a Recurrence Visit for clinical evaluation and a *C. difficile* stool toxin test (central laboratory). The subject should not initiate antibiotic treatment for suspected CDI until instructed to do so by the investigator.

10. STUDY SCHEDULE

The Schedule of Assessments and Procedures is presented in Table 1 and Table 2. Study days are relative to the oral administration of the first dose of study drug on Day 1. Assessments will be performed and noted in each subject's chart or record. In Cohort 1, as necessary for safety of subject, study visits including *Week 2, Week 8, Unscheduled, and Early Termination,* may be conducted at subject's home, with qualified nurse or site personnel who will be appropriately documented on the site's delegation log to perform these Remote Study Visits and associated procedures. If a nurse or site personnel cannot perform the visits, a telephone call or a video conference (Zoom, Skype, FaceTime, etc.) should be conducted in place of the Week 2, Week 8, Unscheduled, and Recurrence / Early Termination visits and must be documented in the source. Any required procedures not performed during a remote visit at the subject's home will be documented as a protocol deviation by site staff. The option of additional home visits was added

to maintain follow-up when subjects were not able to be seen in the clinic for planned study visits due to COVID-19.

In Cohort 2, the Week 8, early termination, and recurrence visits may all be conducted at home or in the clinic. If a nurse or site personnel cannot perform the visits due to COVID-19, a telephone call or a video conference (Zoom, Skype, FaceTime, etc.) should be conducted in place of the visits and must be documented in the source. Any required procedures not performed during a remote visit at the subject's home will be documented as a protocol deviation by site staff.

10.1. Screening Period

For Cohort 2, subjects with symptoms suggestive of RCDI may submit a stool sample for CDI toxin or PCR testing at a Clinical Laboratory Improvement Amendment (CLIA)- certified local laboratory or the central laboratory with a prescreening or screening consent. Stool samples submitted to the central lab for screening will be tested via PCR. ..Subjects who were previously test negative who have a new episode may repeat pre-screening or screening using a new consent and study number.

10.1.1. Clinic Visit (Day -24 to Day -2)

For Cohort 1, screening for this study begins at the Recurrence Visit of Study SERES-012. Assessments performed at the Recurrence Visit in Study SERES-012 do not need to be repeated if performed within the SERES-013 screening window (Day -24 to Day -2) and from the central laboratory of study SERES-012. Cohort 2 subjects will undergo all screening procedures and assessments, with the exception of urine dipstick.

- After a full explanation of the study protocol, have each subject or their legally authorized representative sign an informed consent form (ICF) before performing any study-related activity (including Screening activities).
- Subjects will receive 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg BID]. For Cohort 2, subjects will receive 10 to 42 days of vancomycin or 10 to 25 days of fidaxomicin (200 mg). On Day -1, within 3 days of completion of antibiotic treatment for their CDI, subjects will undergo a bowel cleanse by consuming 10 oz. (~300 mL) of oral magnesium citrate followed by overnight fasting in Cohort 1 only. Cohort 2 will not require fasting after bowel cleanse. Subjects with impaired kidney function who are unable to take magnesium citrate will take 250 mL of GoLytely (polyethylene glycol electrolyte solution).
- Assess each patient to ensure all inclusion criteria are met and no exclusion criteria are met.
- Update medical history. Ensure documentation of most recent CDI episode to include dates, test results, duration, and antibiotic treatment received.
- Perform a physical examination including vital sign measurements (blood pressure, pulse, respiratory rate, and body temperature), and weight.

- For Cohort 2 only, subjects will complete EuroQol 5 Dimension 5 Level (EQ-5D-5L) Visual Analog Scale using a paper questionnaire
- Provide stool collection kits.
- Collect blood and urine samples and ship to the central laboratory for evaluation of:
  - Blood chemistry
  - Hematology
  - Urine dipstick performed at the study site; if positive for nitrates and/or leukocytes, sample may be sent to central laboratory for analysis at the investigator's discretion (Cohort 1 only)
  - Urine pregnancy test, if applicable

    Note: Laboratory values obtained during the screening window and from the central laboratory of Study SERES-012 (e.g., SERES-012 Week 8 visit) do not need to be repeated at the Screening Visit. The SERES-012 Recurrence Visit assessments may be used for the SERES-013 Screening Visit. However, if the Recurrence Visit occurred outside the SERES-013 screening window (Day -24 to Day -2), the required screening procedures for 013 must be repeated within the screening window (Refer to Table 1: Schedule of Assessments and Procedures).
- Obtain information regarding prior medication use within the 8 weeks before anticipated enrollment as well as concomitant medications. Note: For Cohort 1, Comprehensive prior medication and concomitant medicine information from SERES-012 study can be used for this purpose.
- Ensure documentation of recent antibiotic or immunosuppressive medication use that may affect eligibility in the study.
- Assess AEs for Cohort 1 only.
- Register subject in IWRS.
- Instruct subjects that, should they enroll in the study, they will need to meet the following requirements:
  - Probiotic use is prohibited during the study.
  - If currently experiencing an active CDI:
    - On Day -4 to Day -2, subject should take their last dose of antibiotic treatment for their CDI.
    - On Day -1, before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel preparation, they should collect a stool sample.
  - Subjects are to contact the investigator immediately (including on weekends) if they experience diarrheal symptoms or suspect a CDI episode to arrange a Recurrence Visit (see Section 10.3.3) for clinical evaluation and a *C. difficile* stool toxin testing at the Central Laboratory. Advise subjects that antibiotic treatment should be

initiated only after a positive *C. difficile* test, and clinical assessment by the investigator.

10.1.2. Pre-treatment Preparation Phone Call Visit (Day -4 to -2)

Contact subject by phone to:

- Perform diarrhea assessment to ensure that subject's diarrhea has been controlled (< 3 unformed stools per day for 2 consecutive days).
- Assess AEs for Cohort 1 only.
- Review concomitant medications.
- Remind subject to not take antibiotics beyond Day -2.
- For both cohorts, remind subject to collect a stool sample at Day -1. For those required to administer a bowel cleanse, instruct the subject to collect the sample before beginning the magnesium citrate or GoLytely (polyethylene glycol electrolyte solution) bowel cleanse on Day -1.

10.1.3. Pre-treatment Preparation Phone Call Visit (Day -1)

Contact subject by phone to:

- Ensure all inclusion criteria continue to be met and no exclusion criteria are met, including that subject's CDI has responded to antibiotics without diarrhea over the previous 2 days (< 3 unformed stools per day).
- Ensure subject has discontinued antibiotics to control CDI symptoms, and has had their last dose of antibiotic on any day from Day- 4 to Day -2.
  - Remind subject to collect a stool sample. Instruct the subject to collect the sample before beginning the magnesium citrate or GoLytely bowel cleanse.
  - Ensure subject consumes a 10 oz (~300 mL) bottle of magnesium citrate (or, for subjects with impaired renal function, 250 mL of GoLytely and is prepared to fast overnight (no food or drink other than small amounts of water for ≥8 hours) before anticipated receipt of study drug.
- Assess AEs.
- Review concomitant medications.
- Remind subject to bring their Day -1 stool sample collected at home to the study site on Day 1 to be processed for shipment to the central laboratory.
- Remind subjects in Cohort 1 to bring their SERES-012 electronic diary device to the site at their Day 1 visit so it can be collected and a SERES-013 device can be dispensed. An electronic diary will not be used for subjects in Cohort 2.

10.2. Efficacy Period

10.2.1. Clinic Visit (Day 1)

10.2.1.1. Before Administering Study Drug (Pre-dose)

Both Cohort 1 and 2

- Assess subject to ensure all inclusion criteria are met and no exclusion criteria are met. Ensure that subject's CDI has responded to antibiotics without diarrhea for the previous 2 days (< 3 unformed stools per day).
- Review concomitant medications and update information regarding prior medication use. Confirm that subject took their last dose of antibiotic treatment for their CDI on any day from Day -4 to Day -2.
- Ensure subject consumed a 10 oz (~300 mL) bottle of magnesium citrate or 250 mL of GoLytely on Day -1.
- Obtain stool sample from subject's Day -1 at home collection and process, store, and ship per Laboratory Manual.
- Perform a focused history and physical exam. including vital sign measurements (i.e., blood pressure, pulse, respiratory rate, and body temperature
- Urine Pregnancy test, if applicable
- Access the IxRS to obtain the bottle number of SER-109
- Provide stool collection kit

For Cohort 1,

- Ensure subject is undergoing a fast (no food or drink other than small amounts of water) for ≥ 8 hours before anticipated receipt of study drug Collect blood and urine samples for the following laboratory tests:
  - Blood chemistry
  - Hematology
  - Serum for future biomedical research
  - Urine dipstick performed at the study site; if positive for nitrates and/or leukocytes, the sample may be sent to central laboratory for analysis at the discretion of the investigator
- Assess AEs.
- Administer the EQ-5D-5L and CDiff32 questionnaires.
- Collect SERES-012 diarrhea diary device and dispense SERES-013 device with instructions to continue completing the diary daily through week 24.

For Cohort 2 subjects:

- Subjects will not have to undergo a fast prior to study dose

- For subgroup of subjects who consent in Cohort 2, remind subjects to courier or bring their Week 1 stool specimen to the clinic. Provide stool collection kit to these subjects.

10.2.1.2. Administering Study Drug

On Day 1, administer 4 study drug capsules orally with at least 8 oz of water (capsules are to be swallowed, not chewed).

10.2.1.3. After Administering Study Drug (Post-dose):

- Observe subject in the clinic for ≥ 60 minutes.
- Assess vital sign measurements (i.e., blood pressure, pulse, respiratory rate, and oral body temperature) approximately 30 minutes after dosing.
- Assess AEs.
- Provide subject with stool collection kits.
- Provide specific instructions and a reminder card on reporting and follow-up of symptoms including diarrhea and abdominal discomfort, collection of samples, reporting of any concerns, and, in particular, notification of the investigator of the occurrence of diarrhea.
- Ensure subject continues to fast for a total of 1 hour after dosing (post-dose).(Cohort 1 only; no fasting required in Cohort 2)
- Dispense a 2-day supply of study drug to subjects with instructions for proper storage and home-administration on Day 2 and Day 3.
- Release subject from the clinic upon authorization by the investigator.
- Review instructions with subject for recording episodes of diarrhea (See Investigator Site Manual).
- Investigators should manage subjects' expectations, they may have diarrhea early-on after receiving drug in the study.

10.2.2. Phone Call Visit (Day 2)

Contact subject by phone to:

- For cohort 1, confirm administration of 2nd dose (4 capsules) of study drug in the morning before breakfast For cohort 2, confirm administration of 2nd dose (4 capsules) of study drug in the morning. If subject has not yet taken study drug, remind subject to take study drug as soon as possible.
- Inquire about general health.
- Perform diarrhea assessment:
  - Remind subjects they may have diarrhea early-on after receiving drug in the study.
  - Remind subjects to complete the diarrhea log/device (see Investigator Site File).(Cohort 1 only)

- Assess AEs.
- Review concomitant medications.

10.2.3. Phone Call Visit (Day 3)

Contact subject by phone to:

- Confirm administration of 3rd dose (4 capsules) of study drug in the morning before breakfast (fasting for Cohort 1 only).For Cohort 2, confirm administration of 2nd dose (4 capsules) of study drug in the morning. If subject has not yet taken study drug, remind subject to take study drug as soon as possible.
- Inquire about general health.
- Perform diarrhea assessment:
  - Remind subjects they may have diarrhea early-on after receiving drug in the study.
  - Remind subjects to complete the diarrhea log/device (see Investigator Site File).(Cohort 1 only)
- Assess AEs.
- Review concomitant medications.

For Cohort 1,

- Remind subject to bring their Week 1 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site.

For Cohort 2

- A subgroup of subjects who consent will collect a stool specimen at Week 1 and will make arrangements to return it to the clinic or ship directly to the central laboratory.

10.2.4. Phone Call Visit (Week 1)

Contact subject by phone at Week 1 (± 2 days).

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 10.3.3).
- Assess AEs.
- Review concomitant medications.

For Cohort 1,

- The CDiff32 questionnaire will be administered.

- Remind subject to bring their Week 1 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site.

For subgroup of subjects who consent in Cohort 2, remind subjects to courier their Week 1 stool sample collected at home to the study site to be processed for shipment to the central laboratory.

10.2.5. Clinic or Home Visit (Week 2)

For Cohort 1, arrange for a home visit or an in-clinic visit at Week 2 (± 2 days) to:

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 10.3.3).
- Assess AEs.
- Review concomitant medications.
- Collect a serum sample and a stool sample (if ICF for FBMR obtained)

10.2.6. Phone Call Visits (Weeks 3-7)

For Cohort 1, contact subject by phone at Weeks 3-7 (± 2 days).

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, arrange a Recurrence Visit (see Section 10.3.3).
  - Advise subject to continue diarrhea log, and collect stool sample for the Recurrence Visit.
  - Advise subject to not initiate antibiotic treatment for CDI until advised to do so by the study investigator.
- Assess AEs.
- Review concomitant medications.
- Remind subject to bring their Week 8 stool sample collected at home to the study site to be processed for shipment to the central laboratory.

For Cohort 2, Phone call visits will take place from Week 1-7

- For all phone call visits, a standardized script will be used to inquire about general health, AEs, concomitant medications and to perform a diarrhea assessment. There will be no stool collection for Week 8

10.2.7. End of Efficacy Period Clinic Visit (Week 8)

Subjects will be seen in the clinic at the study site or at a home visit at Week 8 (± 2 days). The home visit option for Cohort 1 is only during COVID-19 period, but for Cohort 2 is option throughout study.

- Obtain stool sample from subject's Week 8 at home collection and process, store, and ship per Laboratory Manual (Cohort 1 only).
- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 10.3.3).
- Assess AEs.
- Review concomitant medications.
- Perform a focused history and physical exam.
- Collect blood and urine samples for the following laboratory tests:
  - Blood chemistry
  - Hematology
  - Urine pregnancy test, if applicable
- The EQ-5D-5L and CDiff32 questionnaires will be administered. (for Cohort 1 only)
- The EQ-5D-5L VAS using a paper questionnaire (for Cohort 2 only)
- Bowel Cleanse Patient Satisfaction Survey Measure using a paper questionnaire (for Cohort 2 only)
- Provide subject with stool collection kits as necessary.
- Perform Drug Accountability

10.3. Follow-up Period

10.3.1. Phone Call Visits (Every 4 Weeks)

Contact subject by phone at Weeks 12, 16 and 20 (± 3 days) to:

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, arrange a Recurrence Visit (For Cohort 1) (see Section 10.3.3)

  - Advise subject to continue diarrhea log, and collect stool sample for the Recurrence Visit
  - Advise subject not to initiate antibiotic treatment for CDI until advised to do so by the study investigator.
- Assess AEs.
- Review concomitant medications.
- Remind subject to bring their Week 24 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site (For Cohort 1).

10.3.2. Phone Call Visit - Study Completion (Week 24)

Contact subject by phone at Week 24 (± 3 days) to:

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI, obtain information regarding the day, frequency, and quality of bowel movements during diarrheal episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. Complete Recurrence Visit assessments (see Section 10.3.3).
- Assess AEs.
- Review concomitant medications.
- The EQ-5D-5L questionnaire will be administered. (to be completed in Cohort 1 only)
- Remind subject to bring their Week 24 stool sample collected at home to the study site to be processed for shipment to the central laboratory or make arrangements for a courier for delivery to the study site (Cohort 1 only)
- Remind subject to either send their diarrhea device to the study site with the courier or bring it with them when they drop off their Week 24 stool sample. (Cohort 1 only)

10.3.3. Recurrence and Early Termination (ET) Visits

For Cohort 1, any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day over 2 consecutive days) will be asked to contact the investigator and return to the clinic or have a home visit for a *C. difficile* stool test and evaluation for recurrence of CDI. Additionally, all subjects will be seen in the clinic or have a home visit if the subject withdraws early from the study, whenever possible. These home visits are permitted only during COVID-19.

Perform the following assessments and procedures:

- Obtain stool sample from subject's at-home collection and process, store, and ship sample per Laboratory Manual.
- Perform diarrhea assessment:

  - If subject has a suspected episode of CDI that has not already been reported, obtain information regarding the day, frequency, and quality of bowel movements during diarrhea episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample. To inform subject care, a *C. difficile* test on unformed stool may be performed locally at the study site (see Laboratory Manual); ship stool to the central laboratory for *C. difficile* stool testing (see Laboratory Manual). The subject should not initiate antibiotics for CDI prior to providing a stool sample for the central laboratory stool testing.
  - If the *C. difficile* test on a stool sample determined by a toxin assay is positive and the investigator determines that antibiotic treatment is appropriate per protocol guidelines (see Section 8.7), prescribe standard of care antibiotic regimen to control CDI.
  - Advise subject to continue diarrhea log up until the day of initiation of antibiotic treatment for their CDI.
- Assess AEs.
- Review concomitant medications.
- Perform a focused history and physical exam.
- Collect blood and urine samples for the following laboratory tests:
  - Blood chemistry
  - Hematology
  - Serum for future biomedical research
  - Urine pregnancy test, if applicable
- The EQ-5D-5L and CDiff32 questionnaires will be administered. (*Note: the CDiff32 questionnaire should only be completed for an ET or Recurrence Visit prior to Week 8).

For Cohort 2, any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day over 2 consecutive days) will be asked to contact the investigator and return to the clinic or have a home visit for a *C. difficile* stool test and evaluation for recurrence of CDI. Additionally, all subjects will be seen at home or in the clinic if the subject withdraws early from the study, whenever possible.

- Perform diarrhea assessment:
  - If subject has a suspected episode of CDI that has not already been reported, obtain information regarding the day, frequency, and quality of bowel movements during diarrhea episodes.
  - If subject reports episode(s) of diarrhea (≥ 3 unformed stools per day) lasting 2 or more consecutive days, obtain stool sample to ship to the central laboratory for *C.*

difficile stool testing (see Laboratory Manual). The subject should not initiate antibiotics for CDI prior to providing a stool sample for the central laboratory stool testing.

  - If the *C. difficile* test on a stool sample determined by a toxin assay is positive and the investigator determines that antibiotic treatment is appropriate per protocol guidelines (see Section 8.7), prescribe standard of care antibiotic regimen to control CDI.

- Assess AEs
- Review concomitant medications.
- Perform a focused history and physical exam
- If termination visit precedes 8 weeks, safety laboratory tests, EQ-5D-5L VAS, and Bowel Cleanse Patient Satisfaction Survey Measure will be performed using a paper questionnaire

11. ASSESSMENT OF SAFETY AND ADVERSE EVENT REPORTING

All AEs, SAEs/AESIs, and concomitant medications will be collected from the time of initiation of study drug up to Week 8. From Week 8 up to Week 24, all SAEs/AESIs and SAE/AESI-related data, and any antibiotic medication and its corresponding indication will be collected.

The investigator is responsible for:

- Informing the sponsor in the event that a subject or a subject's partner becomes pregnant during the study. A "Pregnancy Report Form" will be generated and the pregnancy will be captured in the safety database and will be followed through to the outcome.
- Instructing subjects in the self-reporting of selected AEs including diarrhea and abdominal discomfort.
- Evaluating subject safety including assessment of AEs for seriousness, severity, and causality.
- Informing the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) of AEs as required and SAEs as per IRB/IEC guidelines.

For the purpose of this study, an AE is defined as any untoward medical occurrence in a subject who was administered study drug, regardless of its causal relationship to the study drug. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the study drug, whether or not it is considered related to the study drug.

An SAE is any AE regardless of causality that:

- Results in death.

- Is life threatening. Life threatening means that the subject was at immediate risk of death from the adverse event as it occurred. This does not include an event that, hypothetically had it occurred in a more severe form, it might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization; hospital admissions and/or surgical operations scheduled to occur during the study period, but planned before study entry are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not worsen in any unexpected manner during the study (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a subject's ability to conduct normal life functions.
- Is associated with a congenital anomaly/birth defect.
- Is an important medical event. An important medical event is an event that may not result in death, be life threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition of an SAE. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions that do not result in inpatient hospitalization, and the development of drug dependency or drug abuse.

An adverse event of special interest (AESI) (serious or non-serious) is one of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor is appropriate.

In this protocol, an invasive infection (e.g., bacteremia, abscess, meningitis) is designated as an AESI, and as such, will be reported and followed in the same manner as an SAE during the course of the study.

All AEs, including SAEs and AESIs, will be graded for severity by using common terminology criteria for adverse events' (Common Terminology Criteria for Adverse Events v4.0 (CTCAE) Publish Date: May 28, 2009 with the exception of diarrhea. Criteria for diarrhea severity will be as follows:

- mild: 3-4 unformed bowel movements per day
- moderate: 5-6 unformed bowel movements per day
- severe: ≥7 unformed bowel movements per day

In general, the severity of AEs can be assessed using following guidelines:

Severity Description

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living*

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (ADL)**

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. **Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden. Changes in the severity of an AE will be documented, and documentation will include assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent will be documented based on the severity, onset, and duration of each episode.

An abnormal laboratory test finding that meets any of the criteria below will be considered an AE:

- Is associated with accompanying symptoms;
- Requires additional diagnostic testing or medical/surgical intervention;
- Leads to a concomitant drug treatment or any change in a concomitant medication or therapy;
- Is considered an AE by the investigator.

Laboratory results that fall outside the reference range and do not meet one of the criteria above will not be reported as AEs. Repeating a test because of an abnormal result, in the absence of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error will not be reported as an AE.

For all AEs, including SAEs, the investigator will report on the relationship of the AE to the study drug by using the following definitions:

- **Unrelated**: There is little or no chance that the study drug caused the AE; other conditions, including concurrent illnesses, progression or expression of the disease state, or a reaction to a concomitant medication best explain the event
- **Related** or **Possibly Related**: The association of the AE with the study drug is unknown; however, the AE is not clearly due to another condition, or a reasonable temporal association exists between the AE and treatment administration and, based on the investigator's clinical experience, the association of the AE with the study drug seems likely

Adverse events, including local and systemic reactions not considered medically serious, will be recorded. Information to be collected includes event description, time of onset, investigator assessment of severity, relationship to study drug, date of resolution of the event, seriousness, and outcome. Additionally, serious criteria will be collected for all SAEs.

Any medical condition that is present at the time that the subject is screened will be considered as a baseline condition and not be reported as an AE. However, if it worsens at any time during the study, it should be recorded as an AE.

With regards to events of diarrhea, diarrhea that meets the protocol definition of CDI recurrence (≥ 3 unformed stools per day over 2 or more consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, a positive *C. difficile* test on

a stool sample determined by a toxin assay, and assessment by the investigator that treatment is required) should NOT be entered as an AE. Events of diarrhea that are not associated with CDI recurrence (e.g., due to food poisoning or flu), should be reported as an AE (e.g., Diarrhea [Not CDI related]). Other symptoms associated with CDI recurrence, e.g. abdominal pain, abdominal distension, should be reported as adverse events.

When CDI recurrence is deemed serious due to hospitalization, CDI recurrence should be included as an SAE term and recorded as the reason for hospitalization in the Hospitalization CRF page.

11.1. Serious Adverse Event Reporting

The sponsor has requirements for expedited reporting of SAEs meeting specific criteria to worldwide regulatory authorities. Therefore, the sponsor (or sponsor's designee) must be notified immediately regarding any SAE that occurs after administration of the study drug.

All SAEs must be reported to the medical monitor within 24 hours of knowledge of the event at the study site. Refer to the Investigator Site File for detailed instructions.

The study site will transmit an SAE report (SAER) to the sponsor or sponsor's designee by facsimile or email. The study site will be provided with SAER forms wherein the following information is requested:

- Subject identification, investigator name, and study site number
- SAE information: event term, onset date, severity, and causal relationship to study drug
- The outcomes attributable to the event (i.e. serious criteria) (e.g., death, life threatening, inpatient hospitalization, prolongation of existing hospitalization, a congenital anomaly, a persistent or significant disability or incapacity, or other important medical event)
- A summary of relevant test results, pertinent laboratory data, and any other relevant medical history
- The date of study drug administration
- Whether or not the study drug was discontinued
- Supplemental information, which may include the following hospital records: laboratory results, radiology reports, progress notes, admission and emergency room notes, holding and observation notes, discharge summaries, autopsy reports, and death certificates

In addition, relevant eCRF pages should be appended to communicate relevant study drug and subject outcome information.

The SAER should be faxed or emailed within 24 hours with as much of the above information as available at the time. The following minimum information is required for an initial SAE report: subject identification, reporting source (i.e., Site Name and Site Number), and an event or outcome. Supplemental information may be transmitted by using a follow-up report and should not delay the initial report. The sponsor may contact the study site to solicit additional information or follow-up on the event.

The investigator must take all therapeutic measures necessary for resolution of the SAE. Any medications or procedures necessary for treatment of the SAE must be recorded in the appropriate pages of the subject's eCRF.

12. STATISTICAL METHODS

12.1. STUDY ENDPOINTS

12.1.1. Primary Efficacy Endpoint

For Cohort 1, recurrence of CDI or sustained clinical response as determined by a toxin assay up to 8 weeks after initiation of treatment is the primary efficacy endpoint. A recurrence is defined as ≥ 3 unformed stools per day for 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with a positive *C. difficile* test on a stool sample determined by a toxin assay, and assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment.

For Cohort 2, recurrence of CDI or sustained clinical response as determined by a toxin assay up to 8 and 12 weeks after initiation of treatment are the efficacy endpoints.

12.1.2. Secondary Efficacy Endpoints

For Cohort 1, secondary efficacy endpoints are the following:

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay
- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

For Cohort 2, secondary efficacy endpoints are not being sought.

12.1.3. Exploratory Efficacy Endpoints

For Cohort 1, exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment

- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 24 weeks after treatment initiation (for subjects hospitalized)
- Changes from Baseline in Health-Related Quality of Life (HRQoL) and health outcomes as assessed by the EQ-5D-5L from Day 1 through Weeks 8 and 24, and assessed by the Cdiff32 HRQoL from Day 1 to Week 1 and Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

For Cohort 2, exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1 week after initiation of treatment in a subgroup of up to approximately 50 subjects
- Change in the fecal metabolome from Baseline up to 1 week after initiation of treatment in a subgroup of up to approximately 50 subjects.
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 24 weeks after treatment initiation (for subjects hospitalized)
- Change in EQ-5D-5L visual analog score from Screening to Week 8 visit after initiation of treatment
- Assess Bowel Cleanse Patient Satisfaction Survey Measure at Week 8

12.1.4. Safety Endpoints

Safety endpoints are the following:

- Incidence of AEs
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

12.2. Analysis Populations

Three analysis populations will be defined:

- Intent-to-Treat (ITT) Analysis Population. The ITT Population will consist of all enrolled subjects.
- Modified Intent-to-Treat (mITT) Analysis Population. The mITT Population will be composed of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.

- Safety Population. The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

12.3. Determination of Sample Size

Approximately 30 subjects are anticipated to enroll from SERES-012 (Cohort 1). The recurrence rates prior to 8 weeks after initiation of study drug assumed in SERES-012 are 16% in SER109 and 36% in placebo. However, it is expected that only a fraction of subjects who recur in SERES-012 prior to 8 weeks will roll-over to this study, since a follow-up of 8 weeks from start of study drug in earlier protocol versions of SERES-012 (up through and including Amendment 5) was required before rolling over to SERES-013, regardless of when the CDI recurrence occurred in SERES-012. Additionally, 200 subjects with RCDI may enroll in the Open-Label program (Cohort 2) into SERES-013. Approximately 230 subjects total will be assessed for safety and tolerability, and efficacy.

12.4. General Statistical Considerations

Descriptive statistics, including the numbers and percentages for dichotomous or categorical variables, and the numbers, means, standard deviations, medians, minimums, and maximums for continuous variables will be provided. Listings of individual subject data will be produced.

All summary tables will be presented based on the following groups: For Cohort 1, 1) Subjects who were randomized to SER-109 in SERES-012, 2) Subjects who were randomized to placebo in SERES-012, 3) Overall (groups 1 and 2 combined). For Safety and Tolerability and Primary Efficacy, summary tables will include an additional fourth group consisting of Cohort 2 . The safety summary will be presented combining all subjects (pooling cohort 1 and cohort 2).

A comprehensive statistical analysis plan (SAP) will be submitted to regulatory authorities.

12.5. Subject Population and Baseline Characteristics

Enrollment, protocol deviations, and discontinuations from the study will be summarized by group for the ITT Population. Demographics (e.g., age, race, ethnicity, sex), baseline characteristics (e.g., weight,), medical history, and other baseline characteristics will be summarized by group for the Safety, ITT and mITT Populations.

12.6. Study Drug Exposure

SER-109 exposure will be summarized as the total number of capsules taken with counts and percentages of subjects by group. The summary will be presented for the Safety, ITT, and mITT Populations.

12.7. Efficacy Analysis

12.7.1. Primary Efficacy Analysis

For Cohort 1, the primary efficacy endpoint is the recurrence of CDI up to Week 8 in the ITT Population. Subjects will be categorized as having favorable (no CDI recurrence or sustained clinical response) or unfavorable outcomes (had CDI recurrence). The number and percentage of

subjects defined as having favorable (sustained clinical response) and unfavorable (had CDI recurrence) outcomes will be reported with exact 95% confidence intervals (CIs) for each group. The CIs will be derived using the Clopper-Pearson exact method (Clopper and Pearson, 1934). Subjects who are lost to follow-up, terminated the trial early, or died without a recorded recurrence of CDI before Week 8 will be defined as having an unfavorable outcome for the primary analysis. Subjects who miss any contact with the site (phone calls or the Week 2 visit) before Week 8 but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent contact will be defined as having a favorable outcome (or sustained clinical response) for the primary analysis. Additional rules for imputing CDI recurrence status for subjects with at least one component of the CDI recurrence endpoint criteria missing will be provided in the SAP.

For Cohort 2. the efficacy endpoint is the recurrence of CDI up to Week 8 or 12 in the ITT Population. Subjects will be categorized as having favorable (no CDI recurrence or sustained clinical response) or unfavorable outcomes (had CDI recurrence). The number and percentage of subjects defined as having favorable (sustained clinical response) and unfavorable (had CDI recurrence) outcomes will be reported with exact 95% confidence intervals (CIs) for each group. The CIs will be derived using the Clopper-Pearson exact method (Clopper and Pearson, 1934). For the Week 8 analysis, subjects who are lost to follow-up, terminated the trial early, or died without a recorded recurrence of CDI before Week 8 will be defined as having an unfavorable outcome for the efficacy analysis. Subjects who miss any contact with the site (phone calls or the Week 2 visit) before Week 8 but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent contact will be defined as having a favorable outcome (or sustained clinical response) for the analysis. For the Week 12 analysis, subjects who are lost to follow-up, terminated the trial early, or died without a recorded recurrence of CDI before Week 12 will be defined as having an unfavorable outcome for the efficacy analysis. Subjects who miss any contact with the site (phone calls or the Week 2 visit) before Week 12 but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent contact will be defined as having a favorable outcome (or sustained clinical response) for the analysis. Additional rules for imputing CDI recurrence status for subjects with at least one component of the CDI recurrence endpoint criteria missing will be provided in the SAP.

12.7.2. Sensitivity Analysis for the Primary Efficacy Endpoint

Sensitivity analyses of the primary efficacy endpoint as described in Section 12.7.1 will also be conducted as follows:

- For Cohort 1, all subjects who are lost to follow-up, terminated the study prematurely, or died without having a CDI recurrence by Week 8, will be considered to have a favorable outcome (sustained clinical response). Subjects who missed any contact with the site before Week 8 (phone calls or the Week 2 visit) but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent contact will continue to be defined as having a favorable outcome (sustained clinical response) for this analysis, which will be conducted in the ITT Population
- For Cohort 2, all subjects who are lost to follow-up, terminated the study prematurely, or died without having a CDI recurrence by Week 8 or 12, will be considered to have a favorable outcome (sustained clinical response) in the two analyses.

The primary efficacy outcome will also be analyzed for the mITT Population.

12.7.3. Secondary Efficacy Analysis (applies to Cohort 1 only)

Time to recurrence of CDI will be summarized by group for the ITT and mITT Populations using the median, 25th and 75th percentiles from a Kaplan Meier- analysis. The 95% CI for the median will also be provided. Subjects who complete the follow-up period and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminated the study prematurely before experiencing a CDI recurrence will be censored in the analysis on the date of last contact. Subjects who die before having a CDI recurrence will be censored on the date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Subjects who were not dosed will have their time to recurrence measured from their enrollment date.

12.8. Exploratory Analysis

The exploratory endpoints will be summarized descriptively for the mITT populations. Mean, median, standard deviation, maximum and minimum will be presented for continuous variable. Count, Percentage and total will be presented for categorical variables. More details of the exploratory efficacy endpoints will be specified in the SAP.

12.9. Safety Analysis

All safety analyses will be conducted in the Safety Population. All safety summary tables will be presented for the following groups: 1) Cohort 1 2) Cohort 2 3) all subjects pooling Cohorts 1 and 2. Any additional analyses will be detailed in the SAP.

Adverse events will be coded by using the Medical Dictionary for Regulatory Activities (MedDRA). Summary tables of TEAEs will be provided. A TEAE is any AE that newly appeared, increased in frequency, or worsened in severity after initiation of SER-109. A listing of all AEs, including those occurring before the start of study drug, will be provided. The percentage of patients with TEAEs will be tabulated by system organ class and preferred term on Day 10, Week 2, Week 8 and Week 24. The incidence of TEAEs by system organ class and preferred term, by severity, and by relationship to treatment on Day 10, Week 2, Week 8 and Week 24 will also be presented. Tables of any TEAE leading to SER-109 discontinuation and SAEs will also be provided.

An additional analysis will determine the incidence of TEAEs based on the number of days the patients were followed before receiving antibiotics for treatment of recurrent CDI. The incidence of TEAEs per patient for days before receiving antibiotics will be tabulated by system organ class and preferred term, and by severity and relationship to treatment.

Descriptive statistics of the laboratory parameters and vital sign measurements will be presented for all study visits at which they were collected. The change from Baseline to each post-baseline visit and to the overall worst post-baseline value will also be summarized. Laboratory parameters will be defined, as within or outside normal limits, and shift tables from baseline to each postbaseline visit will be provided.

12.10. Handling of Missing Data

Every effort will be made to collect all data at specified times, according to the schedule of study events.

For the primary endpoint in Cohort 1, subjects who are lost-to-follow-up, terminated from the study prematurely, or died without a CDI recurrence before 8 weeks after treatment are defined as having an unfavorable outcome for the primary analysis. Subjects who miss any contact with the site before Week 8 (phone calls or Week 2 visit) but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent telephone contact or by Week 8 will be defined as having a favorable outcome for the primary analysis. If the Week 8 visit is missed, a subject will be considered as having an unfavorable outcome for the primary analysis if he reports 2 or more consecutive days with ≥ 3 unformed stools at the next unmissed telephone contact or visit. If any of the 3 components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then an unfavorable outcome for the primary analysis is imputed. However, if some of the 3 components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a favorable outcome for the primary analysis is imputed. The imputed recurrence status for subjects who have at least one component of the CDI recurrence endpoint criteria missing will be detailed in the SAP.

For the efficacy endpoints determined at 2 timepoints in Cohort 2, subjects who are lost-to-follow-up, terminated from the study prematurely, or died without a CDI recurrence before 8 (or 12) weeks after treatment are defined as having an unfavorable outcome for the primary analysis. Subjects who miss any contact with the site before Week 8 (or 12) (phone calls) but who do not report 2 or more consecutive days with ≥ 3 unformed stools at the subsequent telephone contact or by Week 8 (or 12) will be defined as having a favorable outcome for the primary analysis. If the Week 8 (or 12) visit is missed, a subject will be considered as having an unfavorable outcome for the primary analysis if he reports 2 or more consecutive days with ≥ 3 unformed stools at the next unmissed telephone contact or visit. If any of the 3 components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then an unfavorable outcome for the primary analysis is imputed.

Missing data for the time to CDI recurrence analyses will be handled with censoring by the Kaplan-Meier method. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminate the trial prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before experiencing a CDI recurrence will be censored on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Sensitivity analyses of the time to recurrence endpoint will be provided in the SAP.

No other imputations for missing data will be made (except as detailed in the SAP for missing dates and times).

13. ADMINISTRATIVE REQUIREMENTS

13.1. Good Clinical Practice

This study will be conducted in accordance with the International Conference on Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) and the appropriate regulatory requirements. The investigator will be thoroughly familiar with the appropriate use of the investigational product. Essential clinical documents will be maintained to demonstrate the validity of the study and integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

- The principal investigator has the overall responsibility for the conduct and administration of the study at the study site and for contacts with the sponsor, the IRB/IEC, and local authorities.
- The principal investigator is responsible for ensuring the privacy, health, and welfare of the subjects during and after the clinical study.
- All investigators are responsible for performing the study in accordance with the protocol and the above guidelines and regulations, and for collecting, documenting, and reporting the data accurately.
- All investigators must be familiar with the background and requirements of the study and with the properties of the investigational product as described in the current version of the Investigator's Brochure.
- The principal investigator is responsible for distributing study information and documentation to all appropriate staff members before and during the course of the study as updated information becomes available.

13.2. Trial Governance and Oversight

This study was developed in collaboration with a Clinical Advisory Committee, which comprises both sponsor-employed and independent scientific experts who provide input on study design, interpretation of study results, and subsequent peer reviewed scientific publications.

13.3. Ethical Considerations

This study will be conducted in accordance with ethical principles in the Belmont Report, and in compliance with local IRB/IEC requirements and institutional guidelines.

The investigator must obtain IRB/IEC approval of the protocol, ICF, and other required study documentation before starting the study. It is the responsibility of the investigator to ensure that all aspects of IRB/IEC review are conducted in accordance with current governmental regulations.

A progress report must be submitted to the IRB/IEC at the required intervals and not less than annually. At the completion or termination of the study, the investigator must submit a closeout letter to the IRB/IEC.

13.4. Subject Information and Informed Consent

Before any testing under this protocol, including screening tests and assessments, written informed consent with the IRB/IEC approved ICF must be obtained from the subject or their legally authorized representative (LAR) in accordance with local practice and regulations.

For Cohort 2, a prescreen consent will be available for testing the stool for *C. difficile* toxin or PCR assay either at the local or central laboratory. The central laboratory will test with the PCR assay for eligibility.

The background of the proposed study, procedures, and benefits and risks of the study must be explained to the subject or LAR. The subject or LAR must be given sufficient time to consider whether to participate in the study.

A copy of the ICF, signed and dated by the subject or LAR, must be given to the subject or LAR. Each ICF should contain an authorization allowing the investigator to use and disclose subject health information (i.e., subject identifiable health information) in compliance with local law.

13.5. Subject Confidentiality

Subject confidentiality is held strictly in trust by the investigator and medical and laboratory staff. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating subjects. The investigator will grant a regulatory authority access to the subject's original medical records for verification of data gathered, and to audit the data collection process. The subjects' and donors' confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Subjects will not be identified by name in any study reports, and these reports will be used for research purposes only.

13.6. Protocol Compliance

The investigator will conduct the study in compliance with the IRB/IEC approved protocol without any changes or deviations. Modifications to the protocol will require approval from the sponsor and written IRB/IEC approval before implementation, except when the modification is needed to eliminate an immediate hazard to the subject. Any change, intentional or otherwise, must be reported immediately to the sponsor and to the relevant IRB/IEC and/or regulatory authority as required by guidelines or regulation. Study sites that fail to comply may be terminated.

13.7. Future Use of Stored Specimens

The sponsor may, where permitted by local regulations, conduct future biomedical research on specimens (including serum, and stool) routinely and specifically collected during this clinical study for potential commercial use by Seres Therapeutics, Inc., and specimens may be stored for up to 10 years.

13.8. Study Monitoring

Regular monitoring is defined in ICH Guidance for Industry E6 Good Clinical Practice: Consolidated Guidance, Section 1.38, as "The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, standard

operating procedures, GCP, and the applicable regulatory requirement(s)." The purpose of monitoring is to verify that:

- The rights and well-being of the human subjects are protected.
- The reported study data are accurate, complete, and verifiable from source documents.
- The conduct of the study is in compliance with the currently approved protocol/amendment(s), with GCP, and with the applicable regulatory requirements.

It will be the responsibility of the investigator to ensure that the essential documents are available at the investigator or institutional site. Any or all of these documents may be pertinent to, and should be available for, monitoring by the sponsor or inspection by the regulatory authorities as defined in the monitoring plan.

The sponsor or an authorized sponsor representative will conduct regular study site monitoring visits to review and validate study data as defined in the monitoring plan by reviewing subjects' medical records and eCRFs in accordance with written standard operating procedures, ICH guidelines, GCP, and applicable regulations and guidelines. The investigator will allow representatives of the sponsor or regulatory authorities to inspect facilities and records relevant to this study.

13.9. Case Report Forms and Study Records

Data will be collected for this study by using an eCRF. The investigator and study site staff will receive training and support on the use of the eCRF. All eCRF data are to be completed by the study coordinator or other designated study site personnel. All data entry, modification, or deletion will be recorded automatically in the electronic audit trail. All data changes will be clearly indicated with a means to locate prior values. A unique user identification and password will be assigned to all personnel approved to enter or change data to prevent unauthorized access to the data.

All electronic data entered by the study site (including the electronic audit trail) will be maintained or made available at the study site in compliance with Title 21 Part 11 of the Code of Federal Regulations (CFR) and other applicable retention regulations. The computerized system is able to generate accurate and complete copies of records in paper or electronic form for inspection and review by applicable regulatory authorities, the IRB/IEC/Research Ethics Board, and auditors or other designees authorized by the sponsor.

In addition to capturing the user identification as part of the audit trail for all data entry, the eCRF allows for application of electronic signatures. The investigator or designated sub--investigator, after review of the data in the eCRF, will confirm the validity of each subject's data by electronic signature. This electronic signature will be certified as outlined in 21 CFR Part 11.

The sponsor will retain the original eCRF data and audit trail. An electronic or certified paper copy of all completed eCRF data, including query resolution correspondence, will be provided to the investigator at the end of the study.

13.10. Study Completion

The sponsor requires the following data and materials to be submitted before a study can be considered complete or terminated:

- Laboratory findings, clinical data, and all special test results from the time of informed consent through the End of Study Visit at Week 24
- Electronic CRFs properly completed by appropriate study personnel and signed and dated by the investigator
- Complete study drug accountability records
- Copies of IRB/IEC approval and notification of the original protocol and of any protocol amendments, if appropriate
- A summary of the study prepared by the investigator (an IRB/IEC summary letter is acceptable)

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Statistical Analysis Plan

Protocol Number and Title:	**SERES-013** ECOSPOR IV: An Open-Label Extension of Study SERES-012 Evaluating SER-109 in Adult Subjects with Recurrent *Clostridium difficile* Infection (RCDI)
Protocol Version and Date:	**Amendment 5.0** **9 May 2019**
Author(s):	**Patricia Bernardo, ScD** **Vice President, Biostatistics and Statistical Programming**
SAP Version:	**Version 1.0**
SAP Version Date:	**15 May 2019**

Version: 1.0 Version Date: 15 May 2019

I confirm that I have reviewed this document and agree with the content.

APPROVALS	
Seres Therapeutics	
Patricia Bernardo, ScD Vice President, Biostatistics and Statistical Programming	**Date (dd-Mmm-yyyy)**
Kevin Horgan, MD Executive Vice President and Chief Medical Officer	**Date (dd-Mmm-yyyy)**
Cytel Inc.	
Michael Kutch, M.Sc Director, Biostatistics	**Date (dd-Mmm-yyyy)**

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1. GLOSSARY OF ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical
CDI	*Clostridium difficile* infection
CI	Confidence Interval
cm	Centimeter
CRF	Case Report Form
DSMC	Data and Safety Monitoring Committee
EAIR	Exposure-adjusted Incidence Rate
eCRF	Electronic Case Report Form
EIA	Enzyme Immunoassay
EQ-5D-5L	EuroQol 5 Dimension 5 Level
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
ICF	Informed Consent Form
ICH	International Conference to Harmonisation
ITT	Intent-To-Treat
IXRS	Interactive Voice or Web Response System
kg	Kilogram
K-M	Kaplan-Meier
mg	Milligram
mITT	Modified Intent-to-Treat
mL	Milliliter
mmHg	Millimeters of Mercury
PCR	Polymerase Chain Reaction
PT	Preferred Term
QoL	Quality of Life
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class

Abbreviation	Description
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
TLF	Table, Listing and Figure
UBM	Unformed Bowel Movement
WHO	World Health Organization

2. PURPOSE

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

This SAP is based on SERES-013 Protocol Amendment 5 dated 9 May 2019 and electronic case report form (eCRF) Version 2.0 dated 27 November 2018.

2.1. TIMINGS OF ANALYSES

Unblinded safety and efficacy analyses will be performed after all subjects have completed the 24-week follow-up period, or otherwise terminated from the study.

3. STUDY OBJECTIVES

3.1. PRIMARY EFFICACY OBJECTIVE

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined by a toxin assay, up to 8 weeks after initiation of treatment

3.2. SECONDARY EFFICACY OBJECTIVES

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a PCR algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

3.3. PRIMARY SAFETY OBJECTIVE

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

3.4. EXPLORATORY OBJECTIVES

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109

- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, diagnosis-related group (DRG)-adjusted hospital costs (where available) up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

3.5. BRIEF DESCRIPTION

ECOSPOR IV is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a treatment regimen SER-109 in adult subjects 18 years of age or older with recurrent *Clostridium difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012.

This study will be conducted at approximately 100 study centers in the North America. Subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a treatment regimen of SER 109 or placebo in Study SERES-012, and who have responded to 10 to 21 days of standard-of-care (SOC) antibiotic treatment for CDI (i.e. vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID]) will be eligible to enroll and receive a treatment regimen of SER-109 in Study SERES-013. A treatment regimen of SER-109 is administered orally as 3×10^7 spore colony forming units (SCFUs) in 4 capsules once daily for 3 consecutive days. Approximately 45 eligible subjects with recurrent CDI disease from Study SERES-012 are expected to enroll.

The study duration is approximately 27 weeks, including a ~3-week Screening Period, an 8-week Efficacy Period, and a 16-week Follow-up Period from initiation of treatment on Day 1.

Favorable clinical outcome in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of treatment of study drug, with CDI recurrence defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

3.6. SUBJECT SELECTION

3.6.1. Inclusion Criteria

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study related- procedures:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012.
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES-012 must have met the protocol definition of:
    a. ≥3 unformed stools per day for 2 consecutive days
    b. A positive *C. difficile* stool toxin assay
    c. The requirement of CDI SOC antibiotic therapy (defined as 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg] mg BID).
    An adequate clinical response following SOC antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
4. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

3.6.2. Exclusion Criteria

A subject will not be enrolled if the subject meets any of the following criteria:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.
2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding). Note: nursing homes, rehabilitation, assisted living centers and acute care hospitals are acceptable.
4. Absolute neutrophil count of <500 cells/ml[3]

5. Taking antibacterial therapy other than SOC antibiotics for the most recent episode of CDI during the screening period (a single day- antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.

6. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery. (Bariatric surgery which does not disrupt the gastrointestinal lumen, i.e., restrictive procedures such as banding, are permitted).
7. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past 3 months.
8. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to start of study.
9. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term opiate use is permitted (e.g., for a dental extraction).
10. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
11. Known stool studies positive for ova and/or parasites within the 30 days before enrollment.
12. Poor concurrent medical risks with clinically significant co-morbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
13. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
14. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
15. Any history of immunoglobulin (IgG) replacement therapy within the past 3 months.
16. Any history of fecal microbiota transplantation (FMT) within the past 3 months.
17. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
18. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
19. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives.
20. Life expectancy is 24 weeks or less.

3.7. DETERMINATION OF SAMPLE SIZE

Approximately 45 subjects are anticipated to roll-over to this study from SERES-012. The recurrence rates prior to 8 weeks after initiation of study drug assumed in SERES-012 are 16% in SER-109 and 36% in placebo. However, it is expected that only a fraction of subjects who recur in SERES-012 prior to 8 weeks will roll-over to this study, since a follow-up of 8 weeks from start of study drug in earlier protocol versions of SERES-012 (up to Amendment 5) was required before rolling over to SERES-013, regardless of when the CDI recurrence occurred in SERES-012.

3.8. METHOD OF ASSIGNING SUBJECTS TO STUDY TREATMENT

This is an open-label study. All subjects who qualify for dosing will receive single daily doses of SER-109 (3×107 SCFUs) in 4 capsules administered over 3 consecutive days.

The interactive voice and web response system (IxRS) will assign appropriate bottles of SER-109 that will be available at the site for all subjects on their Day 1 study visit. Subjects who discontinue this study or who have previously received SER-109 in this study will not be permitted to re-enter. Similarly, SER-109 dispensed to a subject may not be re-used, even if the bottle(s) are returned unopened.

3.9. MAINTAINING THE RANDOMIZATION CODES AND BREAKING THE STUDY BLIND

Not applicable. This study is not randomized nor blinded.

3.10. TABLE OF ASSESSMENTS AND PROCEDURES

Please refer to the protocol (Amendment 5.0) for the Table of Assessments and Procedures.

4. ENDPOINTS

4.1.1. Primary Efficacy Endpoint

The primary efficacy endpoint is recurrence of CDI as determined by a toxin assay up to 8 weeks after initiation of treatment. A recurrence is defined as (i) ≥ 3 unformed stools per day for 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with (ii) a positive *C. difficile* test on a stool sample determined by a toxin assay, and (iii) assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment. The requirement that subjects continue to have diarrhea until antibiotic treatment is initiated in (i) is met if the subject has ≥1 UBM each day during the period between having 2 consecutive days of ≥ 3 unformed stools and the start date of the CDI antibiotic treatment.

4.1.2. Secondary Efficacy Endpoints

Secondary efficacy endpoints are the following:

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay
- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

4.1.3. Exploratory Efficacy Endpoints

Exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 24 weeks after treatment initiation (for subjects hospitalized)

- Diagnosis-related group-adjusted hospital costs (for subjects hospitalized when available) after initiation of treatment
- Changes from Baseline in Health-Related Quality of Life (HRQoL) and health outcomes as assessed by the EQ-5D-5L from Day 1 through Weeks 8 and 24, and assessed by the Cdiff32 HRQoL from Day 1 to Week 1 and Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

4.1.4. Safety Endpoints

Safety endpoints are the following:

- Incidence of AEs
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

5. ANALYSIS POPULATIONS

5.1.1. Intent-to-Treat (ITT) Population

The ITT Population will consist of all enrolled subjects.

5.1.2. Modified Intent-to-Treat (mITT) Population

The mITT Population will be composed of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.

5.1.3. Safety Population

The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

5.2. PROTOCOL DEVIATIONS

Protocol deviations are collected in IcoTrial, the Clinical Trial Management System (CTMS), used by ICON Research, the contract research organization employed for this study. Protocol deviations will be assigned to a deviation sub-type within one of the following deviation types: inclusion/exclusion criteria, informed consent form issues, procedures/tests, laboratory, visit schedule, study drug, concomitant medication, and other. The protocol deviations will be further classified as key vs. non-key in ICOTrial and reviewed by the medical monitors on an on-going basis. Protocol deviations will be presented by critical category (critical (key) vs. non-critical (non-key)), deviation type and deviation sub-type and summarized in 2 tables as follows: 1) with frequencies and percentages of subjects with at least one deviation in each deviation sub-type and type. Subjects with multiple deviation sub-types will only be counted once for a given deviation type within the critical/non-critical deviation category and once for the protocol deviation sub-type within a deviation type; and 2) with all incidences of the protocol deviations counted separately in each deviation sub-type, type and critical category. The total count of protocol deviations will be used as the denominator for percentages in this table.

A listing of all protocol deviations by subject, deviation sub-type and type will also be provided, indicating which are critical.

6. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

6.1. GENERAL METHODS

- All analyses and summaries will be produced using SAS® version 9.4 (or higher).
- Unless otherwise specified, summaries will be presented by the randomized treatment arm in SERES-012 (SER-109 and Placebo) and for all subjects (Overall).
- Continuous variables will be summarized using the number of subjects with evaluable data, mean, standard deviation (SD), median, minimum and maximum. The same number of decimal places as in the raw data will be presented when reporting minimum and maximum, 1 more decimal place than in the raw data will be presented when reporting the mean and median, and 2 more decimal places than in the raw data will be presented when reporting the SD.
- Categorical variables will be summarized using the number of observations (n), frequency and percentage of subjects. All percentages will be presented as one-decimal point, unless otherwise specified. Percentages equal to 100 will be presented as 100% and percentages will not be presented for zero frequencies.
- Unless stated otherwise, the percentages will be based on the number of non-missing observations. The header will still contain the number of subjects in the treatment group. There will be a row for the number of non-missing observations in the table (at each time point, if required) for each variable being summarized.
- No formal statistical testing will be performed. All inferential statistics are based on two-sided 95% confidence intervals.
- Any calculated p-values will be presented to 3 decimal places; p-values less than 0.001 will be presented as ‘p<0.001’ and p-values greater than 0.999 will be presented as ‘p>0.999’.
- All relevant subject data will be included in listings and sorted by randomized treatment arm in SERES-012 (SER-109 and Placebo), Subject ID, and visit, as applicable, for all randomized subjects.
- Unscheduled or repeat assessments will not be included in summary tables unless specified otherwise (e.g., unscheduled CDI assessments will be summarized), but will be included in the subject listings.
- All tables, listings and figures will include footers that identify the name of the program that created the item, together with the date and time on which it was created. Headers will include the total number of pages that the presentation contains and, for each page, the number of the page within the presentation.

6.2. KEY DEFINITIONS

6.2.1. Study Day

Study Day 1 is defined as the first day of study drug administration. Subsequent days are numbered consecutively (Day 2, Day 3, etc.). Before the day of study drug administration, study days are numbered sequentially with negative values (i.e., Day -1, Day -2, etc.). There is no Day 0.

6.2.2. Baseline Values

Baseline values will be taken as the last assessments before dosing with study drug. In general,

these will be taken from the pre-dose assessment on Day 1, unless otherwise specified.

6.3. MISSING DATA

Every effort will be made to collect all data required in this study, especially with regards to the primary endpoint. Contact with subjects is made weekly either by telephone or clinic visits up to Week 8, in which all the components of the CDI recurrence endpoint, specifically, (i) 2 or more consecutive days with ≥3 unformed stools, with the requirement that subjects continue to have diarrhea until antibiotic treatment is initiated, (ii) *Clostridium difficile* test on a stool sample determined by a toxin assay and (iii) assessment by investigator that the condition warrants antibiotic treatment, are assessed.

From the date of enrollment to the end of study (Week 24) assessment, subjects are given 24 hours to enter the number of unformed bowel movements (UBMs) from the previous day in the electronic diarrhea log, including recording when no UBMs are experienced on any given day. Subjects are instructed to do this daily until the end of the study. However, some missing data can be expected. Handling of missing data for components of the CDI recurrence endpoint is discussed below.

For the primary endpoint, subjects who are lost-to-follow-up, terminated from the study prematurely, or died without a CDI recurrence before 8 weeks after treatment (Day 58) are defined as having an unfavorable outcome for the primary analysis. Reasons for withdrawal from the study will be recorded on the eCRF.

If the number of unformed bowel movements (UBM) is missing on any day from the date of randomization to the end of the study, then the missing UBM counts will be assumed to be ≥ 3.

If a subject missed entry into the diarrhea log on any day, the site will call the subject to inquire how many UBMs they had on the day entry into the device was missed and remind them to enter their UBM count every day until the end of the study. Subject's response to how many UBMs they had on the day entry was missed will be entered in the EDC database, but not used to assess the primary endpoint, i.e. criteria (i) above will be evaluated based solely on the data entered in the diarrhea log by the subject.

If entry into the device is missed for 1 day and the subject reports ≥3 UBMs for either of the adjacent days, the subject will be contacted by the site. If the subject reports ≥3 UBMs for the missed entry, the subject will be asked to return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI. If the subject reports having <3 UBMs on the missed day, then the site will complete the Suspected CDI Recurrence page in the eCRF, without requiring the subject to come to the site for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI.

If entry into the device is missed for ≥2 consecutive days and the subject reports 2 consecutive days of ≥3 UBMs the next time the site is able to make contact, the subject will be asked to return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI. If entry into the device is missed for ≥2 consecutive days, but the subject reports not experiencing 2 consecutive days of ≥3 UBMs the entire time entry into the diarrhea log was missed at the next contact, then the site will complete the Suspected CDI Recurrence page in the eCRF, without requiring the subject to come to the site for a *C. difficile* stool toxin test and clinical

evaluation for recurrence of CDI.

Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. If the results of the *C. difficile* toxin assay from the central laboratory are missing, then the results of the *C. difficile* toxin test performed by a CLIA-certified local laboratory using an FDA-approved toxin test will be used, if available.

If any of the components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then an unfavorable outcome for the primary analysis is imputed. However, if some of the components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a favorable outcome for the primary analysis is imputed.

Sensitivity analyses of the primary endpoint will be conducted using various methods for handling missing data as detailed in Section 8.1.2.

For the secondary endpoints of recurrence of CDI by Weeks 4, 12, and 24, subjects will be considered as having had a recurrence using similar rules.

Missing data for the time to CDI recurrence analyses will be handled with censoring by the Kaplan-Meier method. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminate the trial prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before experiencing a CDI recurrence will be censored on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event but censored on their last date of contact. The last date of contact will be determined by either the last visit (clinic or phone) date in which the site made contact with the subject or the last entry date in the diarrhea log, whichever is later. A sensitivity analysis of the time to CDI recurrence will be conducted as detailed in Section 8.2.1.3.

6.4. VISIT WINDOWS

For the primary endpoint of CDI recurrence up to 8 weeks after the start of treatment, and secondary endpoints of CDI recurrence up to 4 weeks, 12 weeks, and 24 weeks after last treatment regimen received, CDI recurrences will be included in the analyses for the specified endpoints as follows:

Endpoint	Recurrences Included in Analysis
CDI recurrence up to 4 weeks after treatment	Up to Day 30
CDI recurrence up to 8 weeks after treatment	Up to Day 58
CDI recurrence up to 12 weeks after treatment	Up to Day 87
CDI recurrence up to 24 weeks after treatment	Up to Day 171

The incidence of hospitalization will also be summarized by timepoint (Week 8 and 24) using the same cut-off days specified for the primary endpoint. Observed study visits will be used for other

efficacy analyses, including responses to the questionnaire data. For analyses of vital signs and laboratory data, data collected at an early termination visit will be presented separately.

A summary describing adherence to visit schedules by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall will be provided for the ITT Population. The summary will include the count and percentage of subjects who have discontinued on or prior to the end of the previous clinic visit/phone contact window; and those who are still ongoing at the current visit/phone contact. Ongoing subjects are those who have not discontinued on or prior to the end of the previous clinic visit/phone contact window and are further classified into: (i) those with data collected within the window for the respective visit/phone contact, (ii) those with data collected outside the window for the respective visit/phone contact, (iii) those who experienced a CDI recurrence since the end of the previous clinic visit/phone contact window through the end of the current clinic visit/phone contact window, and (iv) those with missing data, which includes subjects who discontinued for an AE, withdrew consent, were lost to follow-up, had a protocol deviation, died, or other reason (excluding CDI recurrence) after the end of the previous clinic visit/phone contact window and on or prior to the end of the current clinic visit/phone contact window, and subjects that are ongoing but missed the clinic visit/phone contact. The percentages of subjects who have discontinued and are ongoing will be based on the ITT Population, while the percentages of subjects with data collected in and out of window, CDI recurrence and missing data at each visit/phone contact will be based on the number of ongoing subjects at the respective visit/phone contact.

Subject listings of telephone contacts, as well as of clinic visits and visit details will be provided.

6.5. POOLING OF CENTERS

There is no planned pooling of centers.

6.6. MULTIPLICITY ADJUSTMENTS

No adjustments for multiple comparisons will be made.

7. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Summary tables will include columns for randomized treatment arm in SERES-012 (SER-109 and Placebo) and for all subjects (Overall).

7.1. SUBJECT DISPOSITION AND WITHDRAWALS

Summary statistics will tabulate the number and percentage of subjects who are screened, screen failures, enrolled, who completed the study, and who prematurely discontinued the study together with reasons for discontinuation by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall. The number and percentage of subjects included in each of the analysis populations will be presented. No statistical testing will be performed on these data. The number of subjects in the ITT Population for each group will be used as the denominator for percentages.

Subject listings of screen failure subjects, inclusion and exclusion criteria information and subject disposition data will be provided.

7.2. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographics (age, race, ethnicity, sex) and baseline characteristics (weight, height, body mass index (BMI)), number of previous CDI episodes, including the BI/NAP1/027 status when available, and previous history of fecal microbiota transplantation (FMT) will be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall, for the ITT, mITT and Safety Populations.

BMI will be calculated as:

$$\text{BMI } (kg/m^2) = \text{Weight}(kg)/[\text{Height}(m)]^2$$

A subject listing of demographic data will be provided. A separate listing on the previous FMT history information will also be generated.

7.3. MEDICAL HISTORY

A by-treatment summary table of the number and percentage of subjects with medical history by system organ class (SOC) and preferred term (PT) will be produced for subjects in the Safety Population. Medical history will be sorted by highest occurrence in the overall column in decreasing order of SOC and PT using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary, v20.0 (March 2017). For the summary tables, a subject may appear more than once if he has more than one medical history finding coded under different SOC terms or more than one medical history finding with a different PT under the same SOC term. However, the subject will be counted only once in the overall category.

A by-subject listing with coded SOC and PT along with verbatim term will also be provided.

7.4. QUALIFYING CDI EPISODE CHARACTERISTICS AND SEVERITY

Severity characteristics of the qualifying CDI episode will be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall in the ITT population. All information collected on the qualifying CDI episode will be included in separate listings for the ITT Population.

The Bowel Prep data on the Screening Day will also be listed.

7.5. PRIOR AND CONCOMITANT MEDICATION

Prior medications are defined as medications that started before the date of dosing. Any medication that started on the date of dosing will not be considered prior. Concomitant medications are defined as all medications (excluding study treatment) taken on or after the date of dosing. This also includes medications ongoing on the dosing date. Medications that started before the date of dosing and are ongoing after the date of dosing will be considered as both prior and concomitant.

Partial start dates in prior and concomitant medications will be imputed to the first day of the month (if missing day) or the first month of the year (if missing month). Partial end dates in prior and concomitant medications will be imputed to the last day of the month (if missing day) or the last month of the year (if missing month).

A subject listing of prior and concomitant medications use will be provided, coded by using the ATC classification codes and preferred drug name according to the World Health Organization (WHO) Drug Dictionary Enhanced, (Sept. 1, 2016). Separate summary tables will be provided for prior and concomitant medications in the Safety Population, presenting the number and percentage of subjects by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall, and will be sorted by descending frequency of ATC Level 2 and then PT in the overall column. For each subject, the medication will be counted only once within a given ATC level 2 and only once within a given preferred drug name level. A subject may appear more than once if he/she has more than one concomitant medication coded under different ATC categories, however, the subject will be counted only once in the overall category.

7.5.1. Antibiotic Medication

Summary tables for prior and concomitant antibiotic medication use by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall will be provided separately in the Safety Population. A combined subject listing of prior and concomitant antibiotic use will also be generated.

8. EFFICACY

Efficacy is based on CDI recurrence. CDI recurrence will be determined based on the definition below:

- ≥ 3 unformed bowel movements per day over 2 consecutive days and the requirement that patients must continue to have diarrhea until antibiotic treatment is initiated. The requirement that subjects continue to have diarrhea until antibiotic treatment is initiated is met if the subject has ≥1 UBM each day during the period between having 2 consecutive days of ≥ 3 unformed stools and the start date of the CDI antibiotic treatment.
- Positive *Clostridium difficile* test on a stool sample determined by a toxin assay from the central laboratory
- Assessment by the investigator (based on clinical assessment) that the patient's condition warrants antibiotic treatment.

The investigator will use the data collected from stool sample analysis (*C. difficile* stool test as described in protocol).

All summary tables will be presented by the randomized treatment group in SERES-012 (SER-109 and Placebo), accompanied by an overall (all subjects) column.

8.1. PRIMARY EFFICACY ENDPOINT AND ANALYSIS

8.1.1. Primary Analysis of the Primary Endpoint

The primary efficacy outcome is the proportion of subjects who had a CDI recurrence through Day 58 in the ITT Population. Subjects will be categorized as having favorable (no CDI recurrence) or unfavorable outcomes (had CDI recurrence) based on the CDI recurrence data as recorded on the eCRF. For the primary analysis, subjects who are lost to follow up, terminate from the study prematurely, or die without a recorded recurrence of CDI before Day 58 will be defined as having an unfavorable outcome (CDI recurrence). If any of the components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then an unfavorable outcome for the primary analysis is imputed. However, if some of the components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a favorable outcome for the primary analysis is imputed. Details regarding how missing data will be handled are provided in Section 6.3

The number and percentage of subjects in each group defined as having favorable and unfavorable outcomes will be reported with exact 95% confidence intervals (CIs) for each group. The CIs will be derived using the Clopper-Pearson exact method.

Subject listings of all suspected CDI recurrences on study including results for each CDI criteria, as well as subject listings of the severity assessment for these episodes will be provided. Subject listings of local and central laboratory *C. Difficile* test results will be generated. A subject

listing of all subject recurrences, including qualifying and on-study recurrences, will also be provided.

8.1.2. Sensitivity Analyses

Sensitivity analyses of the primary efficacy outcome (as described in Section 8.1.1) in the ITT Population will also be conducted as follows:

- The primary analysis will be repeated with the modification that subjects who are lost to follow-up, terminated the study prematurely, or died without having a CDI recurrence on or before Day 58 will be considered to have a favorable outcome.
- The primary efficacy outcome will also be analyzed as described in Section 8.1.1 for the mITT Population.

All preceding sensitivity analyses will only be conducted for CDI recurrences determined by a toxin assay.

8.2. ANALYSES OF SECONDARY EFFICACY ENDPOINTS

8.2.1.1. Recurrence of CDI up to Week 8 as determined by a PCR Algorithm

The number and percentage of subjects with CDI recurrence determined by a PCR algorithm up to 8 weeks (Day 58) will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall in the ITT and mITT Populations. The number and percentage of subjects in each group defined as having favorable and unfavorable outcomes will be estimated and tested using the same methods as for the primary efficacy assessment at Week 8 (Day 58) in Section 8.1.1.

8.2.1.2. Time to Recurrence of CDI Determined by a Toxin Assay

Time to first recurrence of CDI determined by a toxin assay will be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall for the ITT and the mITT Populations using the median and 25th and 75th percentiles from the Kaplan-Meier (K-M) analyses. The 2-sided 95% CIs for the median will also be provided. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminated the study prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before having a CDI recurrence will be censored on the date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for any of the components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Subjects who were not dosed will have their time to recurrence measured

from their enrollment date.

The plot of the K-M survival function estimates will be provided by treatment for the ITT population.

8.2.1.3. Sensitivity Analysis of Time to Recurrence of CDI Determined by a Toxin Assay

A sensitivity analysis of the time to first recurrence of CDI determined by a toxin assay endpoint will also be conducted using a different censoring rule for missing data in the ITT population. In this analysis, subjects who do not experience a CDI recurrence by the end of the study follow-up period will continue to be censored on the date of last contact. However, subjects who are lost to follow-up or who terminate the trial prematurely prior to experiencing a CDI recurrence will be counted as having a CDI recurrence on the date of last contact. Subjects who die prior to experiencing a CDI recurrence will be counted as having a CDI recurrence on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for any of thecomponents of CDI recurrence will be counted as having a CDI recurrence on the date of the earliest diarrhea, *C. difficile* stool test, assessment of investigator that the subject's condition warrants antibiotics or the date of last contact, whichever is the earliest, in the analysis.

Analyses will be conducted as described in Section 8.2.1.2 above.

Note that the preceding sensitivity analyses will only be conducted for CDI recurrences determined by a toxin assay.

8.2.1.4. Time to Recurrence of CDI Determined by a PCR Algorithm

The same analyses described in Section 8.2.1.2 for time to first CDI recurrence determined by a toxin assay will be conducted for the analysis of time to first CDI recurrence determined by a PCR algorithm. However, the corresponding Kaplan-Meier plots will not be generated.

8.2.1.5. Recurrence of CDI up to 4, 12, and 24 Weeks Post-Treatment Determined by a Toxin Assay

The number and percentage of subjects with recurrence of CDI determined by a toxin assay up to 4 (Day 30), 12 (Day 87), and 24 weeks after treatment (Day 171) will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall in the ITT and mITT Populations.

The same analysis described in Section 8.1.1 will be conducted for CDI recurrences up to 4, 12 and 24 weeks post-treatment.

8.2.1.6. Recurrence of CDI up to 4, 12, and 24 Weeks Post-Treatment Determined by a PCR Algorithm

The same analyses described in Section 8.2.1.5 will be conducted for the recurrence of CDI determined by a PCR algorithm up to 4, 12, and 24 weeks post-treatment.

8.3. EXPLORATORY EFFICACY ANALYSES

8.3.1. Microbiome Outcome Analysis

A separate Microbiome Statistical Analysis Plan will be provided by Seres Therapeutics.

8.3.2. Incidence of All-Cause Mortality

The numbers and percentages of subjects who experience death from any cause through Weeks 8, 12, and 24 will be summarized for the ITT Population. The denominator will be the number of subjects in the ITT Population within the randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

8.3.3. Incidence of Hospitalizations

The numbers and percentages of subjects who are hospitalized for recurrent CDI through Weeks 8, 12, and 24 will be summarized for the ITT Population. Subjects with more than one hospitalization within a time period will be counted only once. The denominator will be the number of subjects in the ITT Population within each randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall. No statistical tests will be conducted.

A similar summary will also be produced for the incidence of all hospitalizations, regardless of reason.

The number of hospitalizations per subject for recurrent CDI and for any reason will be summarized with frequencies and percentages.

Descriptive statistics for the total length of stay (in days) of all hospitalizations through 24 weeks for recurrent CDI and for any reason will be provided. No adjustments for time on study will be made.

Subject listings of the health care utilization information will be provided.

8.3.4. EQ-5D-5L Questionnaire

The EQ-5D-5L was developed by the EuroQol Group. The questionnaire measures health outcomes in 5 dimensions, using 5 levels of responses indicating severity. A visual analog scale (VAS) is also included. The dimensions are: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ VAS records the respondent's overall self-rated health on a scale of 0 to 100.

A unique health state is obtained by combining the levels from each of the 5 dimensions into a 5-digit number – a maximum of 3125 possible health states is possible. For example, 11111 indicates no problems on any dimensions. 12345 indicates no problems with mobility, slight problems with washing or dressing, moderate problems with doing usual activities, severe pain or discomfort, and extreme anxiety or depression. Missing values for a dimension are coded as 9.

The EQ VAS is scored as 0 to 100, where 0 is the worst health you can imagine, and 100 is the best health you can imagine.

An index value for the EQ-5D-5L can be obtained by using the Crosswalk Index Value Calculator, downloadable from the EuroQol website.

Responses on each dimension will be summarized with frequencies and percentages at each visit, including the early termination visit. Shift tables from baseline to each visit, including the early termination visit, will also be constructed.

Summary statistics will be supplied for the Crosswalk Index Value and EQ VAS at each visit, including the early termination visit, and also for the changes from baseline.

Subject listings of the EQ-5D-5L data will be provided.

8.3.5. Cdiff32 Health-care Quality of Life (HRQOL) Questionnaire

The Cdiff32 HRQOL questionnaire is a validated CDI-specific instrument (Garey et al, 2016) developed to assess HRQOL changes related to CDI with a focus on recurrent disease.

The questionnaire comprises 32 questions with 5 possible levels of response for each question. It measures health outcomes in 3 domains (physical, mental and social), and 5 sub-domains (general physical complaints, specific physical complaints, anxiety future, anxiety current, and relationship).

The responses are scored from 0 to 100 for each question, with the most positive response scored as 0 and incrementing by 25 points as the response becomes more negative. For example, for Question 1 ‘Have you had any difficulties and/or disruption carrying out your daily activities?’, the possible responses are scored as follows: Not at all = 0, A little bit = 25, Moderately = 50, Quite a bit = 75, and Extremely = 100.

The overall score for each subject is derived using the average score of the subject’s responses to all 32 questions. Each domain and sub-domain score for each subject is similarly derived by taking the average of all of the subject’s responses to all questions within the domain and sub-domain, respectively. The following items are included in the specified domains and sub-domains:

Domain	Sub-domain	Item Number
Physical	General physical complaints	1–4, 9-10

	Specific physical complaints	11-18
Mental	Anxiety future	5-8, 27
	Anxiety current	19-26, 28
Social	Relationship	29-32

Descriptive statistics of the overall score, as well as the domain and sub-domain scores will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall for all study visits, including the early termination visit, at which they were collected for the ITT population. The change from baseline to each post-baseline visit, including the early termination visit, will also be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

All responses to the Cdiff32 HRQOL questionnaire will be listed.

8.3.6. Diagnosis-Related Group-Adjusted Hospital Costs (for Hospitalized Subjects)

Diagnosis-Related Group (DRG), also known as Medicare Severity Diagnosis-Related Groups (MS-DRG), is a system to classify hospital cases into payment categories for the purpose of reimbursing hospitals for each case in a given category with a fixed fee regardless of the actual costs incurred.

For hospitalized subjects, when DRG codes are available, descriptive statistics of the DRG-adjusted hospitals costs up to 8 and 24 weeks after initiation of treatment will be provided by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall in the ITT population. Specifically, Average Covered Charges, Average Total Payments and Average Medicare Payments corresponding with the primary DRG codes, secondary DRG codes and with the primary and secondary DRG codes both included, will be summarized. The Average Covered Charges, Average Total Payments and Average Medicare Payments from the most current National and State of Inpatient Charge Data from the Centers for Medicaid and Medicare Services website (https://www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/Medicare-Provider-Charge-Data/Inpatient2014.html) available at the time of analysis will be used. No adjustments for time on study will be made.

8.4. SUBGROUP ANALYSES

A summary table of the number and percentage of subjects in each group having favorable (no CDI recurrence) and unfavorable (CDI recurrence) outcomes up to 8 weeks after treatment determined by a toxin assay (Day 58) will be reported with exact 95% confidence intervals (CIs) with columns for randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall, for the following baseline characteristics in the ITT Population:

- Age (<65 years old, ≥65 years old)

- Prior Antibiotic Regimen (Vancomyin, Fidaxomicin)
- Gender (Male, Female)
- Race (White, Black or African American, Asian, Other)
- Region (USA, Canada)
- Bowel Cleanse (Yes, No)
- SER-109 Donor Lot

Side by side forest plots of the proportion of subjects who had a CDI recurrence by Week 8 (Day 58) with the corresponding 95% CI for the different subgroups will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

9. SAFETY

All safety analyses will be conducted in the Safety Population, unless specified otherwise. Safety summaries will be presented with columns for the randomized treatment arm in SERES-012 (SER-109 and Placebo) and for all subjects (Overall).

9.1. EXTENT OF EXPOSURE AND TREATMENT COMPLIANCE

Exposure and compliance will be assessed by the number of capsules taken on each of the 3 dosing days and overall, as well as the percentage of subjects who took each number in the Safety, ITT and mITT Populations.

Subject listings of the study drug administration information will be provided.

9.2. ADVERSE EVENTS

9.2.1. Adverse Events

AEs will be coded using MedDRA v20.0 (March 2017). A listing of all AEs from the time of randomization up to Week 8 will be summarized; from Week 8 up to Week 24, only serious adverse events (SAEs) and adverse events of special interest (AESIs) will be collected and summarized.

An AESI (serious or non-serious) is one of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor is appropriate. In this protocol, an invasive infection (e.g., bacteremia, abscess, meningitis) is designated as an AESI, and as such, will be reported and followed in the same manner as an SAE during the course of the study.

Only treatment-emergent adverse events (TEAE) will be collected and summarized in this study. A TEAE is any AE that newly appeared, increased in frequency, or worsened in severity following initiation of study drug.

The following TEAE summaries will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall on Day 10, Week 2, Week 8 and Week 24 (End of Study):

- An overall summary, including the number and percentage of
    - TEAEs
    - Subjects with At Least One TEAE
    - Subjects with No TEAEs
    - Study Drug Related or Possibly Related TEAEs
    - Subjects with Study Drug Related or Possibly Related TEAEs
    - Serious TEAEs
    - Subjects with Serious TEAEs
    - Treatment-Emergent AESIs
    - Subjects with Treatment-Emergent AESIs
    - Serious TEAEs Related or Possibly Related to Study Drug

  - Subjects with Serious TEAEs Related or Possibly Related to Study Drug
  - Treatment-emergent AESIs Related or Possibly Related to Study Drug
  - Subjects with Treatment-emergent AESIs Related or Possibly Related to Study Drug
  - Severe TEAEs
  - Subjects with Severe TEAEs
  - TEAEs Leading to Study Withdrawal
  - Subjects with TEAE Leading to Study Withdrawal
  - Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Subjects with Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Serious TEAEs Leading to Study Withdrawal
  - Subjects with Serious TEAEs Leading to Study Withdrawal
  - Serious and Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Subjects with Serious and Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Treatment-emergent AESIs Leading to Study Withdrawal
  - Subjects with Treatment-emergent AESIs Leading to Study Withdrawal
  - Related or Possibly Related Treatment-emergent AESIs Leading to Study Withdrawal
  - Subjects with Related or Possibly Related Treatment-emergent AESIs Leading to Study Withdrawal
  - TEAEs Leading to Death
  - Subjects with TEAEs Leading to Death

- TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- Serious TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs Leading to Study Withdrawal by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs by System Organ Class (SOC), Preferred Term (PT) and Maximum Severity
- TEAEs by System Organ Class (SOC), Preferred Term (PT) and Maximum Relationship to Study Drug
- TEAEs Occurring Before Antibiotic Use by System Organ Class (SOC), Preferred Term (PT) and Maximum Severity

The following subject listings will be provided:

- All TEAEs,
- Deaths,
- Serious TEAEs,
- AESIs, and
- TEAEs leading to study withdrawal.

For all TEAE tables summarized by SOC and PT, a subject contributes only once to the count for a given TEAE on the SOC level and on the PT level within SOC.

In the summary by maximum severity, subjects reporting AEs at different severities will be counted only once at the greatest severity reported within an AE level (SOC or PT). Severity categories will include mild, moderate, and severe. Any missing severity will be imputed as severe prior to selecting the report that will contribute to the summary; as a result, a subject would be counted as severe due to a missing severity, even if the subject reported similar events at a lesser degree of severity.

In the summary by maximum relationship, subjects reporting AEs at different relationships will be counted only once at the strongest relationship reported within an AE level (SOC or PT). Relationship categories will include related, possibly related and unrelated. Any missing relationship will be imputed as related prior to selecting the report that will contribute to the summary; as a result, a subject would be counted as having a related AE due to a missing relationship, even if the subject reported similar events at a lesser relationship.

In all summary tables, TEAEs will be sorted in decreasing incidence, first by SOC and then by PT within the SOC, according to the incidence in the overall column. SOCs and PTs occurring at the 7same incidence will be sorted alphabetically, unless specified otherwise.

No statistical tests will be performed.

Additional analyses will determine the exposure-adjusted incidence rates (EAIR) per 100-person years of specific TEAEs occurring before subjects received antibiotics for recurrence of CDI, based on the number of days the subjects were followed up to Week 24/End of Study, including TEAEs for subjects who did not receive antibiotics for treatment of CDI before Week 24/End of Study. Incidence rates per 100-person years will be presented for the following:

1) subjects with at least one treatment-emergent SAE,

2) subjects with at least one treatment-emergent AESI, and

3) subjects with at least one TEAE leading to study withdrawal.

The EAIR per 100 person years will be calculated as (100*number of subjects with events)/total person years, where total person years equals the sum of the following: 1) [(earliest of the date of first antibiotic treatment before Week 24/End of Study or the date of the event of interest) – date of dose + 1]/365.25, summed across subjects who received antibiotics for treatment of CDI; and 2) [(earliest of the date of last contact up to Week 24/End of Study or the date of the event of interest) – date of dose + 1]/365.25, summed across subjects who did not receive antibiotics for treatment of CDI before Week 24/End of Study. A 95% CI obtained using the normal approximation to the Poisson distribution, will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

9.3. LABORATORY EVALUATIONS

All hematology, chemistry, blood screening, and pregnancy laboratory tests will be performed by a central laboratory. Descriptive statistics of the laboratory parameters will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall for all study visits at which they were collected. The change from baseline to each post-baseline visit, including the early termination visit, and to the minimum and maximum post-baseline value will also be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

Laboratory parameters will be defined as within or outside normal range limits and shift tables from baseline to each post-baseline visit will also be provided by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

All laboratory evaluations will be included in the data listings.

9.4. VITAL SIGNS

Vital signs data include measurements of weight (kg), height (cm), blood pressure (mmHg), respiratory rate (breaths/minute), body temperature (Celsius), and Body Mass Index (kg/m^2). Descriptive statistics of the vital signs will be presented by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall for all study visits, including the early termination visit, at which they were collected. The change from baseline to each post-baseline visit, including the early termination visit and to the minimum and maximum post-baseline value, will also be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

All vital signs data will be listed.

9.5. PHYSICAL EXAMINATION

A listing with physical examination findings will be provided.

9.6. OTHER SAFETY

All data in the Diarrheal Assessment Log will be listed. Subject listings will also be generated for data on the central laboratory stool sample and samples for future biomedical research.

10. DATA AND SAFETY MONITORING COMMITTEE

An independent DSMC will review unblinded safety data through review of suspected, unexpected serious adverse reactions (SUSARs) as they occur, as well as monthly review of unblinded SAE and AESI listings.

The roles and responsibilities of the DSMC, including its membership, scope, timing of meetings, and communication plan are defined in the DSMC charter. The DSMC will monitor the study for subject safety throughout the trial. The DSMC may recommend changes to study conduct based on emerging safety information to protect the safety and welfare of clinical study subjects.

11. CHANGES FROM ANALYSIS PLANNED IN PROTOCOL

There are no planned changes from the analyses specified in the protocol (SERES-013 Protocol Amendment 5 dated 09 May 2019).

12. REFERENCE LIST

Garey K, et al. Clin Gastroenterol 2016;00:000. Development and Validation of a *Clostridium difficile* Health-related Quality-of-Life Questionnaire.

13. PROGRAMMING CONSIDERATIONS

All tables, data listings, figures (TLFs), and statistical analyses will be generated using SAS for Windows, Release 9.4 or later (SAS Institute Inc., Cary, NC, USA). Computer-generated table, listing and figure output will follow the Cytel templates and outputspecifications.

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Statistical Analysis Plan

Protocol Number and Title:	**SERES-013** ECOSPOR IV: An Open-Label Extension of Study SERES-012 and Open-Label Program for Evaluating SER-109 in Adult Subjects with Recurrent Clostridioides difficile Infection (RCDI)
Protocol Version and Date:	**Amendment 8.0** **16 February 2021**
Author(s):	**Henry Wu, Ph.D.**
SAP Version:	**Version 2.0**
SAP Version Date:	**13 January 2022**

Version: 2.0 **Version Date: 13 JAN 2022**

I confirm that I have reviewed this document and agree with the content.

APPROVALS	
CR Medicon Research, Inc.	
Henry Wu, PhD Statistician	**Date (DD-MMM-YYYY)**
Seres Therapeutics	
Lisa von Moltke, MD Executive Vice President and Chief Medical Officer	**Date (DD-MMM-YYYY)**
Asli Memisoglu, ScD Executive Director, Biostatistics and Statistical Programming	**Date (DD-MMM-YYYY)**
Cytel Inc.	
Veena Palle, MPH Senior Biostatistician	**Date (DD-MMM-YYYY)**

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1. GLOSSARY OF ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical
CCNA	Cell Cytotoxicity Neutralization Assay
CDI	*Clostridioides difficile* infection
CDiff32	Clostridioides difficile Health-related Quality-of-Life Questionnaire
CI	Confidence Interval
CLIA	Clinical Laboratory Improvement Amendments
cm	Centimeter
CRF	Case Report Form
CTMS	Clinical Trial Management System
EAIR	Exposure-adjusted Incidence Rate
eCRF	Electronic Case Report Form
EIA	Enzyme Immunoassay
EQ-5D-5L	EuroQol 5 Dimension 5 Level
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
HRQOL	Health-Related Quality of Life
IgG	Immunoglobulin
ITT	Intent-To-Treat
IXRS	Interactive Voice or Web Response System
kg	Kilogram
K-M	Kaplan-Meier
m	Meters
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mITT	Modified Intent-to-Treat
mmHg	Millimeters of Mercury
n	Number of observations
PCR	Polymerase Chain Reaction
PT	Preferred Term
RCDI	Recurrent *Clostridioides difficile* infection
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCFU	Spore Colony Forming Units
SD	Standard Deviation
SOC	System Organ Class

Abbreviation	Description
TEAE	Treatment-Emergent Adverse Event
TLF	Table, Listing and Figure
UBM	Unformed Bowel Movement
VAS	Visual Analog Scale
WHO	World Health Organization

2. PURPOSE

The purpose of this statistical analysis plan (SAP) is to ensure that the data listings, summary tables and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives.

This SAP is based on SERES-013 Protocol Amendment 8 dated 16 February 2021 and electronic case report form (eCRF) Version 6.0. dated 9 December 2021.

2.1. Timings of Analyses

Analysis will be performed after all subjects have completed the 24-week follow-up period, or otherwise terminated from the study.

2.2. Summary of Changes from Version 1.0

The table in Appendix A summarizes the changes made to the specified analysis in this version of the SAP (v2.0) compared to the original version (v1.0). These changes largely reflect changes amendments to the study protocol and additions to the previously specified statistical analysis.

3. STUDY OBJECTIVES

3.1. Study Objectives for Cohort 1

3.1.1. Primary Efficacy Objective

- To evaluate SER-109 in the reduction of *Clostridioides difficile* infection (CDI) recurrence rates and increase in sustained clinical response rate, determined by a toxin assay, up to 8 weeks after initiation of treatment

3.1.2. Secondary Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates, determined using a polymerase chain reaction (PCR) algorithm (see Laboratory Manual) up to 8 weeks after initiation of treatment
- To evaluate the time to CDI recurrence, determined by a toxin assay, after initiation of a treatment regimen of SER-109
- To evaluate the time to CDI recurrence, determined using a PCR algorithm, after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined by a toxin assay, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate the proportion of subjects experiencing CDI recurrence, determined using a PCR algorithm, up to 4, 12, and 24 weeks after initiation of a treatment regimen of SER-109

3.1.3. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

3.1.4. Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109

- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes, including Health-Related Quality of Life (HRQOL), by using the EuroQol 5 Dimensions 5 Level (EQ-5D-5L) and the HRQOL survey for CDI (CDiff32) up to 24 and 8 weeks after initiation of a treatment regimen of SER-109, respectively

3.2. Study Objectives for Cohort 2

3.2.1. Primary Safety Objective

- To evaluate the safety and tolerability of SER-109 in adult subjects with recurrent CDI

3.2.2. Efficacy Objectives

- To evaluate SER-109 in the reduction of CDI recurrence rates and increase in sustained clinical response rates, determined by a toxin assay, up to 8 and 12 weeks after initiation of treatment

3.2.3. Exploratory Objectives

- To evaluate changes in the composition of the gut microbiome from Baseline to 1 week after initiation of a treatment regimen of SER-109
- To evaluate changes in the fecal metabolome from Baseline to 1 week after initiation of treatment
- To determine the incidence of mortality from all causes up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of hospitalizations for recurrent CDI up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine the incidence of all hospitalizations up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To determine, for subjects who are hospitalized, the total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after initiation of a treatment regimen of SER-109
- To assess health outcomes using the EQ-5D-5L Visual Analog Scale at Screening and at Week 8
- To assess a Bowel Cleanse Patient Satisfaction Survey Measure at Week 8 on a bowel cleanse administered prior to SER-109

3.3. Brief Description

ECOSPOR IV is an open-label extension of Study SERES-012. This study is designed to evaluate the safety, tolerability, and efficacy of a treatment regimen, SER-109, in adult subjects 18 years of age or older with recurrent *Clostridioides difficile* infection (RCDI), who received a treatment regimen of SER-109 or placebo in Study SERES-012. In Cohort 2 of ECOSPOR IV, the primary study objective is to examine safety and tolerability in a cohort of subjects receiving SER-109 at the dose used in SERES-012.

This study will be conducted at approximately 140 study centers in North America. In Cohort 1, subjects who had a per-protocol recurrence of CDI within 8 weeks of receipt of a treatment regimen of SER 109 or placebo in Study SERES-012, and who have responded to 10 to 21 days of standard-of-care antibiotic treatment for CDI (i.e. vancomycin [125 mg QID] and/or fidaxomicin [200 mg BID]) will be eligible to enroll and receive a treatment regimen of SER-109 in Study SERES-013. A treatment regimen of SER-109 is administered orally as 3×10^7 spore colony forming units (SCFU) in 4 capsules once daily for 3 consecutive days. Approximately 30 eligible subjects with RCDI disease from Study SERES-012 are expected to enroll in Cohort 1. Approximately 200 subjects will be enrolled through the Open-Label program in Cohort 2. For Cohort 2, subjects with one or more recurrences of CDI (including the current episode) who have responded to CDI antibiotic therapy defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg] will be potentially eligible to enroll in Study SERES-013 to receive a SER-109 treatment regimen.

The study duration is approximately 27 weeks, including a ~3-week Screening Period, an 8-week Efficacy Period, and a 16-week Follow-up Period with initiation of treatment on Day 1 after screening. Subjects treated with prolonged or tapering doses of antibiotics after prescreening confirmation of CDI will be screened during the 3-week screening period (Day -24 to Day -2).

In Cohort 1, favorable clinical outcome, or sustained clinical response, in this study will be determined by the absence of CDI recurrence up to 8 weeks after initiation of treatment of study drug, with CDI recurrence defined as ≥ 3 unformed stools per day over 2 consecutive days with a positive *C. difficile* test on a stool sample determined by a toxin assay performed by the central laboratory and a decision by the investigator (based on clinical assessment), that antibiotic treatment is needed. Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. The central laboratory results will be communicated to the investigator and the decision to treat with antibiotics will be based upon the investigator's assessment.

In Cohort 2, favorable clinical outcome, or sustained clinical response, will be determined by the absence of CDI recurrence up to 8 or 12 weeks after initiation of treatment of study drug, with CDI recurrence defined in the same manner as for Cohort 1.

3.4. Subject Selection

3.4.1. Inclusion Criteria

To be eligible for enrollment, a subject must meet all the following criteria before undergoing any study related- procedures. Only the inclusion criteria from the latest study protocol are listed.

For Cohort 1:

1. Previously enrolled in Study SERES-012 and experienced a CDI recurrence within 8 weeks after receipt of a treatment regimen of SER-109 or placebo in Study SERES-012.
2. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
3. The CDI recurrence in Study SERES-012 must have met the protocol definition of:
    a. ≥3 unformed stools per day for 2 consecutive days
    b. A positive *C. difficile* stool toxin assay
    c. The requirement of CDI standard of care antibiotic therapy (defined as 10 to 21 days of treatment with vancomycin [125 mg QID] or fidaxomicin [200 mg] mg BID).
    d. An adequate clinical response following standard of care antibiotic therapy, defined as (<3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
4. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as postmenopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
5. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.

For Cohort 2:

1. Signed informed consent prior to initiation of any study-specific procedure or treatment. The subject, or their legally authorized representative, must be willing to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.
2. ≥ 2 episodes of CDI, inclusive of the current episode, with estimated total number of prior episodes.
3. The CDI recurrence must have met the protocol definition of:

a. ≥ 3 unformed stools per day for 2 consecutive days
b. A positive C. difficile stool toxin or PCR assay (either local or central laboratory).
c. The requirement of CDI antibiotic therapy (defined as 10 to 42 days of treatment with vancomycin or 10 to 25 days with fidaxomicin [200 mg]. It is acceptable if subject was started on metronidazole, switched to vancomycin or fidaxomicin and is treated for a minimum of 10 days of vancomycin or fidaxomicin with a total treatment duration of up to a maximum of 42 days for vancomycin or 25 days for fidaxomicin.
d. An adequate clinical response following antibiotic therapy, defined as <3 unformed stools in 24 hours) for 2 or more consecutive days before initiation of study drug on Day 1.
e. The requirement that the subject can be dosed with study drug within 4 days of antibiotic completion.

4. Male or female subject ≥ 18 years of age.
5. If female, subject is non-lactating, and is either:
    a. Not of childbearing potential, defined as post-menopausal for at least 1 year or surgically sterile due to bilateral tubal ligation, bilateral oophorectomy, or hysterectomy.
    b. Of childbearing potential and is practicing at least 1 highly effective method of birth control including: the barrier method; oral or parenteral contraceptives; a vasectomized partner; or abstinence from sexual intercourse. The investigator will discuss with the subject the option of practicing more than 1 of the above methods for the duration of the study.
6. If male, and partner is of childbearing potential, subject agrees to practice at least 1 highly effective method of birth control for the duration of the study.
7. If currently taking probiotics, must be willing to stop at time of consent, for the duration of the study.

3.4.2. Exclusion Criteria

A subject will not be enrolled if the subject meets any of the following criteria. Only the exclusion criteria from the latest study protocol are listed.

For Cohort 1:

1. Female subjects who are pregnant, breastfeeding, lactating, or planning to become pregnant during the study.
2. Known or suspected toxic megacolon and/or known small bowel ileus.
3. Admitted to or expected to be admitted to an intensive care unit for medical reasons (not just boarding). Note: nursing homes, rehabilitation, assisted living centers and acute care hospitals are acceptable.
4. Absolute neutrophil count of <500 cells/mm^3
5. Taking antibacterial therapy other than antibiotics for the most recent episode of CDI during the screening period (a single day- antibiotic prophylactic regimen is permitted), or projected to receive antibiotics during the 8-week period post-randomization.

6. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months before enrollment (this does not include appendectomy or cholecystectomy), or any history of total colectomy or bariatric surgery. (Bariatric surgery which does not disrupt the gastrointestinal lumen, i.e., restrictive procedures such as banding, are permitted).
7. History of active inflammatory bowel disease (ulcerative colitis, Crohn's disease, microscopic colitis) with diarrhea believed to be caused by active inflammatory bowel disease in the past 3 months.
8. Unable to stop loperamide, diphenoxylate/atropine, or cholestyramine prior to enrollment.
9. Unable to stop opiate treatment unless on a stable dose, including PRN dosing, as of the onset of diarrhea and no increase in dose planned for the duration of the study. Note: Short term opiate use is permitted (e.g., for a dental extraction).
10. Known positive stool cultures for other enteropathogens including, but not limited to, *Salmonella*, *Shigella*, and *Campylobacter* within the 30 days before enrollment.
11. Known stool studies positive for ova and/or parasites within the 30 days before enrollment.
12. Poor concurrent medical risks with clinically significant co-morbid disease such that, in the opinion of the investigator, the subject should not be enrolled.
13. Received a human monoclonal antibody against *C. difficile* toxin within 3 months before study entry.
14. Received an investigational drug or vaccine, or participated in any experimental procedure within 1 month (3 months for monoclonal antibodies) before study entry.
15. Any history of immunoglobulin (IgG) replacement therapy within the past 3 months.
16. Any history of fecal microbiota transplantation (FMT) within the past 3 months.
17. Known active intravenous drug or alcohol abuse or use of other drugs of abuse.
18. Concurrent intensive induction chemotherapy, radiotherapy, or biologic treatment for active malignancy (subjects on maintenance chemotherapy may only be enrolled after consultation with the study medical monitor).
19. Unable to comply with the protocol requirements, including the ability to take oral drugs; or any condition that, in the opinion of the investigator, might interfere with study objectives.
20. Life expectancy is 24 weeks or less.

For Cohort 2, all Cohort 1 exclusion criteria plus number 21, below, apply.

21. Previously enrolled in a Seres Therapeutics clinical study. An exception is made for subjects who screened in SERES-012 who did not receive SER-109 and did not previously roll-over to SERES-013.

3.5. Determination of Sample Size

Approximately 30 subjects are anticipated to roll-over to this study from SERES-012. The recurrence rates prior to 8 weeks after initiation of study drug assumed in SERES-012 are 16% in SER-109 and 36% in placebo. However, it is expected that only a fraction of subjects who recur in SERES-012 prior to 8 weeks will roll-over to this study, since a follow-up of 8 weeks from start of study drug in earlier protocol versions of SERES-012 (up to Amendment 5) was required before rolling over to SERES-013, regardless of when the CDI recurrence occurred in SERES-012. Additionally, approximately 200 subjects with RCDI may enroll in the Open-Label program (Cohort 2) into SERES-013. Approximately 230 subjects total will be assessed for safety and tolerability, and efficacy.

As of December 2021, enrollment for both Cohort 1 and Cohort 2 were completed. Twenty-nine subjects were enrolled in Cohort 1, and 234 subjects were enrolled in Cohort 2.

3.6. Method of Assigning Subjects to Study Treatment

This is an open-label study. All subjects who qualify for dosing will receive single daily doses of SER-109 (3×10^7 SCFU) in 4 capsules administered over 3 consecutive days.

The interactive voice and web response system (IxRS) will assign appropriate bottles of SER-109 that will be available at the site for all subjects on their Day 1 study visit. Subjects who discontinue this study or who have previously received SER-109 in this study will not be permitted to re-enter. Similarly, SER-109 dispensed to a subject may not be re-used, even if the bottle(s) are returned unopened.

3.7. Maintaining the Randomization Codes and Breaking the Study Blind

Not applicable. This study is not randomized nor blinded.

3.8. Table of Assessments and Procedures

Please refer to the protocol (Amendment 8.0) for the Table of Assessments and Procedures for Cohort 1 and Cohort 2.

4. ENDPOINTS

4.1. Primary Efficacy Endpoint

For Cohort 1, the primary efficacy endpoint is recurrence of CDI and sustained clinical response as determined by a toxin assay up to 8 weeks after initiation of treatment. A recurrence is defined as (i) ≥ 3 unformed stools per day for 2 consecutive days and the requirement that subjects must continue to have diarrhea until antibiotic treatment is initiated, with (ii) a positive *C. difficile* test on a stool sample determined by a toxin assay, and (iii) assessment by the investigator that the clinical condition of the subject warrants antibiotic treatment. The requirement that subjects continue to have diarrhea until antibiotic treatment is initiated in (i) is met if the subject has ≥1 UBM each day during the period between having 2 consecutive days of ≥ 3 unformed stools and the start date of the CDI antibiotic treatment.

For Cohort 2, recurrence of CDI and sustained clinical response as determined by a toxin assay up to 8 and 12 weeks after initiation of treatment are the efficacy endpoints.

4.2. Secondary Efficacy Endpoints

For Cohort 1, secondary efficacy endpoints are the following:

- Recurrence of CDI as determined by PCR algorithm up to 8 weeks after initiation of treatment
- Time to recurrence of CDI from initiation of treatment as determined by a toxin assay
- Time to recurrence of CDI from initiation of treatment as determined by PCR algorithm
- Recurrence of CDI, as determined by a toxin assay, up to 4, 12 and 24 weeks after initiation of treatment
- Recurrence of CDI, as determined by a PCR algorithm, up to 4, 12 and 24 weeks after initiation of treatment

For Cohort 2, secondary efficacy endpoints are not being sought. As the information is collected for Cohort 2, the related data summaries will be presented for exploratory purpose.

4.3. Exploratory Efficacy Endpoints

For Cohort 1, exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1, 2, 8, and 24 weeks after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1, 2, and 8 weeks after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for RCDI up to 8 and 24 weeks after initiation of treatment

- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 8 and 24 weeks after treatment initiation (for subjects hospitalized)
- Changes from Baseline in HRQoL and health outcomes as assessed by the EQ-5D-5L up to Week 24, and assessed by the Cdiff32 HRQoL from up to Week 8, or at an ET or Recurrence visit prior to Week 8, after initiation of treatment

For Cohort 2, exploratory endpoints are the following:

- Change in the composition of the gut microbiome from Baseline up to 1 week after initiation of treatment
- Change in the fecal metabolome from Baseline up to 1 week after initiation of treatment
- Incidence of mortality from all causes up to 8 and 24 weeks after initiation of treatment
- Incidence of hospitalizations for RCDI up to 8 and 24 weeks after initiation of treatment
- Incidence of all hospitalizations up to 8 and 24 weeks after initiation of treatment
- Total length of stay (days) of hospitalization, including days in the intensive care unit, up to 24 weeks after treatment initiation (for subjects hospitalized)
- Change in the EQ-5D-5L Visual Analog Scale from Screening to Week 8
- Assess Bowel Cleanse Patient Satisfaction Survey Measure at Week 8

4.4. Safety Endpoints

Safety endpoints for both cohorts are the following:

- Incidence of adverse events (AEs)
- Laboratory evaluation results
- Vital sign measurements
- Physical examination findings

5. ANALYSIS POPULATIONS

5.1. Intent-to-Treat (ITT) Population

The ITT Population will consist of all enrolled subjects.

5.2. Modified Intent-to-Treat (mITT) Population

The mITT Population will be composed of all enrolled subjects who received any amount of SER-109, whose CDI was clinically controlled by antibiotic treatment before receiving SER-109, and who have at least 1 post-baseline evaluation.

For subjects in Cohort 1, subjects must have had RCDI diagnosis that occurred on the SERES-012 trial, as defined below:

Confirmation of the qualifying CDI episode requires a positive *C. difficile* test based on a toxin assay.

Requirements for the qualifying CDI episode to be clinically controlled by antibiotic treatment include:

- ≤2 unformed bowel movements (UBM) for at least 2 days prior to randomization
- Receipt of appropriate antibiotic, including adequate treatment duration, for the qualifying episode to roll over on to the SERES-013 Cohort 1 study

For subjects in Cohort 2, subjects with a RCDI diagnosis should have ≥2 CDI episodes prior to screening, inclusive of the current episode, as defined below: .

Confirmation of the qualifying CDI episode requires a positive *C. difficile* test based on a toxin or PCR assay. Earlier protocol versions (up to Amendment 8) required a positive *C. difficile* test based on a toxin assay only.

Requirements for the qualifying CDI episode to be clinically controlled by antibiotic treatment include:

- ≤2 unformed bowel movements (UBM) for at least 2 days prior to enrollment
- Receipt of appropriate antibiotic, including adequate treatment duration, for the qualifying episode

5.3. Safety Population

The Safety Population will consist of all enrolled subjects who received any amount of SER-109. All safety analyses will be conducted based on the Safety Population.

6. PROTOCOL DEVIATIONS

Protocol deviations are collected in ICOTrial, the Clinical Trial Management System (CTMS), used by ICON Research, the contract research organization employed for this study. Protocol deviations will be assigned to a deviation sub-type within one of the following deviation types: inclusion/exclusion criteria, informed consent form issues, procedures/tests, laboratory, visit schedule, study drug, concomitant medication, and other. The protocol deviations will be further classified as key vs. non-key in ICOTrial per ICON's Protocol Deviation Criteria document and reviewed by the medical monitors on an on-going basis.

The deviations entered in ICOTrial are then transferred into a cumulative protocol deviation listing organized by severity, subject, site, deviation sub-type, and type. This listing of ICON-defined key and non-key protocol deviations is reviewed by a Seres team including the Medical monitor, Clinical operations, Data management, and statistical team to identify deviations as either Major or Minor.

Protocol deviations will be presented by severity category of Major vs. Minor, deviation type and deviation sub-type and summarized as follows: with number and percentages of subjects with at least one deviation in each deviation sub-type and type. Subjects with multiple deviation sub-types will only be counted once for a given deviation type within the major/minor deviation category and once for the protocol deviation sub-type within a deviation type The summaries will be presented for each cohort separately, and also for 2 cohorts combined. Deviations related to the COVID-19 pandemic will be included in protocol deviations listings.

A listing of all protocol deviations by subject, deviation sub-type and type will also be provided, indicating which are major/minor.

7. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

7.1. General Methods

- All analyses and summaries will be produced using SAS® version 9.4 (or higher).
- Unless otherwise specified, summaries will be presented by the following groups:
  - For Cohort 1 subjects, their randomized treatment arm in SERES-012 (SER-109 and Placebo)
  - All subjects in Cohort 1
  - All subjects in Cohort 2
  - Cohort 1 and Cohort 2 combined
- Continuous variables will be summarized using the number of subjects with evaluable data, mean, standard deviation (SD), median, minimum and maximum. The same number of decimal places as in the raw data will be presented when reporting minimum and maximum, 1 more decimal place than in the raw data will be presented when reporting the mean and median, and 2 more decimal places than in the raw data will be presented when reporting the SD.

- Categorical variables will be summarized using the number of observations (n), frequency and percentage of subjects. All percentages will be presented as one-decimal point, unless otherwise specified. Percentages equal to 100 will be presented as 100% and percentages will not be presented for zero frequencies.
- Unless stated otherwise, the percentages will be based on the number of non-missing observations. The header will still contain the number of subjects in the treatment group. There will be a row for the number of non-missing observations in the table (at each time point, if required) for each variable being summarized.
- All relevant subject data will be included in listings. Subjects from Cohort 1 will be sorted by randomized treatment arm in SERES-012 (SER-109 and Placebo), Subject ID, and visit, as applicable, for all randomized subjects. Subjects from Cohort 2 will be sorted by Subject ID and visit, as applicable
- Unscheduled or repeat assessments will not be included in summary tables unless specified otherwise (e.g., unscheduled CDI assessments will be summarized), but will be included in the subject listings.
- All tables, listings and figures will include footers that identify the name of the program that created the item, together with the date and time on which it was created. Headers will include the total number of pages that the presentation contains and, for each page, the number of the page within the presentation.

7.2. Key Definitions

7.2.1. Study Day

Study Day 1 is defined as the first day of study drug administration in the SERES-013 study. Subsequent days are numbered consecutively (Day 2, Day 3, etc.). Before the day of study drug administration, study days are numbered sequentially with negative values (i.e., Day -1, Day -2, etc.). There is no Day 0.

7.2.2. Baseline Values

Baseline values will be taken as the last assessments on or before day of dosing with study drug in this study. In general, these will be taken from the pre-dose assessment on Day 1, unless otherwise specified.

7.3. Missing Data

Every effort will be made to collect all data required in this study, especially with regards to the primary endpoint. Contact with subjects is made weekly either by telephone or clinic visits up to Week 8, in which all the components of the CDI recurrence endpoint, specifically, (i) 2 or more consecutive days with ≥3 unformed stools, with the requirement that subjects continue to have diarrhea until antibiotic treatment is initiated, (ii) *Clostridioides difficile* test on a stool sample determined by a toxin assay and (iii) assessment by investigator that the condition warrants antibiotic treatment, are assessed.

Monitoring diarrheal symptoms differs between Cohort 1 and Cohort 2.

- Cohort 1 subjects will be instructed to complete a daily diarrhea log every day whether or not they experience diarrhea. At all scheduled telephone calls and study site visits, subjects will be queried regarding diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea. Any subject suspected of having an episode of CDI per protocol definition will be asked to come in for an in-clinic visit, where possible, for a *C. difficile* stool toxin test and evaluation for recurrence of CDI.
- Cohort 2: at all scheduled telephone calls and clinic or home visits, subjects will be queried regarding diarrheal symptoms, including the day, frequency, and quality of bowel movements described as diarrhea. Any subject suspected of having an episode of CDI per protocol definition (≥ 3 unformed stools per day lasting ≥ 2 consecutive days) will be asked to arrange for an in-clinic or home visit for a *C. difficile* stool toxin test (to be sent to central laboratory) and evaluation for recurrence of CDI.

Daily diarrhea log is only used by subjects in Cohort 1. From the date of enrollment to the end of study (Week 24) assessment, subjects are given 24 hours to enter the number of UBMs from the previous day in the electronic diarrhea log, including recording when no UBMs are experienced on any given day. Subjects are instructed to do this daily until the end of the study. However, some missing data can be expected. Handling of missing data for components of the CDI recurrence endpoint is discussed below.

- For Cohort 1, if the number of UBM is missing on any day from the date of enrollment to the end of the study, then the missing UBM counts will be assumed to be ≥ 3.
- For Cohort 1, if a subject missed entry into the diarrhea log on any day, the site will call the subject to inquire how many UBMs they had on the day entry into the device was missed and remind them to enter their UBM count every day until the end of the study. Subject's response to how many UBMs they had on the day entry was missed will be entered in the EDC database, but not used to assess the primary endpoint, i.e. criteria (i) above will be evaluated based solely on the data entered in the diarrhea log by the subject.
- For Cohort 1, if entry into the device is missed for 1 day and the subject reports ≥3 UBMs for either of the adjacent days, the subject will be contacted by the site. If the subject reports ≥3 UBMs for the missed entry, the subject will be asked to return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI. If the subject reports having <3 UBMs on the missed day, then the site will complete the Suspected CDI Recurrence page in the eCRF, without requiring the subject to come to the site for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI.
- For Cohort 1, if entry into the device is missed for ≥2 consecutive days and the subject reports 2 consecutive days of ≥3 UBMs the next time the site is able to make contact, the subject will be asked to return to the clinic for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI. If entry into the device is missed for ≥2 consecutive days, but the subject reports not experiencing 2 consecutive days of ≥3 UBMs the entire time entry into the diarrhea log was missed at the next contact,

then the site will complete the Suspected CDI Recurrence page in the eCRF, without requiring the subject to come to the site for a *C. difficile* stool toxin test and clinical evaluation for recurrence of CDI.

For the primary endpoint for both Cohort 1 and Cohort 2, subjects who are lost-to-follow-up, terminated from the study prematurely, or died without a CDI recurrence before 8 weeks after treatment (Day 58) will be defined as having CDI recurrence for the primary analysis. Reasons for withdrawal from the study will be recorded on the eCRF.

Data from the *C. difficile* toxin assay (either enzyme immunoassay [EIA] or cell cytotoxicity neutralization assay [CCNA]), performed at the central laboratory, will be used for the primary endpoint analysis. If the results of the *C. difficile* toxin assay from the central laboratory are missing, then the results of the *C. difficile* toxin test performed by a Clinical Laboratory Improvement Amendments (CLIA)-certified local laboratory using an Food and Drug Administration (FDA)-approved toxin test will be used, if available.

If any of the components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then CDI recurrence for the primary analysis will be imputed. However, if some of the components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a CDI non-recurrence (ie, sustained clinical response) for the primary analysis will be imputed.

For recurrence of CDI by Weeks 4, 12, and 24, subjects will be considered as having had a recurrence using similar rules.

Missing data for the time to CDI recurrence analyses will be handled with censoring by the Kaplan-Meier method. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminate the trial prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before experiencing a CDI recurrence will be censored on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for one or more of the 3 components of CDI recurrence will not be counted as an event but censored on their last date of contact. The last date of contact will be determined by either the last visit (clinic or phone) date in which the site made contact with the subject or the last entry date in the diarrhea log, whichever is later. A sensitivity analysis of the time to CDI recurrence will be conducted as detailed in Section 9.2.3.

Frequency (number and percentage) of subjects with an event (CDI recurrence) and censoring reasons will be presented. Thus censoring rules are summarized as follows: (1) study completion without CDI recurrence; (2) lost to follow-up, premature trial termination or death; (3) CDI recurrence due to missing or incomplete data for one or more of the 3 components and the non-missing components meet the CDI recurrence criteria. A sensitivity analysis will be performed where only subjects who complete the study will be censored with subjects in censoring rule 2 and 3 imputed as CDI recurrence.

7.4. Visit Windows

For the primary endpoint of CDI recurrence up to 8 weeks after the start of treatment, and CDI recurrence up to 4 weeks, 12 weeks, and 24 weeks after last treatment regimen received, CDI recurrences will be included in the analyses for the specified endpoints as follows:

Endpoint	Recurrences Included in Analysis
CDI recurrence up to 4 weeks after treatment	Up to Day 30
CDI recurrence up to 8 weeks after treatment	Up to Day 58
CDI recurrence up to 12 weeks after treatment	Up to Day 87
CDI recurrence up to 24 weeks after treatment	Up to Day 171

The number of subjects with hospitalization and the number of hospitalizations will also be summarized by timepoint (Week 4, 8 and 24) using the same cut-off days specified for the primary endpoint. Observed study visits will be used for other efficacy analyses, including responses to the questionnaire data.

For analyses of vital signs and laboratory data, data collected at an early termination visit will be presented separately.

A summary describing adherence to visit schedules will be provided for the ITT Population. The summary will include the count and percentage of subjects who have discontinued on or prior to the end of the previous clinic visit/phone contact window; and those who are still ongoing at the current visit/phone contact. Ongoing subjects are those who have not discontinued on or prior to the end of the previous clinic visit/phone contact window and are further classified into: (i) those with data collected within the window for the respective visit/phone contact, (ii) those with data collected outside the window for the respective visit/phone contact, (iii) those who experienced a CDI recurrence since the end of the previous clinic visit/phone contact window through the end of the current clinic visit/phone contact window, and (iv) those with missing data, which includes subjects who discontinued for an AE, withdrew consent, were lost to follow-up, had a protocol deviation, died, or other reason (excluding CDI recurrence) after the end of the previous clinic visit/phone contact window and on or prior to the end of the current clinic visit/phone contact window, and subjects that are ongoing but missed the clinic visit/phone contact. The percentages of subjects who have discontinued and are ongoing will be based on the ITT Population, while the percentages of subjects with data collected in and out of window, CDI recurrence and missing data at each visit/phone contact will be based on the number of ongoing subjects at the respective visit/phone contact.

Subject listings of telephone contacts, as well as of clinic visits and visit details will be provided.

7.5. Pooling of Centers

There is no planned pooling of centers.

7.6. Multiplicity Adjustments

No adjustments for multiple comparisons will be made.

8. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

8.1. Subject Disposition and Withdrawals

Summary statistics will tabulate the number and percentage of subjects who are screened, screen failures, enrolled, who completed the study, and who prematurely discontinued the study together with reasons for discontinuation. The number and percentage of subjects included in each of the analysis populations will be presented. No statistical testing will be performed on these data. The number of subjects in the ITT Population for each group will be used as the denominator for percentages.

Subject listings of screen failure subjects, inclusion and exclusion criteria information and subject disposition data will be provided.

8.2. Demographic and Other Baseline Characteristics

Demographics (age, race, ethnicity, sex) and baseline characteristics (weight, height, body mass index (BMI)), number of previous CDI episodes, BI/NAP1/027 status when available, previous history of fecal microbiota transplantation (FMT), and additional subgroups defined in Section 8.4 that are not covered by the previous parameters will be summarized for the ITT, mITT and Safety Populations.

BMI will be calculated as:

- BMI (kg/m^2) = Weight(kg)/$[Height(m)]^2$

A subject listing of demographic data will be provided. A separate listing on the previous FMT history information will also be generated.

8.3. Medical History

A by-treatment summary table of the number and percentage of subjects with medical history by system organ class (SOC) and preferred term (PT) will be produced for subjects in the Safety Population. Medical history will be sorted by highest occurrence in the overall column in decreasing order of SOC and PT using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary, v20.0 (March 2017). For the summary tables, a subject may appear more than once if he has more than one medical history finding coded under different SOC terms or more than one medical history finding with a different PT under the same SOC term. However, the subject will be counted only once in the overall category.

A by-subject listing with coded SOC and PT along with verbatim term will also be provided.

8.4. Qualifying CDI episode Characteristics and Severity

Severity characteristics of the qualifying CDI episode will be summarized in the ITT population. All information collected on the qualifying CDI episode will be included in separate listings for the ITT Population.

The Bowel Prep data on the Screening Day will also be listed.

8.5. Prior and Concomitant Medication

Prior medications are defined as medications that started before the date of dosing. Any medication that started on the date of dosing will not be considered prior. Concomitant medications are defined as all medications (excluding study treatment) taken on or after the date of dosing. This also includes medications ongoing on the dosing date. Medications that started before the date of dosing and are ongoing after the date of dosing will be considered as both prior and concomitant.

Partial start dates in prior and concomitant medications will be imputed to the first day of the month (if missing day) or the first month of the year (if missing month). Partial end dates in prior and concomitant medications will be imputed to the last day of the month (if missing day) or the last month of the year (if missing month).

A subject listing of prior and concomitant medications use will be provided, coded by using the Anatomic Therapeutic Chemical (ATC) classification codes and preferred drug name according to the World Health Organization (WHO) Drug Dictionary Enhanced, (Sept. 1, 2016). Separate summary tables will be provided for prior and concomitant medications in the Safety Population, presenting the number and percentage of subjects, and will be sorted by descending frequency of ATC Level 2 and then PT in the overall column. For each subject, the medication will be counted only once within a given ATC level 2 and only once within a given preferred drug name level. A subject may appear more than once if he/she has more than one concomitant medication coded under different ATC categories; however, the subject will be counted only once in the overall category.

8.5.1. Antibiotic Medication

Summary tables for prior and concomitant antibiotic medication use will be provided separately in the Safety Population. A combined subject listing of prior and concomitant antibiotic use will also be generated.

9. EFFICACY

Efficacy is based on CDI recurrence. CDI recurrence will be determined based on the definition below:

- ≥ 3 unformed bowel movements per day over 2 consecutive days and the requirement that patients must continue to have diarrhea until antibiotic treatment is initiated. The requirement that subjects continue to have diarrhea until antibiotic treatment is initiated is met if the subject has ≥1 UBM each day during the period between having 2 consecutive days of ≥ 3 unformed stools and the start date of the CDI antibiotic treatment.
- Positive *Clostridioides difficile* test on a stool sample determined by a toxin assay from the central laboratory
- Assessment by the investigator (based on clinical assessment) that the patient's condition warrants antibiotic treatment.

The investigator will use the data collected from stool sample analysis (*C. difficile* stool test as described in protocol).

9.1. Primary Efficacy Endpoint and Analysis

9.1.1. Primary Analysis of the Primary Endpoint

For Cohort 1, the primary efficacy endpoint is the proportion of subjects who had a CDI recurrence through Week 8 in the ITT Population. For the primary analysis, subjects who are lost to follow up, terminate from the study prematurely, or die without a recorded recurrence of CDI before Week 8 will be defined as having a CDI recurrence. If any of the components of the CDI recurrence criteria is missing, and the non-missing components meet the CDI recurrence criteria, then a CDI recurrence for the primary analysis is imputed. However, if some of the components of the CDI recurrence criteria are missing, and at least 1 of the non-missing components does not meet the CDI recurrence criteria, then a CDI non-recurrence (ie, sustained clinical response) outcome for the primary analysis is imputed. Details regarding how missing data will be handled are provided in Section 7.3 .

The number and percentage of subjects in each group defined as having CDI non-recurrence (ie, sustained clinical response) and CDI recurrence outcomes will be reported with exact 95% confidence intervals (CIs) for each group. The CIs will be derived using the Clopper-Pearson exact method.

Subject listings of all suspected CDI recurrences on study including results for each CDI criteria, as well as subject listings of the severity assessment for these episodes will be provided. Subject listings of local and central laboratory *C. Difficile* test results will be generated. A subject listing of all subject recurrences, including qualifying and on-study recurrences, will also be provided.

For Cohort 2, the primary efficacy endpoint is the proportion of subjects who had a CDI recurrence through Week 8 and Week 12 in the ITT Population. The same analyses will be conducted for CDI recurrence through Week 8 and Week 12 for Cohort 2 subjects.

9.2. Analyses of Secondary Efficacy Endpoints

Secondary efficacy endpoints are only defined for Cohort 1 in the study protocol. The analysis methodology will be presented in this section. Same analysis outputs for Cohort 2 will also be generated for exploratory purposes.

9.2.1. Recurrence of CDI up to Week 8 as determined by a PCR Algorithm

The number and percentage of subjects with CDI recurrence determined by a PCR algorithm up to 8 weeks (Day 58) will be presented in the ITT Population. The number and percentage of subjects in each group defined as having CDI non-recurrence (ie, sustained clinical response) and CDI recurrence outcomes will be estimated and tested using the same methods as for the primary efficacy assessment at Week 8 (Day 58) in Section 9.1.1.

9.2.2. Time to Recurrence of CDI Determined by a Toxin Assay

Time to first recurrence of CDI determined by a toxin assay will be summarized for the ITT and the mITT Populations using the median and 25th and 75th percentiles from the Kaplan-Meier (K-M) analyses. The 2-sided 95% CIs for the median, calculated using the Greenwood formula,

will also be provided. Subjects who complete the study and do not experience a CDI recurrence by the end of the follow-up period will be censored on the date of last contact. Subjects who are lost to follow-up or who terminated the study prematurely before experiencing a CDI recurrence will be censored on the date of last contact. Subjects who die before having a CDI recurrence will be censored on the date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for any of the components of CDI recurrence will not be counted as an event, but censored on their last date of contact. Subjects who were not dosed will have their time to recurrence measured from their enrollment date.

The plot of the K-M survival function estimates will be provided by treatment for the ITT population.

9.2.3. Sensitivity Analysis of Time to Recurrence of CDI Determined by a Toxin Assay

A sensitivity analysis of the time to first recurrence of CDI determined by a toxin assay endpoint will also be conducted using a different censoring rule for missing data in the ITT population. In this analysis, subjects who do not experience a CDI recurrence by the end of the study follow-up period will continue to be censored on the date of last contact. However, subjects who are lost to follow-up or who terminate the trial prematurely prior to experiencing a CDI recurrence will be counted as having a CDI recurrence on the date of last contact. Subjects who die prior to experiencing a CDI recurrence will be counted as having a CDI recurrence on their date of death. Subjects who were assessed to have a CDI recurrence due to missing or incomplete data for any of the components of CDI recurrence will be counted as having a CDI recurrence on the date of the earliest diarrhea, *C. difficile* stool test, assessment of investigator that the subject's condition warrants antibiotics or the date of last contact, whichever is the earliest, in the analysis.

Analyses will be conducted as described in Section 9.2.2 above.

Note that the preceding sensitivity analyses will only be conducted for CDI recurrences determined by a toxin assay.

9.2.4. Time to Recurrence of CDI Determined by a PCR Algorithm

The same analyses described in Section 9.2.2 for time to first CDI recurrence determined by a toxin assay will be conducted for the analysis of time to first CDI recurrence determined by a PCR algorithm. However, the corresponding Kaplan-Meier plots will not be generated.

9.2.5. Recurrence of CDI up to 4, 12, and 24 Weeks Post-Treatment Determined by a Toxin Assay

The number and percentage of subjects with recurrence of CDI determined by a toxin assay up to 4 (Day 30), 12 (Day 87), and 24 weeks after treatment (Day 171) will be presented in the ITT and mITT Populations.

The same analysis described in Section 9.1.1 will be conducted for CDI recurrences up to 4, 12 and 24 weeks post-treatment.

9.2.6. Recurrence of CDI up to 4, 12, and 24 Weeks Post-Treatment Determined by a PCR Algorithm

The same analyses described in Section 9.2.5 will be conducted for the recurrence of CDI determined by a PCR algorithm up to 4, 12, and 24 weeks post-treatment.

9.3. Exploratory Efficacy analyses

9.3.1. Microbiome Outcome Analysis

A separate Microbiome Statistical Analysis Plan will be provided by Seres Therapeutics.

9.3.2. Incidence of Hospitalizations

Hospitalizations are defined as resource use recorded in the healthcare resource utilization eCRF as "Veterans Affairs Hospital", "Hospital", "Hospice" "Long Term Acute Care" "Rehabilitation Center". The numbers and percentages of subjects who are hospitalized for RCDI through Weeks 4, 8, 12, and 24 will be summarized for the ITT Population. Subjects with more than one hospitalization within a time period will be counted only once. No statistical tests will be conducted.

A similar summary will also be produced for the incidence of all hospitalizations, regardless of reason.

The number of hospitalizations per subject for recurrent CDI and for any reason will be summarized with frequencies and percentages.

Descriptive statistics for the total length of stay (in days) of all hospitalizations through 24 weeks for recurrent CDI and for any reason will be provided. A hospital duration less than 24 hours is assigned 1 day. Hospital duration will be presented as a continuous variable and by categories:1-3; 4-7; 8-14; and >14 days. No adjustments for time on study will be made.

Subject listings of the health care utilization information will be provided. This analysis will be performed for both Cohort 1 and Cohort 2.

9.3.3. EQ-5D-5L Questionnaire

For Cohort 1 subjects:

The full EQ-5D-5L questionnaire was collected for Cohort 1 subjects only. . The questionnaire developed by the EuroQol Group, measures health outcomes in 5 dimensions, using 5 levels of responses indicating severity with higher responses indicating greater severity. A visual analog scale (VAS) is also included. The dimensions are: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ-5D-5L VAS records the respondent's overall self-rated health on a scale of 0 to 100.

A unique health state is obtained by combining the levels from each of the 5 dimensions into a 5-digit number – a maximum of 3125 possible health states is possible. For example, 11111 indicates no problems on any dimensions. 12345 indicates no problems with mobility, slight problems with washing or dressing, moderate problems with doing usual activities, severe pain or discomfort, and extreme anxiety or depression. Missing values for a dimension are coded as 9.

The EQ-5D-5L VAS is scored as 0 to 100, where 0 is the worst health you can imagine, and 100 is the best health you can imagine.

An index value for the EQ-5D-5L can be obtained by using the Crosswalk Index Value Calculator, downloadable from the EuroQol website.

Responses on each dimension will be summarized with frequencies and percentages at each visit, including the early termination visit. Shift tables from baseline to each visit, including the early termination visit, will also be constructed.

Summary statistics will be supplied for the Crosswalk Index Value and EQ-5D-5L VAS at each visit, including the early termination visit, and also for the changes from baseline.

Subject listings of the EQ-5D-5L data will be provided.

For Cohort 2 subjects:

For Cohort 2 subjects, only EQ-5D-5L VAS was collected for sites who enrolled subjects under protocol amendment Version 8.0. Summary statistics will be supplied for EQ-5D-5L VAS at baseline and Week 8 visit, and also for the changes from baseline. Subject listings for this measurement will be provided.

9.3.4. Cdiff32 Health-care Quality of Life (HRQOL) Questionnaire

The Cdiff32 HRQOL questionnaire was collected for Cohort 1 subjects only. It is a validated CDI-specific instrument (Garey et al, 2016) developed to assess HRQOL changes related to CDI with a focus on recurrent disease.

The questionnaire comprises 32 questions with 5 possible levels of response for each question. It measures health outcomes in 3 domains (physical, mental and social), and 5 sub-domains (general physical complaints, specific physical complaints, anxiety future, anxiety current, and relationship).

The original CRF responses are from 1 to 5 for each question. They will be transformed to scores from 0 to 100 for each question, with the worst response scored as 0 and incrementing by 25 points as the response becomes more positive. For example, for Question 1 ‘Have you had any difficulties and/or disruption carrying out your daily activities?’, the original CRF responses are Not at all = 1, A little bit = 2, Moderately = 3, Quite a bit = 4, and Extremely = 5, with the lower score being the best original response. This will be transformed as follows: Not at all = 100, A little bit = 75, Moderately = 50, Quite a bit = 25, and Extremely = 0. For all questions except for Question 19 and 32, they all have lower scores as the best original score and will be transformed using the same way. For Question 19 and 32, since lower original scores are the worst scores, they will be transformed in the other direction. For example, for Question 19 ‘Despite my C. diff infection I can live a normal life.’, the original CRF responses are Totally disagree = 1, Mostly disagree = 2, Don't know = 3, Mostly agree = 4, and Totally agree = 5, with the lower score being the worst original response. This will be transformed as follows: Totally disagree = 0, Mostly disagree = 25, Don't know = 50, Mostly agree = 75, and Totally agree = 100.

The overall score for each subject is derived using the average score of the subject’s responses to all 32 questions. Each domain and sub-domain score for each subject is similarly derived by taking the average of all of the subject’s responses to all questions within the domain and sub-

domain, respectively. The following items are included in the specified domains and sub-domains:

Domain	Sub-domain	Item Number
Physical	General physical complaints	1–4, 9-10
	Specific physical complaints	11-18
Mental	Anxiety future	5-8, 27
	Anxiety current	19-26, 28
Social	Relationship	29-32

Descriptive statistics of the overall score, as well as the domain and sub-domain scores will be presented for all study visits, including the early termination visit, at which they were collected for the ITT population. The change from baseline to each post-baseline visit, including the early termination visit, will also be summarized by randomized treatment arm in SERES-012 (SER-109 and Placebo) and overall.

All responses to the Cdiff32 HRQOL questionnaire will be listed.

9.3.5. Bowel Cleanse Questionnaire

The bowel cleanse questionnaire will be collected for Cohort 2 subjects only. Patient satisfaction with the bowel cleanse is collected in this questionnaire. The questionnaire has 2 domains.

- Domain 1 includes four items assessing the ease or difficulty of consuming the bowel-cleansing preparation, whether the patient is able to consume the entire preparation, the taste of the preparation, and the overall experience when using the preparation. Patients report on their ability to complete the entire preparation with a binary 'yes' or 'no' response. The three other questions in Domain 1 require patients to report satisfaction on a five-point scale. The three items of Domain 1 with a five-point scale are assigned scores from 0 to 4, with 0 representing the most favorable ("Very Easy" or "Excellent") and 4 representing the least favorable ("Very Difficult" or "Bad") answers; the dichotomous item are coded as 0 for yes and 1 for no. The raw score of each of the four items are then transformed to a range from 0 to 100 to standardize items. These values are then summed to generate a total satisfaction score between 0 and 400, where a lower score indicates higher satisfaction.
- Domain 2 included two additional 'yes/no' questions to assess participants' willingness to accept or refuse the same bowel preparation for a future treatment of SER-109.

The following analyses will be conducted for this questionnaire in ITT population. The analyses in this Section follows the similar methodology as Hatoum et al. (2016).

- Reliability
  - Cronbach's alpha will be used to measure the internal consistency of patients' responses to the individual questions of Domain 1 of the satisfaction questionnaire. Values above 0.70 reflect acceptable reliability (internal consistency).

- Validity
  - The validity of the patient satisfaction instrument will be assessed by determining the 'ability to measure change' and the 'predictive validity' of test scores.
  - The ability to measure change will be assessed by examining the extent of floor and ceiling effects, as measured by the percentages of responses at either end of the response range using the raw item scores (an item is considered to have a ceiling effect if most responses fall on its highest value). The number of subjects and the percentage of responses at each level of the raw response to each question will be provided for this summary. All questions in Domain 1 and 2 will be included in this summary.
  - The predictive validity will be assessed by evaluating the relationship between the satisfaction scores (Domain 1) and the acceptability measure on the likelihood to use the same preparation in the future (Question 5 and 6 in Domain 2). Normality test based on Shapiro-Wilk W test will be used to test the Normality of the satisfaction scores. If the satisfaction scores are Normally distributed, T-test will be used to compare the satisfaction scores between the 2 groups of subjects who reported "Yes" or "No" for Questions 5 and 6. If satisfaction scores are not Normally distributed, Wilcoxon rank-sum test will be used for this comparison instead. Descriptive summaries for the satisfaction scores for these 2 groups of subjects will also be presented.
  - The relationship between transformed satisfaction scores (Domain 1) and EQ-5D-5L VAS will also be assessed. Correlation of total score of the four questions in Domain 1 as well as each individual question in Domain 1 with EQ-5D-5L VAS will be assessed. Correlation of individual questions in Domain 2 with EQ-5D-5L VAS will also be assessed.
  - The discriminant validity of the bowel cleanse satisfaction will be assessed by comparing Domain 1 total score with EQ-5D-5L VAS week 8 change from baseline. Mean and median bowel cleanse total score of Domain 1 will be calculated for patients that have no change, five-point improvement, and five-point decline on the EQ-5D-5L VAS. This will be repeated for ten-point change of EQ-5D-5L VAS.
  - The discriminant validity of willingness to use bowel cleanse in the future will be assessed by comparing Domain 2 individual questions with EQ-5D-5L VAS week 8 change from baseline. Percentages of patients with a "Yes" and a "No" answer to Domain 2 questions will be calculated for patients that have no change, five-point improvement, and five-point decline on the EQ-5D-5L VAS. This will be repeated for ten-point change of EQ-5D-5L VAS.

9.4. Subgroup Analyses

A summary table of the number and percentage of subjects in each group having CDI non-recurrence (ie, sustained clinical response) and CDI recurrence outcomes up to 8 weeks after treatment determined by a toxin assay (Day 58) will be reported with exact 95% confidence intervals (CIs), for the following baseline characteristics in the ITT Population:

- Age (<65 years old, ≥65 years old)
- Prior Antibiotic Regimen (Vancomyin, Fidaxomicin)
- Gender (Male, Female)
- Race (White, Black or African American, Asian, Other)
- Region (USA, Canada)
- SER-109 Donor Lot
- # of prior CDI episodes (not including qualifying episode) (1, 2, ≥3). All Cohort 1 subjects will be included in the category of ≥3 recurrences.
- Enrollment prior to Protocol Amendment 8 and post Protocol Amendment 8
- Qualifying episode defined by PCR alone vs toxin with/without PCR

Side by side forest plots of the proportion of subjects who had a CDI recurrence by Week 8 (Day 58) with the corresponding 95% CI for the different subgroups will be presented.

10. SAFETY

All safety analyses will be conducted in the Safety Population, unless specified otherwise.

10.1. Extent of Exposure and Treatment Compliance

Exposure and compliance will be assessed by the number of capsules taken on each of the 3 dosing days and overall, as well as the percentage of subjects who took each number in the Safety Populations. Subject listings of the study drug administration information will be provided.

10.2. Adverse Events

Adverse events will be coded using MedDRA v20.0 (March 2017). A listing of all AEs from the time of enrolment up to Week 8 (defined as up to Day 58) will be summarized; from Week 8 up to Week 24, only serious adverse events (SAEs) and adverse events of special interest (AESIs) will be collected and summarized.

An AESI (serious or non-serious) is one of scientific and medical concern specific to the product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor is appropriate. In this protocol, an invasive infection (e.g., bacteremia, abscess, meningitis) is designated as an AESI, and as such, will be reported and followed in the same manner as an SAE during the course of the study.

Only treatment-emergent adverse events (TEAE) will be collected and summarized in this study. A TEAE is any AE that newly appeared, increased in frequency, or worsened in severity following initiation of study drug.

The following TEAE summaries will be presented by the time interval of AE onset: At any time; Days 1- 58; Days 1-10; Days 11-14; Days 15-58, Days 59-and beyond (End of Study):

- An overall summary, including the number and percentage of
  - TEAEs
  - Subjects with At Least One TEAE
  - Subjects with No TEAEs
  - Study Drug Related or Possibly Related TEAEs
  - Subjects with Study Drug Related or Possibly Related TEAEs
  - Serious TEAEs
  - Subjects with Serious TEAEs
  - Treatment-Emergent AESIs
  - Subjects with Treatment-Emergent AESIs
  - Serious TEAEs Related or Possibly Related to Study Drug
  - Subjects with Serious TEAEs Related or Possibly Related to Study Drug
  - Treatment-emergent AESIs Related or Possibly Related to Study Drug
  - Subjects with Treatment-emergent AESIs Related or Possibly Related to Study Drug
  - Severe TEAEs
  - Subjects with Severe TEAEs
  - TEAEs Leading to Study Withdrawal
  - Subjects with TEAE Leading to Study Withdrawal
  - Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Subjects with Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Serious TEAEs Leading to Study Withdrawal
  - Subjects with Serious TEAEs Leading to Study Withdrawal
  - Serious and Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Subjects with Serious and Related or Possibly Related TEAEs Leading to Study Withdrawal
  - Treatment-emergent AESIs Leading to Study Withdrawal
  - Subjects with Treatment-emergent AESIs Leading to Study Withdrawal

  - Related or Possibly Related Treatment-emergent AESIs Leading to Study Withdrawal
  - Subjects with Related or Possibly Related Treatment-emergent AESIs Leading to Study Withdrawal
  - TEAEs Leading to Death
  - Subjects with TEAEs Leading to Death
- TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs by Preferred Term (PT)
- Serious TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs Leading to Study Withdrawal by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs by System Organ Class (SOC), Preferred Term (PT) and Maximum Severity
- TEAEs by System Organ Class (SOC), Preferred Term (PT) and Maximum Relationship to Study Drug
- TEAEs Occurring Before Antibiotic Use by System Organ Class (SOC), Preferred Term (PT) and Maximum Severity

The following subject listings will be provided:

- All TEAEs,
- Deaths,
- Serious TEAEs,
- AESIs, and
- TEAEs leading to study withdrawal.

For key TEAE tables, key subgroup summaries will also be performed. This includes the following tables:

- Overall TEAE summary
- TEAEs by SOC and PT
- Study Drug Related or Possibly Related TEAEs by SOC and PT

The key subgroups include the following:

- # of prior CDI episodes (not including qualifying episode) (1, 2,≥3). All Cohort 1 subjects will be included in the category of ≥3 recurrences.
- Enrollment prior to Protocol Amendment 8 and post Protocol Amendment 8
- Qualifying episode defined by PCR alone vs toxin and/or PCR
- Donor lot

For all TEAE tables summarized by SOC and PT, a subject contributes only once to the count for a given TEAE on the SOC level and on the PT level within SOC.

In the summary by maximum severity, subjects reporting AEs at different severities will be counted only once at the greatest severity reported within an AE level (SOC or PT). Severity categories will include mild, moderate, severe and missing.

In the summary by maximum relationship, subjects reporting AEs at different relationships will be counted only once at the strongest relationship reported within an AE level (SOC or PT). Relationship categories will include related, possibly related, unrelated and missing.

In all summary tables, TEAEs will be sorted in decreasing incidence, first by SOC and then by PT within the SOC, according to the incidence in the overall column. SOCs and PTs occurring at the 7same incidence will be sorted alphabetically, unless specified otherwise.

No statistical tests will be performed.

Additional analyses will determine the exposure-adjusted incidence rates (EAIR) per 100-person years of specific TEAEs occurring before subjects received antibiotics for recurrence of CDI, based on the number of days the subjects were followed up to Week 24/End of Study, including TEAEs for subjects who did not receive antibiotics for treatment of CDI before Week 24/End of Study. Incidence rates per 100-person years will be presented for the following:

1. subjects with at least one treatment-emergent SAE,
2. subjects with at least one treatment-emergent AESI, and
3. subjects with at least one TEAE leading to study withdrawal.
4. subjects with a TEAE of urinary tract infection

The EAIR per 100 person years will be calculated as (100*number of subjects with events)/total person years, where total person years equals the sum of the following: 1) [(earliest of the date of first antibiotic treatment before Week 24/End of Study or the date of the event of interest) – date of dose + 1]/365.25, summed across subjects who received antibiotics for treatment of CDI; and 2) [(earliest of the date of last contact up to Week 24/End of Study or the date of the event of interest) – date of dose + 1]/365.25, summed across subjects who did not receive antibiotics for treatment of CDI before Week 24/End of Study. A 95% CI obtained using the normal approximation to the Poisson distribution will be presented.

10.3. Laboratory Evaluations

All hematology, chemistry, blood screening, and pregnancy laboratory tests will be performed by a central laboratory. Descriptive statistics of the laboratory parameters will be presented for all study visits at which they were collected. The change from baseline to each post-baseline visit, including the early termination visit, and to the minimum and maximum post-baseline value will also be summarized.

Laboratory parameters will be defined as within or outside normal range limits and shift tables from baseline to each post-baseline visit will also be provided.

All laboratory evaluations will be included in the data listings.

10.4. Vital Signs

Vital signs data include measurements of weight (kg), height (cm), blood pressure (mmHg), respiratory rate (breaths/minute), body temperature (Celsius), and Body Mass Index (kg/m^2). Descriptive statistics of the vital signs will be presented for all study visits, including the early termination visit, at which they were collected. The change from baseline to each post-baseline visit, including the early termination visit and to the minimum and maximum post-baseline value, will also be summarized.

All vital signs data will be listed.

10.5. Physical Examination

A listing with physical examination findings will be provided.

10.6. Other Safety

For Cohort 1, all data in the Diarrheal Assessment Log will be listed.

Subject listings will also be generated for data on the central laboratory stool sample and samples for future biomedical research.

11. CHANGES FROM ANALYSIS PLANNED IN PROTOCOL

Analysis specified in this SAP supersedes those specified in the study protocol (SERES-013 Protocol Amendment 8 dated 16 February 2021). Substantive differences in the analysis specified in the SAP compared to those specified in the study protocol include:

- Sensitivity analyses specified for primary efficacy endpoint were removed
- Analysis of time to CDI recurrence where events were imputed rather than censored was added
- Secondary efficacy analysis was only specified for Cohort 1 in the protocol. These analyses will also be performed for Cohort 2

12. REFERENCE LIST

Garey K, et al. Clin Gastroenterol 2016; 50(8): 631–637. Development and Validation of a *Clostridium difficile* Health-related Quality-of-Life Questionnaire.

Hatoum H, et al. The Patient - Patient-Centered Outcomes Research 2016; 9(1), 27-34. Validation of a Patient Satisfaction Scale in Patients Undergoing Bowel Preparation Prior to Colonoscopy.

13. PROGRAMMING CONSIDERATIONS

Please review Section below and modify as per protocol requirements.

All tables, data listings, figures (TLFs), and statistical analyses will be generated using SAS for Windows, Release 9.4 or later (SAS Institute Inc., Cary, NC, USA). Computer-generated table, listing and figure output will follow the Cytel templates and output specifications.

14. INDEX OF TABLES/LISTINGS/FIGURES

A separate document that contains the Table/Listing/Figure mock-ups will be developed. The index of Tables/Listings/Figures will be included in the document.

APPENDIX A. SUMMARY OF CHANGES

Table 1: Summary of Material Changes to SAP v2.0 compared to v1.0

Section Title **Section Number** (Refers to v2.0 numbering unless otherwise indicated)	**Description of Change**	**Rationale for Change**
Primary Efficacy Objective Section 3.1.1	Revised language for primary efficacy objective	Achieve consistency with latest protocol amendment
Version 1.0 Exploratory Objective Section 3.4	Removed 7th bullet related to DRG-adjusted hospital costs	Achieve consistency with latest protocol amendment
Study Objectives for Cohort 2.0 Section 3.2	Added section for study objectives specific to Cohort 2.0	Achieve consistency with latest protocol amendment
Brief Description Section 3.3	Revised and added text	Achieve consistency with latest protocol amendment
Subject Selection Section 3.4	Revised inclusion and exclusion criteria	Achieve consistency with latest protocol amendment
Determination of Sample Size Section 3.5.	Revised sample size consideration	Achieve consistency with latest protocol amendment. Reflects revised Cohort 1 rollover assumptions and added enrollment of Cohort 2 De Novo subjects.
Table of Assessments and Procedures Section 3.8.	Removed table in SAP and references source table in protocol	Minimize redundancy in documents and use protocol as source for this information
Endpoints Section 4.	Revised language with additions and deletions. Most substantive additions were endpoints relating to Cohort 2.	Reflect latest amendment of the protocol
Modified Intent-to-Treat Population Section 5.2.	Added detail on defining MITT population	Detail was needed to programmatically define MITT population
Protocol Deviations Section 6.	Added detail on analysis and presentation of protocol deviation data	Revisions reflect planned analysis.

Section Title **Section Number** (Refers to v2.0 numbering unless otherwise indicated)	**Description of Change**	**Rationale for Change**
General Methods Section 7.1.	Added detail on presentation groups for analysis tables Removed information on presentation of p-values Added information on presentation of subject data listings	Revisions reflect planned analysis.
Study Day Section 7.2.1.	Clarified definition of study to specify that relevant study for Day 1 is SERES-013	Revisions reflect planned analysis.
Baseline Values Section 7.2.2.	Revised definition of baseline to include assessments made on the same day initiation of study drug	Revisions reflect planned analysis.
Missing Data Section 7.3.	Clarified handling of missing data for CDI recurrence	Revisions reflect planned analysis.
Visit Windows Section 7.4.	Added the Week 4 time for hospitalization analysis	Revisions reflect planned analysis.
Time to Recurrence of CDI Determined by a Toxin Assay Section 9.2.2.	Added detail on how 95% confidence intervals for median is calculated (ie, Greenwood formula)	Revisions reflect planned analysis.
Incidence of Hospitalizations Section 9.3.2.	Added detail on analysis of hospitalization and duration of hospitalizations. Categories of healthcare resource use included to define hospitalizations was added. Treatment of hospitalization durations <24h and categories of hospital duration to be presented were specified.	Revisions reflect planned analysis.
EQ-5D-5L Questionnaire Section 9.3.3.	Added text specifying the analysis of data from Cohort 2 subjects.	Revisions reflect planned analysis of subjects enrolled under latest protocol amendment.
CDiff32 Health-care Quality of Life Questionnaire Section 9.3.4.	Added more detail on the derivation of CDiff32 scores for analysis from CRF entries.	Revisions reflect planned analysis.

Section Title **Section Number** (Refers to v2.0 numbering unless otherwise indicated)	**Description of Change**	**Rationale for Change**
Bowel Cleanse Questionnaire Section 9.3.5.	Added information of analysis of the Bowel Cleanse Questionnaire which was added in Protocol Amendment 8.0 of Cohort 2 De Novo subjects.	Revisions reflect planned analysis of assessment added to latest protocol amendment.
Subgroup Analysis Section 9.4.	Added subgroups to be evaluated for efficacy	
Adverse Events Section 10.2.	Specified time of onset intervals for presentation of adverse event summaries Specified which AE analyses will be conducted for subgroup analysis. Indicated that AEs with missing severity or relatedness information will be presented as missing. Added TEAEs of urinary tract infections for analysis of exposure-adjusted incidence rates.	Revisions reflect planned analysis.
Other Safety Section 10.6.	Removed information on data safety monitoring committee. Specified that data from Diarrheal Assessment Logs, central laboratory stool sample and samples for future biomedical research will be listed.	Revisions reflect planned analysis and revisions made to the protocol.