CLINICAL STUDY VE303-002

Protocol Version 7.0: 15 July 2021

Study Title:	A Double-Blind Placebo-Controlled Phase 2 Study of VE303 for Prevention of Recurrent *Clostridium (Clostridioides) Difficile* Infection
Study Number:	VE303-002
Study Phase:	Phase 2
Product Name:	VE303
IND Number:	017750
Indication:	Prevention of Recurrent *Clostridium (Clostridioides) Difficile* Infection
Sponsor:	Vedanta Biosciences, Inc. 19 Blackstone Street Cambridge, Massachusetts 02139 United States of America Phone: (857) 706-1427 Responsible Medical Officer: [redacted]

SPONSOR SIGNATURE PAGE

Declaration of Sponsor or Responsible Medical Officer

Study VE303-002: A Double-Blind Placebo-Controlled Phase 2 Study of VE303 for Prevention of Recurrent *Clostridium (Clostridioides) Difficile* Infection

This study protocol was subjected to critical review and has been approved by the Sponsor. The information it contains is consistent with the current risk/benefit evaluation 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 and the guidelines on Good Clinical Practice.

[redacted]

Vedanta Biosciences, Inc.

Date

INVESTIGATOR SIGNATURE PAGE

Declaration of the Principal Investigator

Study VE303-002: A Double-Blind Placebo-Controlled Phase 2 Study of VE303 for Prevention of Recurrent *Clostridium (Clostridioides) Difficile* Infection

I have read this study protocol, including all appendices. By signing this protocol, I agree to conduct the clinical study, following approval by an Independent Ethics Committee/Institutional Review Board, in accordance with the study protocol, the current International Council for Harmonisation Guideline for Good Clinical Practice, and applicable regulatory requirements. I will ensure that all personnel involved in the study under my direction will be informed about the contents of this study protocol and will receive all necessary instructions for performing the study according to the study protocol.

Printed Name	Signature	Date

PROTOCOL SYNOPSIS

Title	A Double-Blind Placebo-Controlled Phase 2 Study of VE303 for Prevention of Recurrent *Clostridium (Clostridioides) Difficile* Infection
Protocol Number	VE303-002
Phase	2
Study Sites	Up to 80 sites in the United States and Canada
Objectives and Endpoints	**Objectives** The primary objective is to determine the recommended VE303 Phase 3 dose regimen(s) based on safety and efficacy, as indicated by the *Clostridioides difficile* (*C. difficile)* infection (CDI) recurrence rate. Secondary objectives are to characterize VE303 colonization and changes in the fecal microbiome. **Endpoints** Safety endpoints include the overall safety profile and treatment-emergent adverse events (TEAEs). Efficacy endpoints include: • Proportion of subjects with CDI recurrence before or at Week 8 (i.e., 8 weeks after the first dose of study drug). • Proportion of subjects without CDI recurrence before or at Week 4 (i.e., 4 weeks after the first dose of study drug), Week 12 (i.e., 12 weeks after the first dose of study drug), and Week 24 (i.e., 24 weeks after the first dose of study drug). Pharmacodynamic and pharmacokinetic endpoints include: • Fecal VE303 component bacteria colonization abundance and duration. • Changes in fecal microbiota diversity and taxonomic composition. • Changes in the fecal metabolomic profile, including short chain fatty acids and bile acids.
Study Design	This Phase 2, randomized, parallel-arm, double-blind, placebo-controlled, dose-selection study will evaluate the safety, microbiota changes, and efficacy of VE303 in the prevention of subsequent CDI-associated diarrhea compared with placebo following completion of at least 1 successful course of standard-of-care (SOC) antibiotics for subjects with

	primary *C. difficile* infection (pCDI) at high risk for recurrence or subjects with recurrent *C. difficile* infection (rCDI). Eligible subjects will be randomized into 4 treatment arms in a 2:1:2:1 ratio to receive VE303 high dose (10 capsules):placebo high dose (10 capsules):VE303 low dose (2 capsules):placebo low dose (2 capsules), respectively. Approximately 60 to 80 subjects are anticipated for enrollment. A Data Monitoring Committee (DMC), which will include independent clinicians with knowledge of the target population, a biostatistician, and Sponsor representatives, will be established to oversee subject safety. Investigators and subjects will remain blinded for the duration of the study period. Randomization will be stratified by 1) number of previous CDI episodes at Baseline (0 vs ≥ 1 episodes, excluding the qualifying episode; i.e. Inclusion criterion 2a vs 2b/2c subjects), 2) SOC antibiotic treatment (non-vancomycin [fidaxomicin or metronidazole] or an alternative oral vancomycin dosing regimen vs vancomycin QID [four times a day; this category will include subjects who receive vancomycin QID on at least the last day of treatment]), and 3) results of CDI laboratory diagnostic method (free toxin [enzyme immunoassay (EIA) for Toxin A/B or Cell Cytotoxicity Neutralization Assay (CCNA)] vs other [polymerase chain reaction (PCR)/cytotoxic culture if EIA for Toxin A/B and/or CCNA results are negative or not available]). On the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing, subjects will be randomized and begin daily oral administration of placebo or VE303 (both at low and high doses) for 14 consecutive days. The first dose of the study drug will be administered under the supervision of study personnel at the Day 1 visit. After the 14-day dosing period is completed, follow-up will be performed every 2 to 4 weeks for 24 weeks after the first dose of study drug. **Study-specific Definitions** An *episode of diarrhea consistent with diarrhea due to C. difficile* is characterized by ≥ 3 loose/unformed bowel movements (Bristol Score 5-7, see Appendix 1) within 24 hours for at least 2 consecutive days or > 8 loose/unformed bowel movements within 24 hours, and considered unlikely to have another etiology. The following are specific protocol definitions: A *prior occurrence* of CDI is based on a subject's medical history and defined as an episode of diarrhea consistent with diarrhea due to *C. difficile*, with a stool sample that is positive for *C. difficile* (e.g., PCR, or EIA for Toxin A/B, and GDH antigen, or CCNA). The CDI episode

	must have initially responded to SOC antibiotic treatment, with no other identifiable cause for the diarrhea. A *qualifying* CDI *episode* is defined as an episode of diarrhea consistent with diarrhea due to *C. difficile*, which includes a positive diagnostic stool test for free toxin or toxigenic *C. difficile*. • Diagnostic tests for enrollment may be performed at the local laboratory or at the central laboratory. Subjects with a positive test (either EIA for Toxin A/B or PCR or CCNA or toxigenic culture assay, performed at the local laboratory or at the central laboratory) may be enrolled in the study. Confirmation of a positive local test by central laboratory test is not required for subject enrollment, provided documentation of a local positive test is available. • Subjects' allocation to a proper stratum is dependent on timely reports of laboratory results from both local and central laboratories. To ensure subjects are allocated to a proper stratum, an effort should be made to obtain and send a stool sample to the central laboratory for confirmation of free toxin or toxigenic *C. difficile* prior to randomization. Additional requirements are provided in Inclusion Criteria #3 and #4. A *successful clinical response* to antibiotic therapy by the time of randomization to the study treatment is required for a subject to be eligible. A *successful clinical response* is defined as the symptomatic control of the qualifying/current CDI episode, i.e., < 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days. An *on-study CDI recurrence* is defined as an episode of diarrhea consistent with diarrhea due to *C. difficile* infection, which includes a stool sample positive for free toxin (EIA for Toxin A/B or CCNA) per test performed at the local laboratory or at the central laboratory. An on-study CDI recurrence may occur at any time after administration of the first dose of study drug. • If recurrent CDI is suspected, a stool sample must be collected for laboratory confirmation. Regardless of the test(s) performed at the local laboratory, samples must be collected from all subjects during each episode of diarrhea for CDI testing at a central laboratory. • After an initial sample collected for suspected recurrence, it is required to collect and submit samples on subsequent day(s) if the laboratory test results are not available or negative for free toxin (EIA for Toxin A/B or CCNA) before initiating antibiotic therapy.

Test and Reference Products	VE303 is a live biotherapeutic product consisting of 8 well characterized clonally derived, nonpathogenic, nontoxigenic, commensal strains of Clostridia originally derived from healthy human individuals. VE303 is formulated as a size 0 enteric capsule for oral administration. Each capsule contains approximately 400 mg of a mixture of 1×10^8 colony forming units (CFU) of lyophilized bacteria from each of the 8 clonally derived and distinct Clostridial species for a total bacterial content of 8×10^8 CFU per capsule. Additionally, the capsule contains sucrose, histidine, yeast extract, cysteine, metabisulfite, and microcrystalline cellulose as excipients. Placebo capsules containing microcrystalline cellulose will be visually identical to and not discernible from VE303 capsules. However, placebo capsules will not contain any bacterial strains or formulation buffer used in the VE303 drug product.
Study Regimens	• Placebo "low" dose (2 capsules per day × 14 days) • Placebo "high" dose (10 capsules per day × 14 days) • VE303 low dose (2 capsules per day × 14 days; total of 2.2×10^{10} CFU) • VE303 high dose (10 capsules per day × 14 days; total of 1.1×10^{11} CFU)
Number of Subjects	It is anticipated that approximately 60 to 80 subjects will be randomized.
Study Population	**Inclusion Criteria:** 1. Able and willing to provide written informed consent prior to initiation of any study-specific procedure or study drug administration and understands the potential risks and benefits of study enrollment and study drug administration. When appropriate, informed consent may be provided by a legally-authorized representative (LAR). 2. Subjects with a *qualifying* CDI *episode* in at least one of the following categories: a. ≥ 18 years of age with a second or greater occurrence of CDI within the last 6 months; b. Subjects ≥ 65 years of age with or without prior episodes of CDI who exhibit one or more of the following risk factors for recurrence:

- Have kidney dysfunction, defined as creatinine clearance of < 60 mL/min/1.73 m^2 at the time of the current CDI episode
- Have a history of regular use of proton pump inhibitors (PPIs) within the past 2 months and will be continuing use of PPIs throughout the study
- Have a history of CDI at any time

c. Subjects ≥ 75 years of age.

3. The *qualifying episode* must meet all of the following criteria:
   a. New onset of ≥ 3 loose/unformed bowel movements (i.e., Types 5 to 7 on the Bristol stool scale, see Appendix 1) within 24 hours for 2 consecutive days or > 8 loose/unformed bowel movements within 24 hours
   b. CDI symptoms started within 30 days (inclusive) prior to the day of randomization
   c. Stool sample collected before (or no later than 72 hours after) initiation of SOC antibiotic therapy and positive for CDI laboratory test as defined in the "qualifying CDI episode" section (NOTE: every effort must be made to collect the diagnostic sample before SOC antibiotic therapy or within 24 hours after antibiotic therapy initiation, whenever possible)
   d. Diarrhea considered unlikely to have another etiology.
4. Prior to randomizing a subject to receive the first dose of the study medication, a subject should:
   a. Receive and complete an Investigator's choice SOC antibiotic regimen of a minimum of 10 days and up to 21 days of total duration (NOTE: antibiotic tapering is not allowed)
   b. Meet successful clinical response criteria defined as the symptomatic control of the qualifying/current CDI episode, i.e., < 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days
   c. Have a positive *C. difficile* diagnostic stool test by either EIA for Toxin A/B or PCR or CCNA or toxigenic culture assay performed at the local laboratory or at the central laboratory.
5. Females of childbearing potential must have a negative pregnancy test and must agree to either use a highly effective acceptable form of birth control (e.g., established hormonal birth control plus a barrier method, double barrier method: intrauterine device plus

condom or spermicidal gel plus condom) or remain celibate during the study period and up to 3 months after the last dose of study drug.

6. Able to receive the first dose of study drug on the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing.
7. Able and willing to follow study procedures (e.g., ingest up to 10 size 0 (2 cm) capsules per day for 14 days, comply with study visits, provide stool samples, and record information/interact with study tools).
8. Recovered from any complications of severe or fulminant CDI and be clinically stable by the time of randomization.

Exclusion Criteria:

1. History of diarrhea (defined as 3 or more loose stools per day lasting for at least 4 weeks) that is not related to *C. difficile* infection within the 3 months prior to randomization.
2. Known or suspected toxic megacolon or small bowel ileus at the time of randomization.
3. Contraindication to oral/enteral therapy (e.g., severe reflux, severe nausea/vomiting, or ileus) at the time of randomization.
4. Presence of white blood cell (WBC) count > 15×10^9 cells/L, or body temperature > 38.5 °C at the time of randomization.
5. Prior administration of genetically modified investigational live bacterial/fungal/bacteriophage/viral isolates for CDI-associated diarrhea (Exception: use of yogurt or other food products containing live bacterial cultures or over the counter probiotics is permitted).
6. History of administration of fecally-derived investigational live biotherapeutic products, or fecally-derived live bacterial isolates for CDI-associated diarrhea including fecal microbiota transplantation within the last 6 months.
7. Use of drugs that alter gut motility (e.g., loperamide, diphenoxylate) within 3 days prior to the planned first dose of study drug.
8. Planned administration of oral or parenteral antibacterial therapy for a non-CDI indication after randomization.
9. History of acute leukemia or hematopoietic stem cell transplantation or myelosuppressive chemotherapy within 2 months prior to randomization.
10. Subjects with compromised immune system, including:

	a. Absolute neutrophil count (ANC) of < 0.5 $\times 10^9$ cells/L on 2 consecutive occasions within 7 days prior to randomization or sustained ANC < 1 $\times$ 10^9 cells/L b. Corticosteroid use at daily doses higher than 20 mg of prednisone equivalent within 14 days prior to randomization, with the exception of inhaled or topical corticosteroids, which are permitted. NOTE: subjects with well-controlled human immunodeficiency virus infection and CD4 count > 300 cells/mcL may be enrolled, provided that all other protocol eligibility requirements are met. 11. Current or immediate potential for mechanical ventilation or vasopressors for hemodynamic support. 12. Life expectancy of < 3 months. 13. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months prior to randomization, current ileostomy, or history of total colectomy. NOTE: appendectomy, cholecystectomy, or restrictive procedures, such as banding, may be permitted upon discussion with the Medical Monitor if at least 1 month since surgery has elapsed and upon the subject's full recovery. 14. Anticipated admission to an intensive care unit during or after the study drug administration treatment period. NOTE: nursing homes, rehabilitation units, assisted living centers, and acute care hospitals are acceptable. 15. Female subject who is pregnant or breastfeeding. 16. Known hypersensitivity/allergy/intolerance to any ingredient in the VE303 study formulation (sucrose, histidine, yeast extract, cysteine, metabisulfite, and micro-crystalline cellulose). 17. Clinically significant medical or surgical condition not mentioned in the above criteria that, in the Investigator's opinion, could interfere with the administration of study drug, interpretation of study safety or efficacy data, or compromise the safety or well-being of the subject. 18. History of confirmed celiac disease, inflammatory bowel disease, short gut, gastrointestinal tract fistulas, or ischemia.
Duration of Study Participation	For each subject, the duration of study drug administration will be 14 days and total study participation will be approximately 7 months from Screening through the last follow-up visit.

Criteria for Evaluation	Safety will be evaluated continuously for the duration of a subject's study participation, including assessment of adverse events (AEs) reported by a subject or observed by study personnel from the date of informed consent until the end of the 6-month follow-up period, evaluation of gastrointestinal symptoms, electrocardiograms, physical examination, assessment of vital signs, and clinical laboratory measurements. Adverse events will be coded using the most current version of Medical Dictionary for Regulatory Activities at the time of study initiation, and the severity of AEs and laboratory abnormalities will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0. Stool samples must be collected at the time points specified in Table 7-1 for *C. difficile* testing (e.g., EIA for Toxin A/B, PCR, CCNA, toxigenic culture assay) and for the exploratory analyses (microbiota composition, VE303 component bacteria detection, antibiotic concentrations, metabolomics, culture, detection of antibiotic resistant bacteria (ARB) other than *C. difficile* (e.g., carbapenem-resistant Enterobacteriaceae [CRE], extended spectrum β-lactamase producing Enterobacteriaceae [ESBL], or vancomycin-resistant Enterococcus [VRE]), and calprotectin). Subjects will be continuously monitored for clinical events of diarrhea that may be indicative of a CDI recurrence.
Guidance on Study Drug Discontinuation for Suspected on-Study CDI Recurrence	As per the Primary Efficacy Analysis, the discontinuation of study treatment for subjects with suspected on-study CDI recurrence is based on the results of free toxin test (EIA for Toxin A/B or CCNA) performed at the local or the central laboratory regardless of the results of toxin PCR. If initial results of free toxin test are negative or not available and the symptoms persist, repeated samples should be collected and tested on subsequent day(s). The subjects will be continuously monitored (more than once/day by telephone) to determine if the suspected rCDI persists and the study drug needs to be discontinued. • If a subject has a new *episode of diarrhea consistent with diarrhea due to C. difficile* (Section 4.2) after starting the study drug but the free toxin test (EIA for Toxin A/B or CCNA) is negative or unavailable at the time and the SOC antibiotic is not administered for this episode, the study treatment should be continued. This episode will not be considered a CDI recurrence if free toxin test (EIA for Toxin A/B or CCNA) is negative or not available. • If a subject has a new *episode of diarrhea consistent with diarrhea due to C. difficile* after starting the study drug but the free toxin test (EIA for Toxin A/B or CCNA) is negative or unavailable at the time and the SOC antibiotic is administered for this episode,

	the study treatment should be discontinued. This episode will not be considered a CDI recurrence if free toxin test is negative or not available. The discontinuation of study drug due to SOC antibiotic treatment in such a case will be considered a protocol deviation and should be reported as such in the eCRF. • If the subject has a new *episode of diarrhea consistent with diarrhea due to C. difficile* after starting the study drug and the free toxin test (EIA for Toxin A/B or CCNA) is positive, the study treatment should be discontinued. This episode will be considered a CDI recurrence. Such an event should be reported as confirmed recurrence in the eCRF.
Statistical Considerations	**Final Analysis** Safety and efficacy endpoints will be based on approximately 60 to 80 subjects completing the intended duration of the study (through Week 24/Day 168) with descriptive statistics.
Sponsor	Vedanta Biosciences, Inc.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
°C	degree(s) Celsius
CBC	complete blood count
CCNA	Cell Cytotoxicity Neutralization Assay
CDI	*Clostridioides difficile* infection
CFU	colony-forming unit(s)
CFR	Code of Federal Regulations
CLSI	Clinical and Laboratory Standards Institute
CPK	creatine phosphokinase
CRE	carbapenem-resistant Enterobacteriaceae
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EIA	enzyme immunoassay
ESBL	extended β-lactamase producing Enterobacteriaceae
FAS	full analysis set
FDA	Food and Drug Administration
FMT	fecal microbiota transplantation
GCP	Good Clinical Practice
GDH	glutamate dehydrogenase
HIV	human immunodeficiency virus
IA	interim analysis
ICH	International Council for Harmonisation
IRB	Institutional Review Board
LAR	legally authorized representative
LBP	live biotherapeutic product
MedDRA	Medical Dictionary for Regulatory Activities
NCI	National Cancer Institute
PacBio	Pacific Biosciences
pCDI	primary *Clostridioides difficile* infection
PCR	polymerase chain reaction
PPI	proton pump inhibitor
PPS	per-protocol analysis set
PROMIS®	Patient-Reported Outcomes Measurement Information System
rCDI	recurrent *Clostridioides difficile* infection
QID	four times a day
QTc	QT interval corrected for heart rate
SAE	serious adverse event

Abbreviation	Definition
SAP	statistical anaylysis plan
SOC	standard-of-care
TEAE	treatment-emergent adverse event
US	United States
VRE	vancomycin-resistant Enterococcus
WBC	white blood cell

1.0 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The Principal Investigator is the person responsible for the conduct of the study at the investigational site. A sub-Investigator is any member of the clinical study team designated and supervised by the Principal Investigator to perform critical study-related procedures and/or to make important study-related decisions.

Prior to study initiation, the Principal Investigator at the study site will provide to Vedanta Biosciences, Inc. (also referred to as "Vedanta" or "Sponsor") or its representative/designee (e.g., Contract Research Organization [CRO] personnel) a signed protocol signature page, a fully executed and signed United States (US) Food and Drug Administration (FDA) Form 1572, a current signed curriculum vitae, a medical license, and a signed financial disclosure form. Financial disclosure forms, current curriculum vitaes and medical licenses will also be provided for sub-Investigators listed on Form 1572, who will be directly involved in the evaluation of subjects.

Prior to initiation at study sites in Canada, a No Objection Letter will be received by the Sponsor and appropriate Clinical Trial Site Information Forms for each site/qualified Investigator in Canada will be provided to Health Canada.

The study will be administered and monitored by employees or representatives of Vedanta in accordance with applicable regulations. Clinical research associates will monitor investigative sites on a periodic basis and perform verification of source documentation for each subject. Vedanta or designee will be responsible for ensuring timely reporting of expedited serious adverse event (SAE) reports to regulatory authorities and Investigators.

2.0 INTRODUCTION

2.1 Background of *C. Difficile* Infection

Clostridium difficile (or more recently *Clostridioides difficile* according to the US National Center for Biotechnology Information; *C. difficile*) infection (CDI) is the most common health care-associated infection, with an estimated incidence of 453,000 cases and approximately 29,000 deaths in the US in 2011 [1]. The health care costs related to CDI are estimated to be as high as US $4.8 billion for acute care facilities, which does not account for the increasing reports of CDI treatment occurring outside of acute care facilities, including community settings in which CDI may be diagnosed and treated without hospitalization [1].

C. difficile is a gram-positive, spore forming anaerobic bacillus. The spores are resistant to heat, acid, and antibiotics; *C. difficile* is transmitted among humans via the fecal-oral route. Following oral ingestion, the spores resist the acidity of the stomach and germinate into the vegetative form in the small intestine [2]. Carriage of *C. difficile* occurs in 5% to 15% of healthy adults but may be as high as 84.4% in newborns and healthy infants and up to 57% in residents of long-term care facilities [3]. The two biggest risk factors to infection are exposure to antibiotics and exposure to the organism [4]. Thus, disruption of the commensal flora of the colon, typically through exposure to antimicrobials, allows *C. difficile* to flourish and produce toxins that lead to colitis. The majority of infections occur following iatrogenic nosocomial exposure, although spores are present in low levels in the environment and food supply, allowing for community-acquired infections [5]. More than 400 strains of *C. difficile* have been identified, but only strains producing the exotoxins, Toxin A and Toxin B, are virulent. The toxins are endocytosed by colonic epithelial cells, resulting in damage to the actin cytoskeleton, causing cell death, loss of intestinal barrier function, and neutrophilic colitis [5, 6].

The clinical manifestations of infection with toxin-producing strains of *C. difficile* range from asymptomatic carriage to mild or moderate diarrhea or fulminant colitis. Infection-related mortality has been reported in 5% of patients, with all-cause mortality up to 15% to 20% [5]. Symptoms of CDI usually begin soon after colonization, with a median time to onset of 2 to 3 days for the most common symptoms of diarrhea, fever, cramping, abdominal discomfort, and peripheral leukocytosis [7]. Patients with severe disease may develop colonic ileus or toxic dilation and present with abdominal pain and distension but minimal or no diarrhea. Complications of severe *C. difficile* colitis include dehydration, electrolyte imbalance, hypoalbuminemia, toxic megacolon, bowel perforation, hypotension, renal failure, systemic inflammatory response syndrome, sepsis, and death.

A prerequisite for developing disease in infected individuals is disruption of the normal flora. Factors associated with CDI vary across infected persons and include host susceptibility, the virulence of the infecting *C. difficile* strain, and the nature and extent of prior antimicrobial exposure. The BI/NAP1/027 strain is associated with severe *C. difficile* disease and is characterized by a mortality rate 3 times as high as that associated with less virulent strains [5]. Advanced age, antineoplastic chemotherapy, and severe underlying diseases, particularly those that lead to altered immune responses, also contribute to susceptibility [5]. The risk of CDI and its severity increases as age increases, even in healthy individuals.

2.1.1 Current Management of *C. Difficile* Infections

Treatment guidelines for CDI have been published by the Society for Healthcare Epidemiology of America in collaboration with the Infectious Disease Society of America and by the American Society of Gastroenterology [4, 7]. Cessation of the inciting antibiotic agent, if medically appropriate, is often sufficient for complete recovery of mild disease [8]. For more severe disease, antimicrobial therapy directed against *C. difficile* is necessary, with standard therapies including vancomycin and fidaxomicin. Metronidazole can be used in cases of non-severe CDI when access to vancomycin or fidaxomicin is limited. The antibiotics are considered equivalent for the treatment of mild to moderate disease, while vancomycin is more effective for severe disease, including patients infected with the BI/NAP1/027 strain [5]. In addition, a Phase 3 randomized controlled trial and an analysis of a subset of patients with first recurrence have confirmed the noninferiority of fidaxomicin 200 mg administered orally twice daily for 10 days compared with vancomycin 125 mg administered orally 4 times daily (QID) for 10 days for the treatment of first occurrence of mild to moderate CDI and first recurrence, with a primary outcome of clinical cure (88% vs 86% and 88% vs 87% for the 2 trials, P = not significant). Fidaxomicin had a lower recurrence rate compared with vancomycin, and the sustained clinical response (i.e., cure without recurrence) was superior with fidaxomicin versus vancomycin at 25 days after the end of treatment in both trials (70% vs 57%, $P = 0.0011$; 72% vs 57%, $P = 0.0004$) [9, 10]. There are no data on fidaxomicin compared with metronidazole for similar CDI populations.

While most patients with initial CDI respond successfully to antibiotic treatment, approximately 20% to 25% will have at least one recurrence of *C. difficile*-related diarrhea following discontinuation of antibiotic treatment [1, 7, 11]. Recurrent disease can be attributed to relapse or re-infection and the mechanisms are similar in clinical practice. Diagnosis and treatment of relapse and re-infection are the same.

The proportion of patients who respond to treatment declines with multiple recurrences. The ability to cure subsequent recurrences is difficult because of the persistence of spores in the bowel or environment, inability to revert to a more normal eubiotic gastrointestinal microbiota, and the inability of the patient to mount an effective immune response to *C. difficile* toxins. Risk factors for recurrent CDI (rCDI) include administration of inciting antibiotics after initial successful treatment of CDI; defective humoral anti-toxin immune responses; underlying comorbidities; older age; and use of proton-pump inhibitors [7]. Symptoms can recur following re-exposure of patients to *C. difficile* spores transmitted from other patients or from the environment. Almost one-half of recurrences have been shown to be caused by reinfection by a different bacterial strain rather than by relapse [12, 13].

The most common therapy for rCDI is a second course of metronidazole or oral vancomycin; however, the 2018 IDSA guidelines favor the use of vancomycin regimens or fidaxomicin over metronidazole [7]. The estimated efficacy of antibiotic therapy for a first recurrence is 50% [5]. Second recurrences can be treated with fidaxomicin or a tapering and/or pulsed regimen of oral vancomycin. The 2018 IDSA guidelines consider the latter recommendation to be of weak strength and based on a low Quality of Evidence (7). In addition, for a first recurrence, fidaxomicin appears to be non-inferior to vancomycin. However, use of fidaxomicin has been

limited by its high-cost relative to oral vancomycin. Management of patients who do not respond to treatment or experience a further relapse is challenging. Use of other antibiotics with activity against *C. difficile*, such as rifaximin, nitazoxanide, ramplanin, teicoplanin, and tigecycline, is limited by the lack of efficacy data, high cost, unfavorable safety profile, and potential for development of resistance. Probiotics have reported preliminary efficacy, but none have shown efficacy in controlled studies. Additionally, patients with fulminant colitis may require emergency colectomy, which increases their risk for mortality by up to 80% [5].

Given that disruption of the indigenous gastrointestinal microbiota is a major risk for CDI, particularly for recurrent infection, there has been increasing interest in fecal microbiota transplantation (FMT). The procedure involves the transoral or rectal transplantation of feces from a healthy, pretested donor, and cessation of antibiotic use in the recipient. Clinical experience with FMT indicates that this procedure is effective, although the precise components of the gastrointestinal microbiota that provide resistance against *C. difficile* remain undetermined. Disease resolution has been reported to be as high as 89% following a single FMT administration to patients, with demonstrated safety of the FMT itself, even in immunocompromised hosts, in whom there were no SAEs or infectious complications related to the FMT and in whom several procedure-related adverse events (AEs) were reported, with one death due to aspiration during the sedation administered for colonoscopy [14,15]. FMT is also limited by the need to screen healthy donors for transmissible agents, and, in addition, the procedure lacks standardization [7]. Thus, while the FMT approach has provided the clinical evidence that changes to the gastrointestinal microbiota can be effective, there is a strong need for a defined uniform product that can be produced and administered in a standardized and safe manner to a broader spectrum of patients and circumvent the safety issues associated with FMT, which include potential transmission of pathogens.

2.2 VE303 Composition and Characterization

The VE303 Drug Product is a live biotherapeutic product (LBP) consisting of 8 well-characterized clonally derived, nonpathogenic, nontoxigenic, commensal strains of Clostridia, including 5 strains from cluster XIVa, 2 strains from IV, and 1 strain from XVII (Table 2-1). All strains were originally derived from healthy human individuals. The composition of VE303 was rationally selected based upon individual strain and consortia-specific properties. Strains were evaluated for their association with healthy human intestinal microbiomes, absence or decrease in *C. difficile*-related dysbiosis, and lack of overt pathogenic features, and whole genome analysis indicated the absence of virulence and toxin genes in all 8 strains. Results from *in silico* studies predicted that use of this LBP would not result in antibiotic resistance and *in vitro* evaluation confirmed that all strains of the VE303 consortia are susceptible to a number of clinically relevant antibiotics of different anti-bacterial mechanisms. The production of short-chain fatty acids by the bacterial strains of the VE303 LBP, its direct suppression of *C. difficile* growth both *in vitro* and in an *in vivo* murine model demonstrating superior reproducible protection compared with other unique LBPs, and its equivalent efficacy to that provided by a human fecal preparation (as judged by the protective benefit in the cefoperazone mouse model of CDI) all provide evidence that this LBP may be of benefit to patients with CDI.

Illumina and Pacific Biosciences (PacBio) -based whole genome sequence analysis of all 8 strains of Clostridia in VE303 identified the most closely related species for each strain using the One Codex algorithm and whole genome alignment [16].

Table 2-1: VE303 Strain Identity

Strain	Cluster Designation	Closest Relative as Determined by Whole Genome Sequencing[a]
DS-0001	XIVa	*Clostridium bolteae* 90A9
DS-0002	IV	*Anaerotruncus colihominis* DSM 17241
DS-0003	XIVa	*Sellimonas intestinalis*
DS-0004	XIVa	*Clostridium symbiosum* WAL-14163
DS-0005	XIVa	Clostridia bacterium UC5.1-1D4
DS-0006	XIVa	*Dorea longicatena* CAG:42
DS-0007	XVII	*Erysipelotrichaceae bacterium* 21_3
DS-0008	IV	*Clostridium orbiscindens* 1_3_50AFAA

[a] Illumina whole genome sequencing One Codex assignment 25 May 2017

There is evidence of at least 6 mechanisms of action potentially contributing to healthy gut maintenance and resistance to *C. difficile* by bacteria from Clostridium clusters IV, XIVa, and XVII:

1. Conversion of primary bile acids to secondary bile acids which, in turn, may suppress *C. difficile* colonization [17, 18].

2. Niche and nutrient competition within the intestinal environment, which limits the outgrowth of pathogenic microbes, such as *C. difficile* [19, 20].

3. Prevention of inflammation from *C. difficile* toxins [21].

4. Short-chain fatty acid production, which has been reported to be important for maintenance of barrier function [22, 23].

5. Induction of T-regulatory cells, which play a critical role in maintaining intestinal homeostasis by controlling inflammation [24, 25].

6. Direct inhibition of *C. difficile* growth *in vitro* (Vedanta internal report).

2.2.1 Nonclinical Studies

2.2.1.1 *In Vitro* Characterization of VE303

Predictions of the presence of antibiotic resistance genes, mobile genetic elements, and virulence genes have been performed from the high, quality whole-genome VE303 sequences obtained using the Pacific Biosciences platform.

Determination of the minimum inhibitory concentration values of the 8 strains of VE303 was performed employing the Clinical and Laboratory Standards Institute (CLSI) agar dilution method for anaerobe susceptibility testing, M11-A8, using the interpretative criteria for susceptibility and resistance based on CLSI M100S. The following controls were included: all antibiotics were tested against quality control strains, sterility of medium was tested, and growth of all strains was confirmed on medium lacking antibiotic. An assessment of VE303 strains for the presence of antibiotic resistance genes was also performed by analyzing PacBio--derived whole genome sequences. Antibiotic resistance genes were predicted by searching each assembled genome for genes using the Comprehensive Antibiotic Resistance Database Resistance Gene Identifier software (v3.1.1) [26].

Based on the susceptibility testing performed to date and despite the presence of potential antibiotic resistance genes, the 8 bacterial strains in VE303 are susceptible to at least 5 clinically relevant antibiotics that include different antimicrobial classes (piperacillin/tazobactam, amoxicillin clavulanate, imipenem, metronidazole, and tigecycline). Therefore, it is expected that these antibiotics could be used to treat any of the VE303 strains if a rescue therapy is required.

To evaluate the risk of the transfer of antibiotic resistance genes to commensal bacteria, the Sponsor searched for the presence of mobile genetic elements and plasmids in the PacBio-derived VE303 genomes and cross-referenced their presence with the location of predicted antibiotic resistance genes. To date, clinically relevant antibiotic genes have not been associated with mobile genetic elements or plasmids.

The whole genomic sequences of the 8 strains of VE303 were also evaluated for the presence of potential orthologs to known Clostridium toxin and virulence factor genes using the Victors Virulence Factor database (http://www.phidias.us/victors/index.php). An analysis of the 8 PacBio-derived VE303 genomes did not identify any known toxin genes or virulence factors. Although the presence of novel virulence genes cannot be ruled out, these data are consistent with the *in vivo* analyses performed and suggest that the VE303 strains pose a low virulence risk.

Refer to the VE303 Investigator's Brochure for additional details.

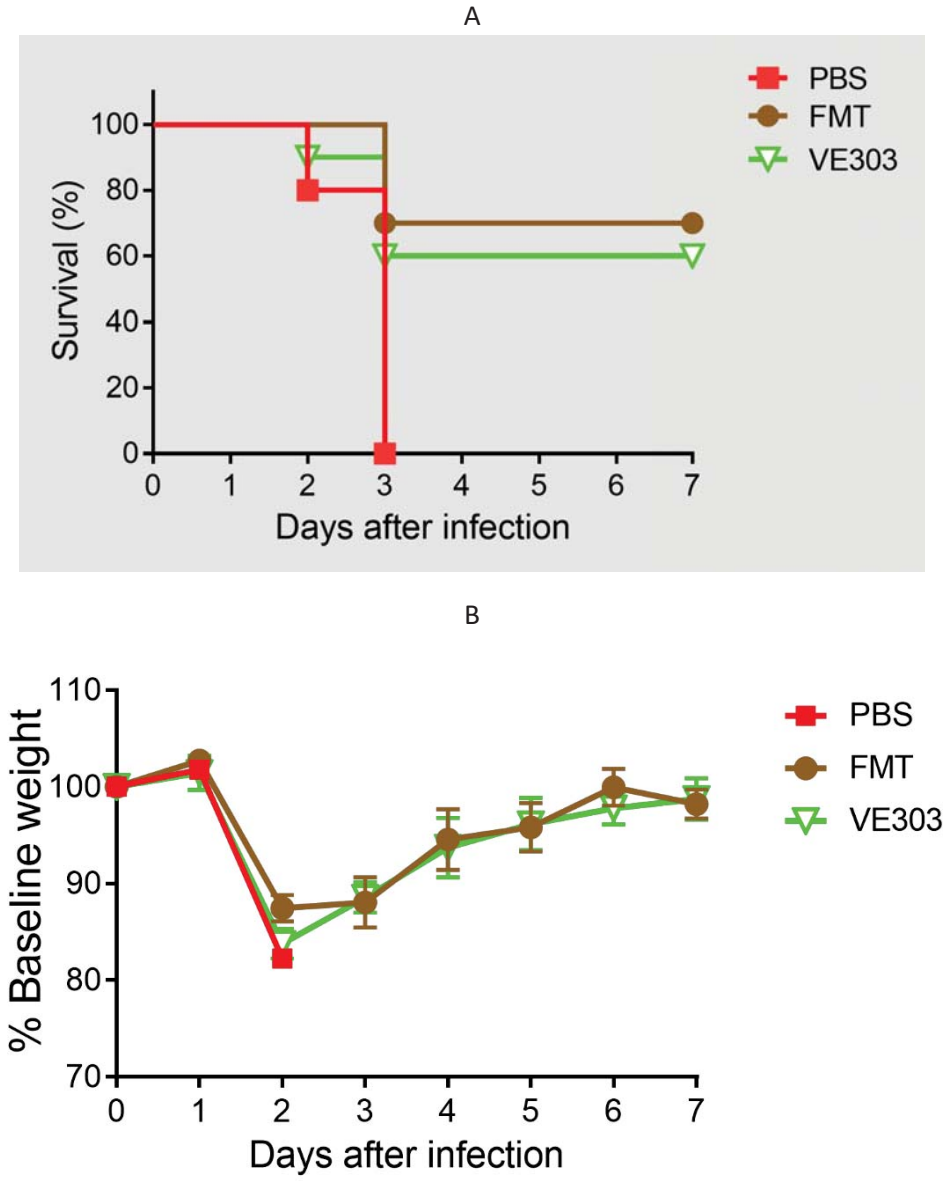
2.2.1.2 *In Vivo* Characterization of VE303

Animal models have been established to study CDI, the most common of which is the hamster model [27]. Recently, mouse models have been increasingly used and, for a variety of reasons, have become the preferred CDI model [28, 29]. Therefore, the Sponsor chose a commonly used mouse model [30] to approximate human CDI in the evaluation of the efficacy of 7 distinct live bacterial products, including VE303.

The initial experiments were performed using female C57Bl/6/J mice treated with the broad-spectrum antibiotic, cefoperazone (500 µg/mL) in the drinking water for 10 days followed by fresh sterile water for 2 days. Animals were then treated with normal saline (negative control) or oral delivery of one of the distinct live bacterial products formulations followed by oral inoculation with *C. difficile*. In these experiments, VE303 demonstrated superior survival compared with 4 live bacterial products comprising a similar bacterial diversity, and equivalent survival compared with 2 live bacterial products comprising a more varied and larger consortium of bacteria.

In a further experiment to confirm the efficacy of VE303 in the mouse model, a VE303 dose of approximately 10^8 colony-forming units (CFU) of total bacteria per mouse (as determined by optical density) was administered to cefoperazone-induced mice as outlined above. Separately, approximately 10^8 CFU of bacteria from a 10% human fecal donor sample was administered to a control group of mice. Following challenge by oral inoculation with *C. difficile*, both FMT and VE303-treated mice were significantly protected against mortality ($P = 0.002$ and $P = 0.038$, respectively) and were not statistically different from each other (Mantel-Cox log-rank test; Figure 2-1A). Weight loss was not different between FMT and VE303 and weight returned to near baseline levels after 6 days in both treatment groups (Figure 2-1B).

Figure 2-1: Survival Benefit and Recovery from Weight Loss Conferred by VE303 and a Human FMT Preparation in the Mouse Cefoperazone-induced CDI Model



Abbreviations: CDI = *Clostridium difficile* infection; FMT = fecal microbiota transplantation; PBS = phosphate-buffered saline

In summary, VE303, a defined preparation containing 8 strains of Clostridia isolated from heathy donors, demonstrated reproducible protection in a mouse model of acute CDI. Six other combinations of live Clostridia bacteria were not as effective using this same model and when tested in the same experiment alongside VE303, even those with greater number and diversity than VE303. Furthermore, VE303 delivered equivalent protection when compared with a human fecal preparation.

Refer to the VE303 Investigator's Brochure for additional information.

2.2.2 Clinical Studies

A Phase 1a/1b, first-in-human, open-label, single-center, dose-escalation study to evaluate the safety and microbiota changes induced by ingestion of VE303 with or without pretreatment with oral vancomycin (Study VE303-01 – "A Phase 1a/1b, First-in-human, Open-label Study of Escalating Doses of VE303 in Healthy Adult Volunteers after Vancomycin to Evaluate Safety, Dosing, and Pharmacodynamics") has been completed and final results can be found in the VE303 Investigator's Brochure.

The primary objective is to characterize the highest safe and well tolerated dose regimen of VE303 by assessing AEs, including gastrointestinal symptoms, physical examinations, vital signs, and changes in clinical laboratory measurements. Secondary objectives include evaluation of gut colonization with VE303 component bacteria, changes in the intestinal microbiota because of VE303 dosing, and metabolomic changes in stool. As of 21 September 2018, thirty-four (34) healthy adult subjects were enrolled and treated with vancomycin (Vancomycin Only cohort [N = 5]) and/or VE303 at escalating single doses (Cohorts 1 [N = 4], 2 [N = 3], and 3 [N = 3]) and multiple doses (Cohorts 4 [N = 6], 5 [N = 8], and 6 [N = 5]) according to pre-defined dose-escalation guidelines at a total daily dose ranging from 1.6×10^9 to 1.7×10^{11} CFU in up to 9 planned dose cohorts, with exploration of intermediate dose levels permitted if necessary. Dose-escalation decisions were determined using pre-specified guidelines by a Safety Review Committee.

Prior to VE303 administration, subjects in Cohorts 1 through 5 received a 5-day course of oral vancomycin at a dose of 125 mg administered QID to modulate the intestinal microbiota and to determine the potential effect of residual vancomycin in the stool on VE303 colonization. In addition, 1 cohort of subjects received only oral vancomycin to serve as a control group (N = 5). Subjects in Cohort 6 were not pretreated with vancomycin and received VE303 at the Cohort 5 dose level for 21 consecutive days. Subjects were admitted to an inpatient unit for baseline assessment, vancomycin dosing and washout (if applicable), for the duration of VE303 dosing, and for 5 days postdose to closely monitor the safety and tolerability of VE303 administration (Cohort 6 did not undergo inpatient postdose monitoring but attended a modified postdose follow-up with increased outpatient monitoring visits). The total duration of inpatient monitoring ranged from 13 to 26 days depending on cohort assignment. Subsequently, postdose follow-up visits are being performed every 2 to 4 weeks for approximately 3 months after initiation of dosing, with continued telephone for approximately 6 months following the first ingestion of study drug. For all VE303-treated subjects who agree and provide written informed consent, additional visits for stool collection are scheduled approximately 6 and 12 months after initiation of VE303 dosing.

Preliminary draft results are available. Demographics for the 34 subjects showed that 20 subjects were male and 14 were female, with a median age of 34 (range 25 to 59) years; 22 subjects were Black or African American, 11 were White, and 1 was mixed/other race. One subject in Cohort 1 discontinued early prior to receiving VE303 due to an inability to tolerate the vancomycin dosing, and 1 subject from Cohort 5 discontinued VE303 dosing early due to an inability to

swallow intact capsules. The remaining 32 subjects (28 of whom received at least 1 dose of VE303) completed dosing assignments as scheduled.

Among the 28 subjects who received at least 1 dose of VE303 at the time of data cutoff, the most frequently reported treatment-emergent adverse events (TEAEs) were largely gastrointestinal in nature and included soft feces (21%), abdominal distension (18%), diarrhea (14%), and abdominal pain, hard feces, headache, and increased lipase (each 11%). Most AEs (regardless of causal relationship to study treatment) were Grade 1 or 2 in severity, with 4 of 28 VE303-treated subjects (14%) experiencing Grade 3 or higher TEAEs on study (all considered not related to VE303), including Grade 4 increased lipase in 2 subjects (7%); Grade 3 increased lipase in 1 subject (4%); and Grade 4 acute kidney injury and Grade 4 increased blood creatine phosphokinase (CPK) in 1 subject (4%).

Among the 28 subjects who received at least 1 dose of VE303, TEAEs were considered by the Investigator to be related to VE303 for 10 subjects (36%), 9 of whom were enrolled in the multiple dose cohorts. The TEAEs that were most frequently attributed to VE303 treatment included abdominal distension and diarrhea (each 11%) and increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), and soft feces (each 7%). All VE303-related TEAEs were Grade 1 in severity.

Among the 29 subjects who received at least 1 dose of vancomycin, TEAEs were considered by the Investigator to be related to vancomycin for 14 subjects (48%). The TEAEs that were most frequently attributed to vancomycin treatment included abdominal distension and soft feces (each 17%), nausea (14%), abdominal pain and diarrhea (each 10%), and decreased appetite, discolored feces, dysgeusia, increased ALT, increased AST, and increased lipase (each 7%). Most vancomycin related TEAEs were Grade 1 or 2 in severity, with Grade 3 vancomycin-related events including increased lipase (7%) and hypotension (3%).

No deaths were reported. One subject in Cohort 2 experienced SAEs of Grade 4 acute kidney injury and Grade 4 increased blood CPK (> 60,000 U/L) that were judged to be unrelated to VE303 and occurred after discharge from the 5-day inpatient follow-up.

Preliminary draft VE303 detection and colonization data are available for Cohorts 1 to 6. In the cohorts that received vancomycin, all 8 of the VE303 component bacteria were detectable in the stool within 2 days after dosing and colonized in a dose-dependent manner. The multiple dose cohorts (Cohorts 4 and 5) had more subjects colonize with more strains for a longer time period; however, durable colonization was observed even in the single-dose cohorts (2, 5, and 10 capsules × 1 day). In many individuals, VE303 bacteria were detectable for at least 12 weeks after the last dose. VE303 bacteria appeared to favorably enhance subjects' microbiota recovery after vancomycin ingestion. VE303 component bacteria colonization was reduced when subjects were not administered vancomycin. When compared with the vancomycin-only control cohort, subjects who ingested VE303 had earlier recovery of beneficial taxa (e.g., Bacteroides and Firmicutes) and reduction in potentially inflammatory taxa (e.g., Proteobacteria).

In summary, these preliminary results demonstrate that single and multiple doses of VE303 ranging up to 1.7×10^{11} total CFU administered over 14 days were well tolerated and safe. VE303 demonstrated early, robust, and durable colonization with early recovery of a normal microbiota in a dose-dependent manner following vancomycin administration.

2.3 Rationale and Dose Selection for the Study

There is no accepted standard of care (SOC) to prevent rCDI. After a first recurrence, the incidence of subsequent CDI recurrence increases, with rates of up to 45% to 65% reported in some studies [31]. Thus, an unmet need exists for the patients who present with their second episode (first recurrence) of CDI, as they are at a higher risk of additional recurrences. Risk factors for multiple recurrent CDI include age, use of antibiotics within 90 days before CDI diagnosis, use of proton pump inhibitors (PPIs) within 90 days of CDI diagnosis, chronic kidney disease, and diagnosis of CDI in a nursing home, among others [32]. This Phase 2 study will be conducted to determine the recommended dose, evaluate the safety, microbiota changes, and efficacy of VE303 compared with placebo following completion of a standard course of antibiotics for subjects with CDI. A double-blind design will be implemented in conjunction with a placebo control in an attempt to mitigate any bias that may be otherwise inherent to evaluating the effects of an investigational drug if compared only with historical controls. Because no SOC exists for the prevention of CDI recurrence after antibiotic treatment, use of a placebo group is not considered to pose any additional risks to subjects compared with the risks they may face in the standard treatment setting outside of a clinical trial. Furthermore, study subjects will be closely monitored for stool changes and diarrhea-like events, particularly those that may signal a recurrence of CDI.

The doses of VE303 to be evaluated in this Phase 2 trial are based on the preliminary safety and VE303 detection and colonization results from the Phase 1 vancomycin-treated normal healthy volunteer trial. The Phase 2 doses will include the higher safe and well tolerated doses from Study VE303-01 (the Cohort 5 dose of 10 capsules per day for 14 days; total of 1.1×10^{11} CFU) and the low dose (2 capsules per day for 14 days 2.2×10^{10} CFU).

Study drug administration will begin on the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing, in order to colonize the subject's intestinal tract as soon as possible. After a 14-day study drug administration period, subjects will be followed at 2 weeks after the last dose and then approximately every 4 weeks through 24 weeks after the last dose of study drug to monitor safety and *C. difficile* and VE303 colonization and clearance. This follow-up period will also provide sufficient time to allow for identification and characterization of any CDI recurrences.

3.0 STUDY OBJECTIVES AND ENDPOINTS

3.1 Objectives

The primary objective is to determine the recommended VE303 Phase 3 dose regimen(s) based on safety and efficacy, as indicated by the CDI recurrence rate.

Secondary objectives are to characterize VE303 colonization and changes in the fecal microbiome.

3.2 Endpoints

Safety endpoints include the overall safety profile and TEAEs.

Efficacy endpoints include:

- Proportion of subjects with CDI recurrence before or at Week 8 (i.e., 8 weeks after the first dose of study drug).
- Proportion of subjects without CDI recurrence before or at Week 4 (i.e., 4 weeks after the first dose of study drug), Week 12 (i.e., 12 weeks after the first dose of study drug), and Week 24 (i.e., 24 weeks after the first dose of study drug).

Pharmacodynamic and pharmacokinetic endpoints include:

- Fecal VE303 component bacteria colonization abundance and duration
- Changes in fecal microbiota diversity and taxonomic composition
- Changes in the fecal metabolomic profile, including short chain fatty acids and bile acids

4.0 STUDY DESIGN

4.1 Study Design Overview

This Phase 2, randomized, parallel-arm, double-blind, placebo-controlled, dose-selection study will evaluate the safety, microbiota changes, and efficacy of VE303 in the prevention of subsequent CDI-associated diarrhea compared with placebo following completion of at least 1 successful course of SOC antibiotics for subjects with primary *C. difficile* infection (pCDI) at high risk for recurrence or subjects with rCDI. Eligible subjects will be randomized into 4 treatment arms in a 2:1:2:1 ratio to receive VE303 high dose (10 capsules):placebo high dose (10 capsules):VE303 low dose (2 capsules):placebo low dose (2 capsules), respectively.

The Sponsor study clinical operations team that interacts with the sites and the database will remain blinded to administrative IA results, as well as the treatment assignments, until the final analysis dataset is cleaned, locked and unblinded.

During the execution of the study, which coincided with the COVID-19 pandemic, changes to the study design were introduced, which included deleting the sample size re-estimation, adding 3 administrative IAs, and modifying the overall sample size to approximately 60 to 80 subjects. Three administrative IAs will be performed after at least 24 subjects complete 8 weeks of the study, after at least 39 subjects complete 8 weeks of the study, and after at least 54 subjects complete 8 weeks of the study.

Per protocol version #6, randomization will be stratified by 1) number of previous CDI episodes at Baseline (0 vs ≥ 1 episodes, excluding the qualifying episode i.e., Inclusion criterion 2a vs 2b/2c subjects), 2) SOC antibiotic treatment (non-vancomycin [fidaxomicin or metronidazole] or an alternative oral vancomycin dosing regimen vs vancomycin QID [four times a day; this category will include subjects who receive vancomycin QID on at least the last day of treatment]), and 3) results of CDI laboratory diagnostic method (free toxin [enzyme immunoassay (EIA) for Toxin A/B or Cell Cytotoxicity Neutralization Assay (CCNA)] vs other [polymerase chain reaction (PCR)/cytotoxic culture if EIA for Toxin A/B and/or CCNA results are negative or not available]).

On the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing, subjects will be randomized and begin daily oral administration of placebo or VE303(both at low and high doses) for 14 consecutive days. The high dose of VE303 is 10 capsules per day administered orally for 14 days, for a maximum VE303 daily dose of 8.0×10^9 CFU and a maximum total dose over 14 days of 1.1×10^{11} CFU. The low dose of VE303 is 2 capsules per day administered orally for 14 days, for a maximum VE303 daily dose of 1.6×10^9 CFU and a maximum total dose over 14 days of 2.2×10^{10} CFU. The first dose of the study drug will be administered under the supervision of study personnel at the Day 1 visit. After the 14-day dosing period is completed, follow-up will be performed every 2 to 4 weeks for 24 weeks after the first dose of study drug.

Safety will be evaluated continuously for the duration of a subject's study participation, including assessment of AEs reported by a subject or observed by study personnel from the date of informed consent until the end of the 6-month follow-up period, evaluation of gastrointestinal symptoms, electrocardiograms, physical examination, assessment of vital signs, and clinical

laboratory measurements. Stool samples must be collected for *C. difficile* testing (e.g., GDH, EIA for toxin A/B, *C. difficile* PCR, CCNA) and for the exploratory analyses (microbiota composition, VE303 component bacteria detection, antibiotic concentrations, metabolomics, culture, detection of antibiotic resistant bacteria other than *C. difficile* (e.g., carbapenem-resistant Enterobacteriaceae [CRE], extended spectrum β-lactamase producing Enterobacteriaceae [ESBL], or vancomycin-resistant Enterococcus [VRE]), and calprotectin). Subjects will be continuously monitored for clinical events of diarrhea that may be indicative of a CDI recurrence.

For each subject, the duration of study drug administration will be 14 days and the total study participation will be approximately 7 months from Screening through the last follow-up visit.

4.2 Study-Specific Definitions

An *episode of diarrhea consistent with diarrhea due to C. difficile* is characterized by ≥ 3 loose/unformed bowel movements (Bristol Score 5-7, see Appendix 1) within 24 hours for at least 2 consecutive days or > 8 loose/unformed bowel movements within 24 hours, and considered unlikely to have another etiology.

The following are specific protocol definitions:

A *prior occurrence* of CDI is based on a subject's medical history and defined as an episode of diarrhea consistent with diarrhea due to *C. difficile*, with a stool sample that is positive for *C. difficile* (e.g., PCR, EIA for Toxin A/B, and GDH antigen, or CCNA). The CDI episode must have initially responded to SOC antibiotic treatment, with no other identifiable cause for the diarrhea.

A *qualifying* CDI *episode* is defined as an episode of diarrhea consistent with diarrhea due to *C. difficile*, which includes a positive diagnostic stool test for free toxin or toxigenic *C. difficile*.

- Diagnostic tests for enrollment may be performed at the local laboratory or at the central laboratory. Subjects with a positive test (either EIA for Toxin A/B or PCR or CCNA or toxigenic culture assay, performed at the local laboratory or at the central laboratory) may be enrolled in the study. Confirmation of a positive local test by central laboratory test is not required for subject enrollment, provided documentation of a local positive test is available.
- Subjects' allocation to a proper stratum is dependent on timely reports of laboratory results from both local and central laboratories. To ensure subjects are allocated to a proper stratum, an effort should be made to obtain and send a stool sample to the central laboratory for confirmation of free toxin or toxigenic *C. difficile* prior to randomization.
- Additional requirements are provided in Inclusion Criteria #3 and #4.

A *successful clinical response* to antibiotic therapy by the time of randomization to the study treatment is required for a subject to be eligible. A *successful clinical response* is defined as the symptomatic control of the qualifying/current CDI episode, i.e., < 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days.

An *on-study CDI recurrence* is defined as an episode of diarrhea consistent with diarrhea due to *C. difficile* infection, which includes a stool sample positive for free toxin (EIA for Toxin A/B or CCNA) per test performed at the local laboratory or at the central laboratory. An on-study CDI recurrence may occur at any time after administration of the first dose of study drug.

- If recurrent CDI is suspected, a stool sample must be collected for laboratory confirmation. Regardless of the test(s) performed at the local laboratory, samples must be collected from all subjects during each episode of diarrhea for CDI testing at a central laboratory.
- After an initial sample collected for suspected recurrence, it is required to collect and submit samples on subsequent day(s) if the laboratory test results are not available or negative for free toxin (EIA for Toxin A/B or CCNA) before initiating antibiotic therapy.

4.3 Study Dosing Regimens

On the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing, eligible subjects will be randomized into 4 treatment arms in a 2:1:2:1 ratio to receive 1 of 2 placebo doses or 1 of 2 VE303 doses daily for 14 consecutive days as outlined in Table 4-1. Each study dose will comprise 2 or 10 capsules per day administered orally (see Section 6.3). Subjects will ingest the first dose in the clinic on Day 1 and subjects will remain at the study site for at least 30 minutes after the first dose for monitoring of potential AEs.

Table 4-1: Planned Study Arms

Study Arm	VE303 Daily Dose	VE303 Total Dose Over 14 Days	Number of Capsules Ingested Per Day (Active/Placebo)
VE303 (low)	1.6×10^9 CFU	2.2×10^{10} CFU	2 (2/0)
VE303 (high)	8.0×10^9 CFU	1.1×10^{11} CFU	10 (10/0)
Placebo (“low”)	Not applicable	Not applicable	2 (0/2)
Placebo (“high”)	Not applicable	Not applicable	10 (0/10)

Abbreviations: CFU = colony-forming unit(s)

4.3.1 Subject Monitoring

Study personnel will telephone outpatient subjects daily during the 14-day study drug administration period to ensure dosing compliance; explore any concerns or issues with study drug ingestion, administration, handling or storage; capture potential AEs; review potential *C. difficile*-associated symptoms and assess the need for an unscheduled visit or stool test for CDI; remind subjects to collect stool to bring to their next clinic visit (if applicable); and answer any questions that the subject may have. If a subject is inpatient or attending a site visit on a scheduled telephone follow-up day, this contact will be performed in person, when possible. Telephone follow-up may be omitted on weekends or public holidays if this is necessary for logistical reasons.

After completion of the study drug administration period (Days 1 through 14, inclusive), postdose monitoring visits will be performed for 24 weeks after the first dose of study drug. In

addition, telephone follow-up will be performed at Week 12/Day 84, Week 16/Day 112, and Week 20/Day 140 to monitor safety and recurrence of diarrhea.

Any clinical event of diarrhea or loose/watery stools will be recorded as an AE. Subjects who are suspected of having a possible recurrence (e.g., subjects who have ≥ 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days, or > 8 loose/unformed bowel movements within 24 hours, or diarrhea of concern to the subject or Investigator) will contact study personnel as promptly as possible for assessment and to arrange for collection of a stool sample, as further detailed in Section 9.3.1.

4.4 Guidance on Study Drug Discontinuation for Suspected On-Study CDI Recurrence

As per the Primary Efficacy Analysis, the discontinuation of study treatment for subjects with suspected on-study CDI recurrence is based on the results of free toxin test (EIA for Toxin A/B, or CCNA) performed at the local or the central laboratory regardless of the results of toxin PCR. If initial results of free toxin test are negative or not available and the symptoms persist, repeated samples should be collected and tested on subsequent day(s). The subjects will be continuously monitored (more than once/day by telephone) to determine if the suspected rCDI persists and the study drug needs to be discontinued.

- If a subject has a new *episode of diarrhea consistent with diarrhea due to C. difficile (Section 4.2)* after starting the study drug but the free toxin test (EIA for Toxin A/B or CCNA) is negative or unavailable at the time and the SOC antibiotic is not administered for this episode, the study treatment should be continued. This episode will not be considered a CDI recurrence if free toxin test (EIA for Toxin A/B or CCNA) is negative or not available.
- If a subject has a new *episode of diarrhea consistent with diarrhea due to C. difficile* after starting the study drug but the free toxin test (EIA for Toxin A/B or CCNA) is negative or unavailable at the time and the SOC antibiotic is administered for this episode, the study treatment should be discontinued. This episode will not be considered a CDI recurrence if free toxin test is negative or not available. The discontinuation of study drug due to SOC antibiotic treatment in such a case will be considered a protocol deviation and should be reported as such in the eCRF.
- If the subject has a new *episode of diarrhea consistent with diarrhea due to C. difficile* after starting the study drug and the free toxin test (EIA for Toxin A/B or CCNA) is positive, the study treatment should be discontinued. This episode will be considered a CDI recurrence. Such an event should be reported as confirmed recurrence in the eCRF.

4.5 Data Monitoring Committee

The DMC will comprise independent clinicians with knowledge of the target population, a biostatistician, and Sponsor representatives. Meeting frequency, procedures for halting study enrollment and study drug administration, and details regarding the data to be reviewed are described in the DMC Charter.

5.0 SUBJECT POPULATION

5.1 Selection of Subjects

Subjects who are candidates for enrollment will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria outlined in Sections 5.1.1 and 5.1.2 have been satisfied and that the subject is eligible.

5.1.1 Inclusion Criteria

Subjects must meet all of the following to be eligible:

1. Able and willing to provide written informed consent prior to initiation of any study-specific procedure or study drug administration and understands the potential risks and benefits of study enrollment and study drug administration. When appropriate, informed consent may be provided by a legally authorized representative (LAR).

2. Subjects with a *qualifying* CDI *episode* in at least one of the following categories:

    a. ≥ 18 years of age with a second or greater occurrence of CDI within the last 6 months

    b. Subjects ≥ 65 years of age with or without prior episodes of CDI who exhibit one or more of the following risk factors for recurrence:

        - Have kidney dysfunction, defined as creatinine clearance of < 60 mL/min/1.73 m^2 at the time of the current CDI episode
        - Have a history of regular use of PPIs within the past 2 months and will be continuing use of PPIs throughout the study
        - Have a history of CDI at any time

    c. Subjects ≥ 75 years of age

3. The *qualifying episode* must meet all of the following criteria:

    a. New onset of ≥ 3 loose/unformed bowel movements (i.e., Types 5 to 7 on the Bristol stool scale, see Appendix 1) within 24 hours for 2 consecutive days or > 8 loose/unformed bowel movements within 24 hours

    b. CDI symptoms started within 30 days (inclusive) prior to the day of randomization

    c. Stool sample collected before (or no later than 72 hours after) initiation of SOC antibiotic therapy and positive for CDI laboratory test as defined in the "qualifying CDI episode" section (NOTE: every effort must be made to collect the diagnostic sample before SOC antibiotic therapy or within 24 hours after SOC antibiotic therapy initiation, whenever possible)

d. Diarrhea considered unlikely to have another etiology

4. Prior to randomizing a subject to receive the first dose of the study medication, a subject should:

   a. Receive and complete an Investigator's choice SOC antibiotic regimen of a minimum of 10 days and up to 21 days of total duration (NOTE: antibiotic tapering is not allowed)
   b. Meet successful clinical response defined as the symptomatic control of the qualifying/current CDI episode, i.e., < 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days
   c. Have a positive *C. difficile* diagnostic stool test by either EIA for Toxin A/B or PCR or CCNA or toxigenic culture assay performed at the local laboratory or at the central laboratory

5. Females of childbearing potential must have a negative pregnancy test and must agree to either use a highly effective acceptable form of birth control (e.g., established hormonal birth control plus a barrier method, double barrier method: intrauterine device plus condom or spermicidal gel plus condom) or remain celibate during the study period and up to 3 months after the last dose of study drug.

6. Able to receive the first dose of study drug on the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing.

7. Able and willing to follow study procedures (e.g., ingest up to 10 size 0 (2 cm) capsules per day for 14 days, comply with study visits, provide stool samples, and record information/interact with study tools).

8. Recovered from any complications of severe or fulminant CDI and be clinically stable by the time of randomization.

5.1.2 Exclusion Criteria

Subjects meeting any of the following are not eligible:

1. History of diarrhea (defined as 3 or more loose stools per day lasting for at least 4 weeks) that is not related to *C. difficile* infection within the 3 months prior to randomization.

2. Known or suspected toxic megacolon /or small bowel ileus at the time of randomization.

3. Contraindication to oral/enteral therapy (e.g., severe reflux, severe nausea/vomiting, or ileus) at the time of randomization.

4. Presence of white blood cell (WBC) count > 15×10^9 cells/L, or body temperature > 38.5 °C at the time of randomization.

5. Prior administration of genetically modified investigational live bacterial/fungal/bacteriophage/viral isolates for CDI-associated diarrhea (Exception: use of yogurt or other food products containing live bacterial cultures or over the counter probiotics is permitted).

6. History of administration of fecally-derived investigational live biotherapeutic products, or fecally-derived live bacterial isolates for CDI-associated diarrhea including FMT within the last 6 months.

7. Use of drugs that alter gut motility (e.g., loperamide, diphenoxylate) within 3 days prior to the planned first dose of study drug.

8. Planned administration of oral or parenteral antibacterial therapy for a non-CDI indication after randomization.

9. History of acute leukemia or hematopoietic stem cell transplantation or myelosuppressive chemotherapy within 2 months prior to randomization.

10. Subjects with compromised immune system, including:

    a. Absolute neutrophil count (ANC) of < 0.5×10^9 cells/L on 2 consecutive occasions within 7 days prior to randomization or sustained ANC < 1×10^9 cells/L

    b. Corticosteroid use at daily doses higher than 20 mg of prednisone equivalent within 14 days prior to randomization, with the exception of inhaled or topical corticosteroids, which are permitted.

    NOTE: subjects with well-controlled human immunodeficiency virus (HIV) infection and CD4 count > 300 cells/mcL may be enrolled, provided that all other protocol eligibility requirements are met.

11. Current or immediate potential for mechanical ventilation or vasopressors for hemodynamic support.

12. Life expectancy of < 3 months.

13. Major gastrointestinal surgery (e.g., significant bowel resection or diversion) within 3 months prior to randomization, current ileostomy, or history of total colectomy. NOTE: appendectomy, cholecystectomy, or restrictive procedures, such as banding, may be permitted upon discussion with the Medical Monitor if at least 1 month since surgery has elapsed and upon the subject's full recovery.

14. Anticipated admission to an intensive care unit during or after the study drug administration treatment period. NOTE: nursing homes, rehabilitation units, assisted living centers, and acute care hospitals are acceptable.

15. Female subject who is pregnant or breastfeeding.

16. Known hypersensitivity/allergy/intolerance to any ingredient in the VE303 study formulation (sucrose, histidine, yeast extract, cysteine, metabisulfite, and micro-crystalline cellulose).

17. Clinically significant medical or surgical condition not mentioned in the above criteria that, in the Investigator's opinion, could interfere with the administration of study drug, interpretation of study safety or efficacy data, or compromise the safety or well-being of the subject.

18. History of confirmed celiac disease, inflammatory bowel disease, short gut, gastrointestinal tract fistulas, or ischemia.

6.0 INVESTIGATIONAL MEDICINAL PRODUCT AND CONCOMITANT MEDICATIONS

Within this protocol, "study drug" refers to either VE303 or placebo.

Detailed instructions for the storage, handling, preparation, and administration of study drug are provided in the Pharmacy Manual.

6.1 VE303 and Placebo Supply

VE303 is formulated as a size 0 enteric capsule for oral administration. Each capsule contains approximately 400 mg of a mixture of 1×10^8 CFU of lyophilized bacteria from each of 8 clonally derived and distinct Clostridial species for a total bacterial content of 8×10^8 CFU per capsule. Additionally, the capsule contains sucrose, histidine, yeast extract, cysteine, metabisulfite, and microcrystalline cellulose as excipients.

The enteric capsule is designed to begin disintegration from 0 to 100% over a 2-hour period at a pH of 6.8. Thus, the capsule will not disintegrate in the pH environment present in the empty stomach (pH of an empty stomach is 1-2, pH after a meal is up to 5), but will begin to disintegrate in the upper small intestine (pH of 6.15 to 7.35).

Capsules are packaged 2 or 10 per 30 mL sealed high-density polyethylene bottle. Each bottle will contain the appropriate number of VE303 and/or placebo capsules for 1 full day of dosing. The study drug will be packaged as 14 bottles per kit. Each subject will receive 1 kit for a total of 14 days of dosing.

Placebo capsules containing microcrystalline cellulose will be visually identical to and not discernible from VE303 capsules. However, placebo capsules will not contain any bacterial strains or formulation buffer used in the VE303 drug product.

Labeled, packaged VE303 will be shipped to the study site(s) by a Good Manufacturing Practice compliant cold chain supply organization.

6.2 Study Drug Dispensing

Randomization will be performed using an Interactive Web Response System. Study drug kits will be dispensed to subjects by the clinical site. Documentation of each bottle and information on the label will be recorded in the drug dispensing record. Drug accountability and compliance will be recorded in the drug dispensing record and subject source documents.

6.3 Study Drug Administration

6.3.1 General Administration

The first dose of study drug (2 or 10 capsules) will be administered on study Day 1 under the supervision of study personnel, and subjects will remain at the study site for at least 30 minutes after the first dose for monitoring of potential AEs.

Study drug (2 or 10 capsules per day, depending on cohort assignment, from a single study drug bottle) will be ingested orally daily at approximately the same time each day and prior to 4 PM.

If the subject has difficulty taking all capsules at the same time, the subject can take with some time intervals, but all capsules should be taken prior to 4 PM each day. If a study drug dose is missed and discovered before 4 PM, the dose may be taken that day, as long as the subject has not eaten for at least 1 hour prior to the delayed dose and will not eat for at least 1 hour after the dose following the administration guidelines.

All capsules should be ingested with a beverage of choice that contains no solid food substances (i.e., water is ideal, but any clear or carbonated beverage may be used). Beverages should be at room temperature, cool, or warm (not hot) to avoid any effect on study drug contents.

Anyone who touches or handles the study drug capsules (not necessary if only handling the plastic bottles) should wash their hands after handling.

6.3.2 Missed or Partial Dosing

All efforts should be made to ensure that a subject takes all assigned capsules each day for 14 days. However, if a study drug dose is missed on a given study day, and this is discovered before 4 PM, the dose may be taken that day as long as the subject has not eaten for at least 1 hour prior to the delayed dose and will not eat for at least 1 hour after the dose following the administration guidelines in Section 6.3.1.

If the missed dose is discovered after 4 PM, the dose should be skipped and the assigned once daily dosing regimen should resume the next day according to the original schedule (i.e., the dose should not be doubled the next day). If study drug is missed or interrupted on any study day(s), the end of the study drug administration period will remain fixed at a maximum of 14 days after starting therapy.

The same instructions as above are to be followed if a study drug dose has only been partially ingested prior to 4 PM on a given study day. Subjects will be instructed to make every effort to consume all assigned capsules before 4 PM. If a subject misses or only partially completes a dose, the subject will be instructed to contact study site personnel to review the requirements for compliance with the dosing guidelines outlined in Section 6.3.1.

6.4 Accountability and Dosing Compliance

Subjects will be instructed to return all study drug supplies (including empty, partially empty, and full bottles) at the end of the treatment period for accountability and compliance review by study personnel. Study personnel will reinforce dosing instructions and answer subject questions at each clinic visit and during telephone follow-up.

6.5 Antibiotic Rescue Therapy Guidance

If systemic antibiotics are deemed necessary during this study for a presumed infection, and VE303 component bacteria may be implicated, consideration should be given to the use of one or more of the 5 following broad spectrum antibiotics to which all of the bacteria contained in VE303 are susceptible (based on *in vitro* testing – refer to the VE303 Investigator's Brochure for more details), according to institutional standard of care guidelines regarding antibiotic dose, route, and frequency:

- piperacillin/tazobactam

- amoxicillin clavulanate
- imipenem
- metronidazole
- tigecycline

This list of antibiotics, in addition to the list of VE303 component bacteria, must be reported to health care personnel if admission to a hospital or emergency department is warranted and reported in the electronic case report form (eCRF).

NOTE: If VE303 component bacteria are implicated in an intestinal infection and an oral antibiotic is desired, oral metronidazole or amoxicillin clavulanate should be considered. Oral vancomycin is not recommended, as 2 of the bacterial strains in VE303 are considered resistant to vancomycin based on established susceptibility breakpoints. Oral fidaxomicin guidance cannot be provided, as susceptibility breakpoints have not been established for fidaxomicin.

7.0 STUDY PROCEDURES AND ASSESSMENTS

Before recruitment of subjects, written Institutional Review Board (IRB)/Ethics Committee (EC) approval of the protocol, informed consent documents, advertising and subject-facing documents will be obtained.

Study procedures and assessments are summarized in Table 7-1 (Schedule of Events).

Detailed instructions for the processing, handling, packaging, and shipping of hematology, clinical chemistry, and all other specimens will be outlined in the study manuals provided to each investigative site.

7.1 Informed Consent

Informed consent must be obtained before any study-specific procedure is performed. This study may implement a Pre-Screening process (when approved by IRB/IEC and permitted by related institution) to determine preliminary subject eligibility in advance of the formal Screening process. For subjects who sign a Screening written informed consent form but are not randomized, the reason for screen failure will be recorded.

7.1.1 Optional Pre-Screening Informed Consent

Once identified as potential study candidates, where applicable, subjects or their LAR will sign a Pre-screening informed consent form to enable collection of a stool sample, which may be collected at home by a qualified home healthcare provider, to confirm the qualifying event of CDI recurrence in accordance with eligibility criteria. A history of *C. difficile* infection and medications may also be reviewed.

7.1.2 Screening

Once identified as potential study candidates, subjects or their LAR will sign an informed consent form to enable collection of a stool sample to confirm the qualifying event of pCDI high risk for recurrence or rCDI in accordance with eligibility criteria. The stool collected on this day will also be used for baseline evaluations of stool microbiota composition, VE303 component bacteria detection, antibiotic concentrations, metabolomics, culture, detection of antibiotic resistant bacteria other than *C. difficile* (e.g., CRE, ESBL, or VRE), and calprotectin. Physical examination findings, vital signs, safety laboratory measurements, medical history, and medications will also be reviewed (see Table 7-1).

Results from Screening evaluations, including laboratory results and CDI confirmation, are required to confirm eligibility and must be available before randomization and study drug administration.

7.2 Randomization and Blinding

After all Screening procedures have been performed and the SOC antibiotic therapy completed, eligibility has been confirmed on Day 1, a centralized interactive web response system will assign each subject a randomization number and subjects will receive study drug.

Investigators, study subjects and the Sponsor study clinical operations team that interacts with the sites and the database will remain blinded to IA results, as well as the treatment assignments, for the full duration of the study period until the final analysis dataset is cleaned, locked and unblinded. Select members of the Sponsor's senior management team, who do not interact with study sites and are not involved in execution of the study, will be unblinded at the treatment group level according to the approved Data Access and Unblinding Plan for development and future planning purposes only. This unblinding will have no impact on the ongoing study. In addition, as spelled out in the Data Access and Unblinding Plan, relevant members of the Systems Biology Department will be unblinded at the subject level to enable effective analysis of subject samples.

7.2.1 Breaking the Blind

Unblinding of study drug assignment may be requested (see contact information on page 1) in an emergency if unblinding is considered necessary for the medical management of a subject. The Investigator will discuss the circumstances for the requested unblinding with the Sponsor's Medical Monitor. Emergency unblinding without the Medical Monitor's input should occur only if knowledge of study drug assignment will affect the immediate workup or treatment of an AE. Communications related to unblinding will be documented, including the date, time, and reason for the requested unblinding.

7.3 Safety Assessments

Safety will be assessed throughout the study and the safety data will include reported AEs, SAEs, gastrointestinal symptoms, clinical laboratory data, electrocardiograms (ECGs), concomitant medications, physical examinations, and vital signs.

7.3.1 Demographics and Medical History

Subject demographics and past and present medical history will be recorded at Screening. Ongoing medical conditions and signs and symptoms observed prior to the time a subject signs the informed consent form will be recorded as medical history.

In addition to general medical history, specific information relative to the qualifying CDI episode at presentation will include:

- Date of onset of clinical symptoms of the qualifying CDI episode
- Whether the subject was inpatient (e.g., in an acute care hospital) or outpatient at the time of onset of clinical symptoms of the qualifying CDI episode
- Method(s) of local laboratory diagnosis of *C. difficile* for the qualifying CDI episode
- Whether the qualifying CDI episode represented the first, second, or later, and time since previous CDI episode
- Antibiotic/treatment regimen for the qualifying episode
- Total number of unformed (loose or watery) stools within a 24-hour period for the qualifying CDI episode

- Highest blood WBC count associated with the qualifying CDI episode
- Date of a successful clinical response defined as the symptomatic control of the qualifying/current CDI episode, i.e., < 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days.

Dates of onset and antibiotic/treatment regimens used for prior CDI episodes within the 6 months before the day of randomization also will be recorded.

7.3.2 Prior and Concomitant Medications

All medications and supplements, including those administered for treatment of the qualifying CDI episode, administered within 60 days prior to Screening through the end of the study will be recorded in the eCRFs using generic drug names when possible. Prior medications are those administered prior to study drug administration, and concomitant medications are those administered from the time of the first dose of study drug through the end of the study.

Concomitant medications may be administered at the Investigator's discretion to conform to standard practice, including routine medications a subject will continue during study participation as well as newly prescribed medications, except for those excluded medications described in Section 7.3.2.2. Overall, VE303 has a low likelihood for eliciting clinically relevant drug-drug interactions; however, no formal drug-drug interaction studies have been done and the Investigator will need to use caution when prescribing concomitant medications. The Investigator is strongly encouraged to contact the Medical Monitor when unsure of a possible drug-drug interaction.

Initially, if clinically appropriate, gastrointestinal symptoms or signs should be observed to determine whether they resolve without therapy. For routine treatment of constipation, a glycerin suppository per rectum is recommended as the initial treatment (other agents may cause interference with the colonization and detection of VE303 component bacteria) but other agents may be used at the Investigator's discretion.

7.3.2.1 Concomitant Antacids and Acid Reducers

Use of medications changing the gastric acidity may potentially reduce the effectiveness of VE303. Use of these medications should be restricted to the following:

- Subjects should not consume food or fast-acting antacids (e.g., calcium-, aluminum-, or magnesium-containing products such as TUMS® or Maalox®) for at least 1 hour before and at least 1 hour after study drug ingestion.
- Subjects should not receive a histamine$_2$-receptor antagonist (ranitidine, famotidine, etc.) within the 12 hours before or 1 hour after study drug ingestion.
- Subjects should not receive a proton-pump inhibitor (omeprazole, pantoprazole, etc.) within the 12 hours before or 1 hour after study drug ingestion.

7.3.2.2 Prohibited Therapies and Substances

The following therapies are prohibited during the 14-day study drug administration period, unless otherwise specified or necessary for management of a medical condition. If any of the

following are medically indicated and prescribed, these should be entered in the eCRF as concomitant medications:

- Drugs that alter gut motility (e.g., loperamide, diphenoxylate)
- Oral or parenteral antibacterial therapy for a non-CDI indication
- Corticosteroids at daily doses higher than 20 mg of prednisone equivalent, with the exception of inhaled or topical corticosteroids, which are permitted.
- Genetically modified investigational live bacterial/fungal/bacteriophage/viral isolates for CDI-associated diarrhea, with the exception of live-bacteria-containing food products (such as yogurt) or over the counter probiotics.
- Fecally-derived investigational live biotherapeutic products, or fecally-derived live bacterial isolates for CDI-associated diarrhea including FMT
- Other investigational products (through Week 8/Day 56)

7.3.3 Adverse Events

Adverse events will be assessed by direct observation and subject assessments/interviews from the time a subject signs the informed consent form through Week 24/Day 168. Complete details on the definition, reporting, and management of AEs, including AEs of special interest, are provided in Section 9.0.

7.3.4 Gastrointestinal Symptoms Self-Administered Questionnaire

Subjects will be evaluated for gastrointestinal symptoms using the self-administered Patient-Reported Outcomes Measurement Information System (PROMIS®) scale questionnaire in order to assess 8 gastrointestinal symptoms (belly pain, bowel incontinence, constipation, diarrhea, disrupted swallowing, gas/bloating, nausea/vomiting, and gastroesophageal reflux; see Appendix 2). The questionnaires are intended to be filled out by the subject or LAR after training by the site staff. To obtain an adequate baseline, questionnaires must be completed before the first dose of study drug on Day 1. Subsequently, questionnaires should be completed on Days 7 and 14. All attempts must be made to have questionnaires completed at approximately the same time of day at each time point outlined in the protocol.

7.3.5 Vital Signs

Vital signs (pulse, blood pressure, and body temperature) will be collected at Screening and on Days 1, 7, 14, 28, 56, and 168 (end of study) as well as at unscheduled times as medically indicated. Subjects are required to remain in the supine position for at least 3 minutes prior to obtaining vital signs. On Day 1, vital signs will be measured prior to administration of study drug.

Abnormal vital sign measurements will be recorded as AEs only if they are considered to be clinically significant by the Investigator.

7.3.6 Physical Examination

Physical examinations will be performed by trained medical personnel. A complete physical examination will be performed at Screening. On Days 1, 14, 28, 56, and 168 (end of study), physical examinations will include abdominal examinations, with limited symptom-directed examinations performed for other organ systems. Abnormal physical examination findings will be recorded as AEs only if they are considered to be clinically significant by the Investigator.

Height will be collected at Screening only. Weight will be collected at Screening and on Days 1, 14, 28, 56, and 168 (end of study). Weight is to be measured consistently in the same manner each time and noted in the source –without heavy items/shoes/bulky clothing, etc.

7.3.7 Laboratory Tests

The following laboratory tests will be performed:

- Hematology (performed at Screening and on Days 1, 7, 14, 28, 56, and 168 [end of study]): Complete blood count (CBC) with differential.
- Serum chemistry (performed at Screening and on Days 1, 7, 14, 28, 56, and 168 [end of study]): sodium, potassium, chloride, albumin, alkaline phosphatase, direct and total bilirubin, ALT, AST, gamma-glutamyl transferase, blood urea nitrogen, calcium, creatinine, phosphorus, glucose, lactate dehydrogenase, amylase, and lipase.
- Urinalysis (performed at Screening and on Day 14) in accordance with the Laboratory Manual.
- Pregnancy testing (performed at Screening [serum] and Day 1 [urine]) for females of childbearing potential. Day 1 pregnancy test results must be negative prior to initiation of study drug dosing.

The diagnosis corresponding to clinically significant abnormalities (including abnormalities requiring treatment/intervention) observed after a subject signs the informed consent will be recorded as AEs.

7.3.8 12-Lead Electrocardiogram

Single 12-lead ECGs will be performed at Screening, Day 28, and at unscheduled time points as medically indicated. ECG parameters to be evaluated include the respiration rate, QT interval corrected for heart rate (QTc), QRS, and PR intervals. Post-baseline abnormal ECG measurements will be recorded as AEs if they are considered to be clinically significant by the Investigator.

7.4 Stool Analyses

Stool samples must be collected at the time points specified in Table 7-1 for *C. difficile* testing (e.g., GDH, toxin A/B, *C. difficile* PCR, CCNA) and for exploratory analyses (microbiota composition, VE303 component bacteria detection, antibiotic concentrations, metabolomics, culture, detection of antibiotic resistant bacteria other than *C. difficile* (e.g., CRE, ESBL, or VRE), and calprotectin. Whenever possible, stool sample collection should occur at the study site. However, because it is important to collect a stool sample at scheduled time points or while

a subject is symptomatic, subjects will be provided with a kit and instructions for stool sample collection at home, if necessary. Applicable instructions will be provided with the Laboratory Manual.

7.4.1 Stool *C. Difficile* and Other Stool Testing

A stool sample must be collected and submitted for *C. difficile* testing as soon as a potential qualifying CDI episode is identified based on the criteria outlined in Section 4.2. Ideally, this stool sample will be obtained prior to starting SOC antibiotics for CDI; however, if SOC antibiotics have been started, the stool sample will be collected no later than 72 hours after the first antibiotic administration. Every effort must be made to collect the diagnostic sample before SOC antibiotic therapy or within 24 hours after the start of SOC antibiotic therapy.

Throughout the study, stool samples collected at every scheduled time point will be sent to a central laboratory for exploratory analyses, and aliquots of each sample will be stored at the central laboratory. Under the direction of the Sponsor, stored stool samples may be used for additional testing, specifically, characterization of other stool bacteria and pathogen analysis.

On-study CDI recurrences, as defined in Section 4.2, will require laboratory confirmation using free toxin test (see Section 9.3.1 for further details).

7.5 Exploratory Serum Analyses

Serum samples must be collected at Screening and on Days 14, 56, and 168 (end of study) and will be stored for future exploratory analyses, including cytokines and other soluble factors that may influence the efficacy and/or safety of study drug.

7.6 Hygiene for *C. Difficile*-Associated Diarrhea and Study Drug Handling

To avoid the potential transmission of *C. difficile*, study subjects will be instructed to thoroughly wash their hands with soap and water after handling stool samples or after each fecal elimination. Study personnel will wear gloves and wash their hands after handling subject stool samples following standard hand-washing requirements for *C. difficile* contact at their institution, if applicable. NOTE: The physical action of washing and rinsing hands is recommended versus alcohol-based antiseptic cleaning, as alcohol-based antiseptics have demonstrated poor activity against *C. difficile* spores.

In addition, these hygiene techniques will be implemented by study subjects and study personnel when handling or administering study drug to mitigate the possible transmission of VE303 component bacteria, which may be present on the outside of the capsules (but not on the drug kits or plastic bottles containing the capsules) (see Section 6.3.1 for more details). Subjects will also be informed that they may remain colonized with VE303 strains (and possibly transmit the bacteria to others) even after discontinuation of study drug dosing.

Table 7-1: Schedule of Events

	Screening: Start of SOC Antibiotics[a, b]	**Study Drug Administration Period**					**Postdose Follow-Up Period**					
Study Day **Assessment[c]**	-22	1[d]	2–6[q]	7	8–13[q]	14 (+ 1 day)/ EOT	28 (± 3 days) (Week 4)	56 (± 3 days) (Week 8)	84 (± 7 days) (Week 12)	112 (± 7 days) (Week 16)	140 (± 7 days) (Week 20)	168 (± 7 days) (Week 24/EOS/ET)
Informed consent	X											
Confirm eligibility criteria	X	X[k]										
Medical history	X											
Medications[e]	X											
Physical examination[f]	X	X				X	X	X				X
Height	X											
Weight[g]	X	X				X	X	X				X
Vital signs[h]	X	X		X		X	X	X				X
12-lead ECG[i]	X						X					
Hematology/Chemistry	X	X		X		X	X	X				X
Exploratory serum	X					X		X				X
Urinalysis	X					X						
Pregnancy test[j]	X	X										
Randomization[k]		X										
Study drug dosing[c, l]		X										
C. difficile testing[m, n]	X[m]											
Stool microbiota composition[n]	X	X		X		X	X	X				X
VE303 detection[n]	X	X		X		X	X	X				X
Antibiotic resistance gene detection[n]	X	X		X		X	X	X				X
Stool antibiotic concentrations[n]		X		X								
Stool metabolomics evaluation[n]	X	X		X		X		X				
Glycerol-preserved stool culture[n]	X	X				X		X				X
Stool culture for whole genome sequencing[n]	X	X				X	X	X				X
Stool/fecal calprotectin extraction[n]		X				X	X	X				X

	Screening: Start of SOC Antibiotics[a, b]	**Study Drug Administration Period**					**Postdose Follow-Up Period**					
Study Day **Assessment**[c]	-22	1[d]	2–6[q]	7	8–13[q]	14 (+ 1 day)/ EOT	28 (± 3 days) (Week 4)	56 (± 3 days) (Week 8)	84 (± 7 days) (Week 12)	112 (± 7 days) (Week 16)	140 (± 7 days) (Week 20)	168 (± 7 days) (Week 24/EOS/ET)
PROMIS® questionnaire [o]		X		X		X						
Adverse events [p]	X											
Telephone follow-up		X [q]							X	X	X	

Abbreviations: AE = adverse event; CDI = *C. difficile* infection; ECG = electrocardiogram; EOS = end of study; EOT = end of treatment; LAR = legally authorized representative; PCR = polymerase chain reaction; SOC = standard of care; ET = Early Termination

a Once identified as potential study candidates, subjects or their LAR will sign an informed consent form to enable collection of a stool sample to confirm the qualifying event of CDI recurrence in accordance with eligibility criteria.

b Upon confirmation of CDI recurrence, subjects will be evaluated for eligibility criteria.

c All tests/assessments scheduled on in-clinic dosing days will be performed prior to study drug administration.

d Day 1 (i.e., the day of the first dose of study drug) is the beginning of the study drug administration period, which will begin on the last planned day of SOC antibiotic administration for a qualifying CDI episode, or no later than 1 day after completion of antibiotic dosing. Day 1 tests/assessments will be considered baseline. The Screening results must be available for confirmation of eligibility. Vital signs and physical examinations should be performed on Day 1; tests/assessments must be performed prior to study drug administration.

e Medications administered within 14 days prior to Screening and/or on study will be recorded continuously for the duration of each subject's study participation.

f A complete physical examination will be performed during Screening; subsequent physical examinations must include abdominal examinations performed by the study physician, with limited symptom-directed examinations performed for other organ systems.

g To be collected in the same manner each time – without heavy items/shoes/bulky clothing.

h Includes pulse, blood pressure, and body temperature. Subjects are required to remain in the supine position for at least 3 minutes prior to obtaining vital signs. On Day 1, vital signs will be measured prior to administration of study drug.

i Additional ECGs may be performed if clinically indicated.

j For females of childbearing potential, serum pregnancy testing will be performed during Screening; a rapid urine pregnancy test will be performed at the study site on Day 1 prior to administration of study drug.

k After all Screening assessments and procedures have been performed and eligibility has been confirmed on Day 1, subjects will be randomized and receive the first dose of blinded study drug.

l The study drug administration period is from Day 1 to Day 14, inclusive. On Day 1, study drug will be administered under the supervision of study personnel, and subjects will remain at the study site for at least 30 minutes after the first study drug dose for monitoring of potential AEs. On Day 1, the subject diary will be given to each subject and the subject will be instructed to complete the diary for study drug accountability and compliance.

m A stool sample or documentation of recent *C. difficile* testing must be collected and/or submitted as soon as a potential qualifying CDI episode is identified. Ideally, this sample will be obtained prior to starting SOC antibiotics for CDI; however, if SOC antibiotics have been started, the stool sample must be collected no later than 72 hours after the first antibiotic administration. Every effort must be made to collect the diagnostic sample before SOC antibiotic therapy or within 24 hours after SOC antibiotic therapy initiation, whenever possible. Samples collected as part of SOC within this window may be submitted for the purpose of *C. difficile* diagnosis. Diagnostic tests may be performed at the local laboratory or at the central laboratory. Subjects with a positive test (either EIA for Toxin A/B or PCR or CCNA or toxigenic culture assay performed at the local laboratory or at the central laboratory) may be enrolled in the study. Confirmation of a positive local test by the central laboratory test is not required for subject enrollment,

provided documentation of a local positive test is available. To ensure subjects are allocated to a proper stratum, an effort should be made to obtain and send a stool sample to the central laboratory for confirmation of toxin or toxigenic C. *difficile* prior to randomization. .

n Stool samples must be sent to a central laboratory. The Day 1 stool sample must be collected prior to administration of any study drug. This sample should be collected predose on Day 1, if possible; however, if a subject cannot produce a stool sample predose on Day 1, a sample from up to 24 hours prior to Day 1 may serve as the Day 1 sample. Thereafter, if a subject cannot produce a stool sample on the planned day of a clinic visit or at the time of diarrhea that may indicate CDI recurrence (see Section 9.3.1), then a stool sample may be obtained within ± 24 hours of the visit/diarrhea event. However, if CDI recurrence is suspected after ingestion of the first dose of study drug, all attempts should be made to collect a stool sample before administration of antibiotics to treat the recurrence.

o PROMIS® gastrointestinal symptom questionnaires (see Appendix 2) are to be completed by each subject or LAR before the first dose of study drug on Day 1. Subsequently, questionnaires should be completed on Days 7 and 14. All attempts must be made to have questionnaires completed at approximately the same time of day at every time point.

p AEs will be collected continuously from the time of informed consent through Week 24/Day 168.

q Study personnel will telephone outpatient subjects daily during the 14-day study drug administration period to ensure dosing compliance; explore any concerns or issues with study drug ingestion, administration, handling or storage; capture potential AEs; review potential *C. difficile*-associated symptoms and assess the need for an unscheduled visit or stool test for CDI; remind subjects to collect stool to bring to their next clinic visit (if applicable); and answer any questions that the subject may have. If a subject is inpatient or attending a site visit on a scheduled telephone follow-up day, this contact will be performed in person, when possible. Telephone follow-up may be omitted on weekends or public holidays if this is necessary for logistical reasons. If a subject is discovered to have had an SAE or a recurrence of diarrhea at any time, an unscheduled visit must be arranged as soon as possible.

8.0 STUDY DISCONTINUATION

8.1 Sponsor Discontinuation Criteria

This study may be discontinued at any time due to safety concerns, failure to meet expected enrollment goals, administrative reasons, or at the discretion of the Sponsor. Should the study be terminated prematurely, the Sponsor will provide written notification to the Investigator and regulatory authorities and will specify the reason(s) for early termination. The Investigator must inform the IRB/EC promptly and provide the reason(s) for the termination as well as subject status of all participants at their site.

8.2 Study Discontinuation for Individual Subjects

Subjects may withdraw their consent at any time for any reason without prejudice to their future medical care by the physician or at the institution. If a subject withdraws consent, the date and stated reason for consent withdrawal will be documented. Subject data, including samples (e.g., blood, serum, stool), collected up to the date of consent withdrawal (i.e., early termination) will be included in the analyses.

Wherever possible, the tests and evaluations listed for the End of Treatment Visit (same as Day 14 [+ 1 day]) will be carried out at the time of discontinuation of study drug. Subjects who discontinue study drug prematurely for reasons other than withdrawal of consent, lost to follow-up, or AE event with an outcome of death will continue to be followed for the intended duration of the study (i.e., through Week 24/Day 168). The Sponsor will be notified of all study withdrawals.

Subjects meeting any of the following criteria must discontinue study drug dosing:

- Subject experiencing related Grade 3 or higher AE and/or any related SAE
- Any allergic reaction during study drug administration, regardless of grade, should prompt study drug discontinuation unless there is a clear alternative etiology for the event. The study drug may be restarted if a clear alternative etiology for the event is identified
- Withdrawal of consent/early termination
- Pregnancy
- Lost to follow-up
- Discretion of the Investigator (e.g., protocol violations, noncompliance, continued study drug administration not in the subject’s best interest)
- Study termination by the Sponsor

9.0 ADVERSE EVENTS

9.1 Adverse Event

An AE is any untoward medical occurrence, including the exacerbation of a pre-existing condition, in a subject administered a pharmaceutical product or study medication regardless of causality.

Adverse events will be recorded in the eCRF during the time periods specified in Section 9.4, including documentation of start and stop dates, outcome, severity, seriousness, relationship to study drug, action taken with respect to study drug, and medication required to manage the event. Adverse events will be reported in accordance with the guidelines set forth in 21 Code of Federal Regulations (CFR) 312.32 and International Council for Harmonisation (ICH) E6R2.

9.1.1 Assessment of Toxicity Grade

The toxicity grade of an AE refers to its severity. The severity of AEs will be categorized using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 5.0 [33], which is appropriate for grading and assessing the range of potential AEs that may be observed in the targeted study population of subjects with rCDI-associated diarrhea. This is because most of these subjects will be elderly, have multiple co-morbidities, and be taking a variety of prescription medications appropriate for the management of their comorbid conditions. For any term that is not specifically listed in the CTCAE scale, severity must be assigned a grade of 1 through 5 using the following CTCAE guidelines:

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

Grade 4: Life-threatening consequences; urgent intervention indicated

Grade 5: Death related to AE

9.1.2 Assessment of Causality

Medical judgment will be used to determine the cause of the AE, considering all relevant facts such as (but not limited to) the underlying study indication, comorbidities, concomitant medication(s), medical history, pattern of the AE (continuous, intermittent, etc.), temporal relationship to the study drug, and de-challenge or re-challenge.

The Investigator will be responsible for selecting “Yes” or “No” as detailed below for the relationship of each AE to the study drug.

Yes (i.e., possibly, probably, or definitely related): there is a reasonable possibility that there is a causal relationship to the study drug and one or more of the following criteria apply:

- The event follows a reasonable temporal sequence from the time of administration of the study drug.
- The event could not be reasonably attributed to the known characteristics of the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject.
- The event follows a known pattern of response to the study drug.
- The event disappears or decreases on cessation or reduction in dose. (It should be noted that in some situations an AE will not disappear or decrease in intensity upon discontinuation of study dosing despite other clear indications of relatedness).

No (i.e., unlikely, probably not related, or definitely not related): there is no reasonable possibility that the study drug caused the event; one or more of the following criteria apply:

- The event does not follow a reasonable temporal sequence from administration of the study drug to the time of the AE.
- The event could be reasonably attributed to the known characteristics of the subject's clinical state, concurrent illness, environmental or toxic factors, or other modes of therapy administered to the subject.
- The event does not follow a known pattern of response to the study drug.
- The event does not disappear or decrease on cessation, and it does not reappear or worsen when dosing is resumed.

9.1.3 Action Taken with Study Drug

For each AE reported, the action taken with the study drug as a result of the AE will be recorded as one of the following:

- Drug withdrawn
- Dose interrupted
- Dose not changed
- Unknown
- Not applicable (i.e., for AEs occurring prior to first or after the last study drug administration)

9.1.4 Outcome of Adverse Event

The outcome of each AE at the time of last observation will be reported as one of the following:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved/ongoing (e.g., for irreversible congenital anomalies)

- Recovered/resolved with sequelae (e.g., for other irreversible medical conditions)
- Fatal (i.e., death is at least possibly related to the AE)
- Unknown

9.2 Serious Adverse Event

An SAE is an untoward medical occurrence in the view of the Investigator or Sponsor that meets any of the following criteria:

- Results in death.
- Is immediately life-threatening (refers to an event in which the subject is at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Based on appropriate medical judgment, represents an important medical event that may jeopardize the subject or may require intervention to prevent one of the other outcomes described above.

9.2.1 Clarification of Serious Adverse Event Definition

- Adverse event seriousness is not necessarily dependent on event severity. AEs that are Grade 3 or 4 in severity do not constitute SAEs unless SAE criteria are met, and events that are Grade 1 or 2 in severity may meet SAE criteria.
- Death is an outcome of an SAE and not an SAE in itself. When death is an outcome, the event(s) resulting in death should be reported (e.g., “pulmonary embolism” with a fatal outcome). The appropriate diagnosis or term should be recorded and assigned severity Grade 5.
- In instances of death due ultimately to an underlying disease, the cause of death should be indicated as the specific event or condition resulting in death to the extent possible. If no appropriate term with a Grade 5 severity in the CTCAE can be identified, then a term should be selected from the CTCAE category “Death”.
- “Life-threatening” means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity. Grade 4 events (e.g., thrombocytopenia) are not always serious unless they have life-threatening consequences or result in hospitalization.
- Pre-planned or elective hospitalizations including social and/or convenience situations (e.g., respite care) are excluded from SAE reporting unless they meet another SAE criterion.

- Overage in dosing of either the study drug or a concomitant medication without overdose signs or symptoms unless the event meets SAE criteria (e.g., hospitalization) are excluded from SAE reporting; however, such events should still be recorded on the appropriate eCRF page.

9.2.2 Serious, Unexpected, Suspected Adverse Reactions

In accordance with regulatory requirements, the Sponsor or designee will immediately notify regulatory authorities and the Investigators, who will in turn notify their IRB/EC as necessary, of any AE associated with study drug administration or study procedures that is a serious, unexpected, suspected adverse reaction or any finding from tests in laboratory animals that suggests a significant risk for human subjects, including reports of mutagenicity, teratogenicity, or carcinogenicity. An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been previously observed.

9.3 Adverse Events of Special Interest

The following are considered AEs of special interest due to the indication under study (CDI) and the nature of the investigational product (live bacteria) and potential for gastrointestinal or extraintestinal AEs:

- Any clinically significant (Grade 2 or higher) gastrointestinal AE considered related to study drug.
- Any clinically significant (Grade 2 or higher) bacterial infection considered related to study drug.

These AEs will be tabulated and analyzed separately from other AEs.

9.3.1 Management of Diarrhea or Loose/Watery Stools

Any clinical event of diarrhea or loose/watery stools (including events reported as *C. difficile* infection/recurrence) will be recorded as an AE. In addition to recording the standard AE information, the Investigator will record the following information:

- Maximum number of unformed (loose or watery) stools within a 24-hour period that are associated with the event.
- Whether a stool sample was collected for this event; if so, the date of collection, method of delivery/collection (produced at study site or brought to study site), and type of *C. difficile* test(s) performed (with results) will be recorded along with protocol-mandated evaluations.
- Whether colon endoscopy or surgery was performed for the event and whether pseudomembranes were present.
- Whether there was another likely cause of the diarrhea (instead of or in addition to CDI) in the opinion of the Investigator, including, but not limited to, food/diet, non-study medication, or other infection.

Subjects who have ≥ 3 loose/unformed bowel movements within 24 hours for at least 2 consecutive days or > 8 loose/unformed bowel movements within 24 hours (or those with any diarrhea of concern to the subject or Investigator) will be instructed to contact study personnel as promptly as possible for clinical assessment and to arrange for collection of a stool sample. Whenever possible, collection of the stool sample should occur at the study site. However, because it is important to collect a stool sample while a subject is symptomatic, subjects will be provided with a kit and instructions for stool sample collection at home, if necessary. These stool samples may be collected within 24 hours of diarrhea onset but must be obtained before administration of any antibiotics or no later than on the day after antibiotics were started. Stool samples collected in association with an AE of diarrhea or loose/watery stools will be tested for *C. difficile* at the central laboratory and in accordance with the Schedule of Events (Table 7-1) and Section 7.4.1. All other aspects of management of subjects with diarrhea or loose/watery stools (including local laboratory testing and any treatment for diarrhea/CDI) will occur at the discretion of the Investigator based on clinical presentation. Subjects should remain in the study (at the discretion of the Investigator) and continue to be followed per protocol. Use of any antibacterial treatments administered for CDI will be recorded (see Section 7.3.2). If a subject develops diarrhea (as defined above) during the 14-day study treatment period and recurrent CDI diagnosis is confirmed by a positive EIA for Toxin A/B or CCNA test, the study treatment should be discontinued (See Section 4.4). Such an event should also be reported as confirmed recurrence in the eCRF.

9.4 Reporting of Adverse Events

All AEs, serious and nonserious, occurring from the time a subject signs the informed consent form through Week 24/Day 168 must be fully recorded on the appropriate eCRF.

Abnormal clinical laboratory values and vital sign and ECG measurements will be recorded as AEs only if they are considered to be clinically significant by the Investigator. If a laboratory abnormality is the sole potential AE, the abnormality must be confirmed (if possible) with a repeat laboratory test performed as soon as possible.

All SAEs, regardless of relationship to study drug, must be reported to the CRO within 24 hours of the Investigator's knowledge. This will be done by faxing or emailing the completed SAE Report Form to the CRO at the number provided on the SAE Report Form.

Contact information for safety reporting is as follows:

Investigators must follow subjects with SAEs for a minimum of 30 days or until event resolution or stabilization, withdrawal of consent, subject is lost to follow-up, or death, whichever occurs first.

9.5 Pregnancy

Female subjects of childbearing potential must provide a negative serum pregnancy test at Screening and a negative urine pregnancy test prior to initiation of study drug dosing. Pregnancies occurring on study that involve a subject must be brought to the attention of the treating physician immediately, and pregnant subjects must discontinue study dosing. Site personnel will notify the Sponsor within 24 hours of the Investigator's knowledge of the pregnancy using a Pregnancy Notification Form. Such pregnancies will be followed to outcome, as possible.

9.6 Clinical Laboratory Abnormalities

It is the responsibility of the Investigator to assess the clinical significance of all abnormal laboratory values as defined by the appropriate reference range(s). All abnormal values assessed to be of clinical concern and at least possibly related to the study drug or of uncertain causality will be repeated. Persistent abnormal values or changes of possible clinical concern that remain within the normal range will be followed up and further evaluated at the discretion of the Investigator.

An abnormal laboratory value that is not already associated with an AE is to be recorded as an AE if:

- a repeat test is performed, when possible, to confirm the abnormality
- an action on study drug dosing is made as a result of the abnormality
- an intervention for management of the abnormality is required
- or the Investigator considers the abnormality to be AE

Abnormal test results must not be recorded as AEs unless they fulfill the aforementioned criteria. All abnormal laboratory values, even if reported as an AE, will be categorized by the Investigator as not clinically significant or clinically significant and a severity grade will be included in the eCRF.

9.7 Review of Safety Data

The designated Medical Monitor will be responsible for the ongoing review and evaluation of safety data, including AEs, clinical laboratory data, and any other safety evaluations (see Section 7.3).

10.0 STATISTICAL METHODS

10.1 Study Design and Primary Analysis

As designed, VE303-002 is a Phase 2, randomized, parallel-arm, double-blind, placebo-controlled, dose-selection study to evaluate the safety, microbiota changes, and efficacy of VE303 in the prevention of subsequent CDI-associated diarrhea compared with placebo following completion of a successful course of SOC antibiotics for subjects with pCDI at high risk for recurrence or subjects with rCDI.

The 3 administrative IAs are planned when at least 24 subjects reach study Week 8, when at least 39 subjects reach study Week 8, and when at least 54 subjects reach study Week 8.

The primary endpoint is the proportion of subjects with CDI recurrence before or at Week 8 (i.e., 8 weeks after the first dose of study drug). The difference in the proportion of subjects with CDI recurrence before or at Week 8 between each VE303 treatment group and placebo will be analyzed using a stratified Cochran-Mantel-Haenszel method. Stratification will involve the stratification factors as determined from the clinical database, i.e., number of previous CDI episodes at Baseline (0 vs ≥1 episodes, excluding qualifying episodes), SOC antibiotic treatment (non-vancomycin [fidaxomicin or metronidazole] or an alternative oral vancomycin dosing regimen vs vancomycin QID), and results of CDI laboratory diagnostic method (free toxin or other).

10.2 Analysis Populations

The following subject populations (i.e., analysis sets) will be evaluated and used for presentation of the data:

- Full Analysis Set (FAS): All subjects who were randomized and received at least 1 dose of study drug. Subjects will be classified according to the planned study drug assignment. The FAS will be the default analysis set for all efficacy analyses, unless otherwise specified.
- Per-Protocol Analysis Set (PPS): All subjects in the FAS who had no major protocol deviations. The PPS will be used for the primary and secondary efficacy endpoint analyses.
- Safety Analysis Set: All subjects who were randomized and received at least 1 dose of study drug. Subjects will be classified according to the actual study drug assignment. The Safety Analysis Set will be the primary set for the analysis of safety data.

10.2.1 Sensitivity Efficacy Analyses

Sensitivity analyses will be conducted on the following efficacy variables for the FAS and PPS:

- Sensitivity Analysis I: In addition to the CDI recurrence definition for the primary efficacy endpoint, the CDI recurrence definition for Sensitivity I will also include any recurrence that was diagnosed with a positive PCR test (including cytotoxicity assay) and treated with an antibiotic that targets *C. difficile.*

- Sensitivity Analysis II: In addition to the CDI recurrence definition for the primary and Sensitivity I efficacy endpoints, the Sensitivity II CDI recurrence definition will include any recurrence, in the absence of laboratory confirmation, that is treated with an antibiotic that targets *C. difficile*.

10.3 Procedures for Handling Missing, Unused, and Spurious Data

In general, missing values for the primary efficacy endpoint will not be imputed. However, as a sensitivity analysis, subjects who discontinued prior to the Week 8 time point will be imputed as having a CDI recurrence at Week 8. Details will be provided in a separate statistical analysis plan (SAP).

10.4 Administrative Interim Analyses

Three IAs are planned for study administrative purposes only. These 3 administrative IAs will provide input to Vedanta senior management regarding development strategy for future investigations. These IAs will have no impact on the ongoing study. The administrative IAs will be performed after at least 24 subjects complete 8 weeks of the study, after at least 39 subjects complete 8 weeks of the study, and after at least 54 subjects complete 8 weeks of the study. Further details of these administrative IAs will be described in the SAP, as appropriate.

10.5 General Methods

Continuous variables will be summarized with descriptive statistics (arithmetic mean, standard deviation, median, minimum, and maximum) by study arm. Categorical data will be summarized with frequency counts and percentages by study arm.

In addition to the frequency (%), descriptive statistics, and confidence intervals, the hypothesis test for CDI recurrence rate (no treatment effect versus at least 1 dose is effective) will be conducted using a stratified Cochran-Mantel-Haenszel test (stratified for the stratification factors used in randomization).

The primary efficacy endpoint is the incidence of recurrences that meet the protocol defined recurrence definition (Section 4.2); however, a supplemental analysis will be conducted for the incidence of recurrences that are both Toxin positive and Toxin negative/unknown. The null hypothesis is that the proportion of VE303 subjects with *C. difficile* recurrence is greater than or equal to that of placebo. The alternative hypothesis is that the proportion of VE303 subjects with *C. difficile* recurrence is lower than that of placebo.

This study may be prematurely terminated with a reduced sample size if, in the opinion of the Sponsor, there is sufficiently reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

10.5.1 Disposition of Subjects

A tabulation of the disposition of subjects will be presented, including the number of subjects who fail Screening after signing informed consent, are randomized, receive study drug, and complete or prematurely discontinue, and the reasons for study discontinuation. Entry criteria eligibility and protocol deviations will be listed.

10.5.2 Baseline Comparisons

Demographic and baseline disease characteristic data summaries will be generated to descriptively assess the comparability of dose groups. Data to be tabulated will include sex, age, race, and ethnicity, as well as disease-specific information such as CDI episode number and prior antibiotic or other CDI treatment.

10.5.3 Safety Analysis

Subjects who receive at least 1 dose of study drug will be included in safety analyses.

Adverse events will be coded using the most current version of Medical Dictionary for Regulatory Activities (MedDRA) at the time of study initiation, and the severity of AEs and laboratory abnormalities will be graded using the NCI CTCAE, version 5.0 [33]. A by-subject AE data listing, including verbatim term, preferred term, system organ class, study drug arm, severity, and relationship to study drug, will be provided. The number of participants experiencing TEAEs and the number of individual TEAEs will be summarized by study arm, system organ class, and preferred term. TEAEs will also be summarized by severity and by relationship to study drug.

Clinical laboratory evaluations and other safety assessments will be summarized by study arm and protocol-specified collection time point. A summary of changes from Baseline will also be presented for each protocol-specified time point.

10.5.4 Stool and Other Exploratory Analyses

Results from stool microbiota composition and VE303 detection analyses will be presented in by-subject listings and summarized by study arm and study day, including absolute values and changes from Baseline.

Tabular and/or graphical summaries for the microbiome and VE303 detection analyses will be produced for VE303 strain presence, community composition (relative abundance of the top 10 species and VE303 strain abundance), community diversity (Shannon Index, Simpson Index, and Species Richness calculations), and relative abundance of all species detected in the sample. In addition, an analysis will be performed to evaluate the longitudinal microbiome composition and VE303 strains within each subject and within each study arm, including changes in diversity over time, changes in microbiome species over time, and changes in VE303 strains over time (both presence/absence and relative abundance).

The Sponsor will also collect serum and stool samples (for stool antibiotic concentrations, metabolomics, antibiotic resistant bacteria other than *C. difficile* [e.g., CRE, ESBL, or VRE], and culture) from clinical study participants and may analyze them for possible exploratory purposes and biomarkers.

10.5.5 Procedures for Reporting Deviations to the Statistical Analysis Plan

All deviations from the statistical analysis plan will be provided in the clinical study report.

10.6 Changes in the Conduct of the Study or Planned Analysis

Only the Sponsor may modify the protocol. Amendments to the protocol will be made only after consultation and agreement between the Sponsor and the Investigator. The only exception is when the Investigator considers that a subject's safety may be compromised without taking immediate action. In these circumstances, the Investigator must inform the Sponsor and the full IRB/EC within 1 working day after the safety event occurred. All amendments that have an impact on subject risk or the study objectives or require revision of the informed consent documents must receive approval from the IRB/EC prior to implementation.

11.0 DATA RECORDING, RETENTION AND MONITORING

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Investigator. In accordance with the ICH and Good Clinical Practice (GCP) guidelines, the Investigator will maintain complete, accurate, legible, timely, and easily retrievable data, and will allow personnel authorized by the Sponsor or recognized regulatory agency to access all study data at any time. Such data shall also be secured in order to prevent loss of data and protect the privacy of the subject.

11.1 Case Report Forms

Medical records and/or study visit worksheets will be used by the Investigator or designee as source documents for recording data specified in the protocol for each subject enrolled. Data recorded in the eCRF derived from source documents must be consistent with the data recorded on the source documents. All source documents must be completed in a neat, legible manner to ensure accurate interpretation of data. Additional data will be collected through clinical laboratories.

Clinical data derived from source documents will be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system at the clinical sites. The data system will include password access, consistent backup, and an audit trail. Internal and external quality checks will be applied to identify data that appear inconsistent, incomplete, or inaccurate. Data not captured on eCRFs will be received and stored as external files by the Sponsor or designee.

Study plans will describe internal and external quality checks and cross functional data monitoring to identify and address data issues via a risk-based approach. Needed queries will be issued by the system (some fields may require immediate correction for form submission), CRO, or authorized Sponsor staff in an attempt to clarify and/or correct missing, incomplete, or illogical data. Changes or corrections to eCRFs will be made by the Investigator or an authorized member of the study staff.

Data entered and compiled for interim analysis and/or DMC review will be monitored as closely as possible for accuracy and quality but may not undergo final data cleaning and verification.

It is the Investigator’s responsibility to ensure eCRFs are complete and accurate, regardless of whether this responsibility has been delegated in whole or in part. Following review and approval, the Investigator or designee will electronically sign and date the pages, which certifies that the Investigator has thoroughly reviewed and confirmed all data on the eCRF.

A read-only copy of the eCRFs will be provided to study sites after the study has ended and the EDC system has been locked.

11.2 Data Retention

Data retention practices will follow ICH guidelines, which note that essential documents must be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the

investigational product. However, these documents must be retained for a longer period if required by the applicable legal requirements.

11.3 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, ICH GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. Deviations must be reviewed by the site, clinical research associate, Sponsor, and designee, as appropriate for potential corrective actions development. Corrective actions will be implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, Section 5.1.1
- 5.20 Noncompliance, Sections 5.20.1, and 5.20.2

It is the responsibility of the Investigator to use continuous vigilance to identify and promptly report deviations. All deviations must be addressed in study source documents and recorded within the Protocol Deviation eCRF. Protocol deviations must be sent to the reviewing IRB/EC per their policies. The Investigator is responsible for knowing and adhering to IRB/EC requirements.

11.4 Data Monitoring

This study will be closely monitored by representatives of the Sponsor or designee throughout its duration. Monitoring will include personal visits with the Investigator and study staff as well as appropriate communications by telephone, fax, mail, email, or use of the EDC system, as applicable. It is the monitor's responsibility to inspect all eCRFs at regular intervals throughout the study to verify the completeness, accuracy, and consistency of the data and to confirm adherence to the study protocol and to GCP guidelines. The Investigator agrees to cooperate with the monitor to ensure that any problems detected are resolved promptly. The Investigator and site will permit study-related monitoring, audits, IRB/EC review, and regulatory inspection, including direct access to source documents.

It is understood that study monitors and any other personnel authorized by the Sponsor may contact and visit the Investigator and will be permitted to inspect all study records (including eCRFs and other pertinent data) on request, provided that subject confidentiality is maintained, and that the inspection is conducted in accordance with local regulations.

Every effort will be made to maintain the anonymity and confidentiality of subjects. However, because of the experimental nature of the investigational product, the Investigator agrees to allow representatives of the Sponsor and authorized representatives of regulatory authorities to inspect the facilities used in the conduct of this study and to inspect, for purposes of verification, the hospital or clinic records of all enrolled subjects.

11.5 Quality Control and Quality Assurance

Quality control procedures will be conducted according to the Sponsor and CRO's internal procedures. The study site may be audited by a quality assurance representative of the Sponsor. All necessary data and documents will be made available for inspection along with any required study staff.

12.0 REGULATORY, ETHICAL, AND LEGAL OBLIGATIONS

The study will be performed in accordance with the requirements of the US FDA, any other governing regulatory bodies, and will also meet all of the requirements of ICH GCP guidance. Amendments to the protocol will be submitted to FDA/other governing regulatory body prior to implementation in accordance with applicable regulations.

12.1 Good Clinical Practice

The study will be performed in accordance with the protocol, guidelines for GCP established by the ICH, and applicable local regulatory requirements and laws.

12.2 Institutional Review Board/Independent Ethics Committee

The Investigator must inform and obtain approval from the IRB/EC for study conduct at named sites, the protocol, informed consent documents, and any other written information that will be provided to the subjects and any advertisements that will be used. Written approval must be obtained prior to recruitment of subjects and shipment of study drugs.

Proposed amendments to the protocol (see Section 10.6) and aforementioned documents must be submitted to the Sponsor for review and approval, then to the IRB/EC. Amendments may be implemented only after a copy of the approval letter from the IRB/EC has been transmitted to the Sponsor.

Per GCP guidelines, the Investigator will be responsible for ensuring that an annual update is provided to the IRB/EC until the study is completed (i.e., finalization of the clinical study report) to facilitate continuing review of study conduct and that the IRB/EC is informed about the end of the study. Copies of the update, subsequent approvals, and final letter must be sent to the Sponsor.

In addition to IRB/EC and regulatory authority approval, all other required approvals (e.g., approval from the local research and development board or scientific advisory committee) will be obtained prior to recruitment of subjects and shipment of study drugs.

12.3 Informed Consent

Informed consent is a process that is initiated prior to the subject's agreeing to participate and continues throughout the subject's study participation. It is the Investigator's (or designee's) responsibility to obtain written informed consent from each subject or their LAR after adequate explanation of the aims, methods, anticipated benefits, and potential hazards before any study procedures are initiated. Each subject or LAR must be given a copy of the signed informed consent documents and associated materials. The original copy of the signed and dated informed consent documents must be retained at the site and is subject to inspection by representatives of the Sponsor or regulatory authorities. If any amendments affect the informed consent form (e.g., when new study procedures or assessments have been added), all active subjects and LARs must be reconsented using the same process for the initial consent.

12.4 Subject Confidentiality

The Investigator must ensure that the subject's privacy is maintained. On eCRFs and other documents submitted to the Sponsor, subjects will be identified by their assigned subject number only and any other information that may reveal the subject's identity must be redacted (e.g., first, and last name). Documents that are not submitted to the Sponsor (e.g., signed informed consent documents) must be kept in a confidential file by the Investigator.

The Investigator shall permit authorized representatives of the Sponsor, regulatory authorities, and IRBs/ECs to review the portion of the subject's medical record that is directly related to the study. As part of the required content of informed consent documents, subjects must be informed that their medical records will be reviewed in this manner.

12.5 Disclosure of Information

Information concerning the study, patent applications, processes, scientific data, or other pertinent information is confidential and remains the property of the Sponsor. The Investigator may only use this information for the purposes of the study.

It is understood by the Investigator that the Sponsor will use information obtained in this study in connection with the clinical development program, and therefore may disclose it as required to other clinical Investigators and to regulatory authorities. In order to allow the use of the information derived from this study, the Investigator understands the obligation to provide complete test results and all data obtained to the Sponsor.

Verbal or written discussion of results prior to study completion and full reporting must be undertaken only with written consent from the Sponsor.

12.6 Publication of Study Data

The Sponsor encourages the scientific publication of data from clinical research studies. However, an Investigator(s) many not present or publish partial or complete study results without participation and written agreement of the Sponsor. The Investigator and the Sponsor may propose appropriate scientific manuscripts or abstracts from the study data. All proposed publications must be reviewed and commented on by the Sponsor before submission for publication. The detailed procedures for the review of publications are set out in the clinical trial agreement entered into with the Sponsor in connection with this study. These procedures are in place to ensure coordination of study data publication and adequate review of data for publication against the validated study database for accuracy.

Qualification of authorship will follow the requirements of the International Committee of Medical Editors (www.icmje.org). The names of Investigators and Sponsor representatives responsible for designing the study and analyzing the results will be included in the publication(s). This custom can be adjusted upon mutual agreement of the authors and Vedanta. In addition, other than clinical pharmacology studies in healthy volunteers or Phase 1 trials, all clinical trials must be registered with ClinicalTrials.gov.

12.7 Ethical Standards

The Sponsor is committed to designing, implementing, conducting, analyzing, and reporting this trial in compliance with the highest ethical and scientific standards. Protection of subject safety is the overriding concern in the design of clinical trials. In all cases, Vedanta clinical trials will be conducted in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

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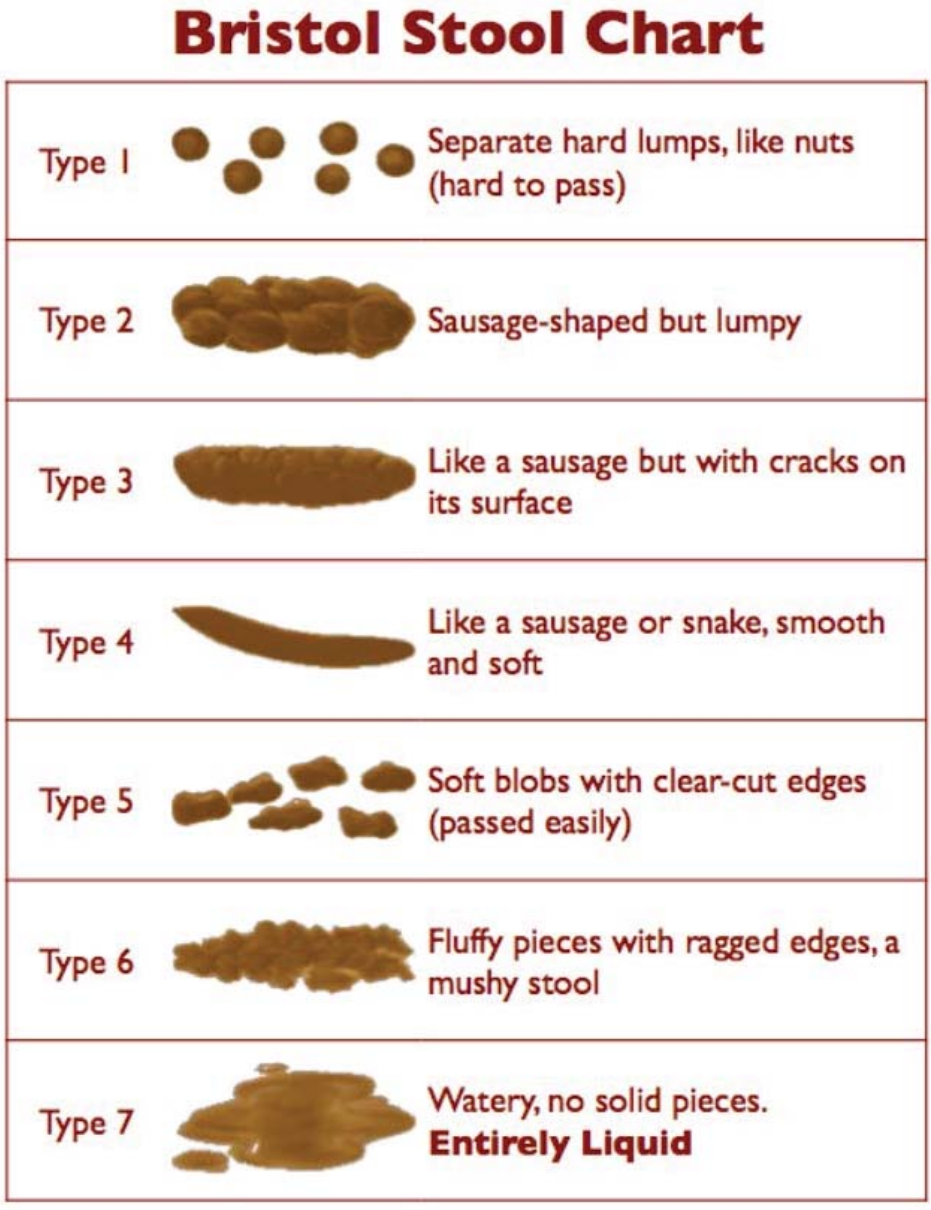
APPENDIX 1: BRISTOL STOOL SCALE

The Bristol Stool Scale is a general measure of stool consistency. This scale classifies stools into 7 types (see below), according to their appearance as seen in the toilet. Subjects will be instructed to use this scale as a guide to assess bowel movements; Types 5, 6 or 7 on this scale represent loose or watery stools.



Source: Lewis SJ, Heaton KW. Stool form as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997; 32(9):920-24.

Picture adapted from: https://www.continence.org.au/pages/bristol-stool-chart.html

APPENDIX 2: PROMIS® SCALE QUESTIONNAIRES

Patient-Reported Outcomes Measurement Information System (PROMIS®) questionnaires (v1.0, dated 01 September 2016/v1.1 dated 01 October 2018) will be administered to subjects at the time points specified in the protocol.

The following best practices for administration of the PROMIS® questionnaires must be followed:

- PROMIS® self-reported measures are intended to be completed by the respondent without help from anyone else.
- If respondents are unable to answer on their own, have someone else ("proxy") report on their behalf. Respondents requiring a proxy may include: young children, people in the early stages of dementia who may not recognize the extent of their impairment, people with cognitive or communication deficits, and people with severe disease burden.
- Keep respondents' privacy in mind, but have staff readily available to help with any issues that may arise.
- It is acceptable for staff to define a term (e.g., "nausea"), but not to define a concept where the respondent's subjective interpretation is the goal of the question (e.g., "quality of life").
- Utilize the same method (e.g., computer, telephone, or paper) and mode (e.g., self vs. interviewer) of administration at every time point for every subject, when possible.
- Provide respondents with the optimal time needed to capture the most relevant perspective and complete data (e.g., before/after clinician visit or in between visits).

PROMIS Scale v1.0 – Gastrointestinal Belly Pain 5a

Belly Pain

Please respond to each question or statement by marking one box.

In the past 7 days…

1 GISX79 How often did you have belly pain?

- ☐ 1 Never → **If Never, go to #5**
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

2 GISX79 At its worst, how would you rate your belly pain?

- ☐ 1 Not bad at all
- ☐ 2 A little bad
- ☐ 3 Somewhat bad
- ☐ 4 Quite bad
- ☐ 5 Very bad

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PROMIS Scale v1.0 – Gastrointestinal Belly Pain 5a

In the past 7 days…

3 GISX90 How much did belly pain interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

4 GISX91 How much did belly pain bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

5 GISX92 How often did you have discomfort in your belly?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 1 September 2016

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PROMIS Scale v1.0 – Gastrointestinal Bowel Incontinence 4a

Gastrointestinal Bowel Incontinence

Please respond to each question or statement by marking one box.

In the past 7 days…

		No days	1 day	2-3 days	4-5 days	6-7 days
GISX45	How often did you have bowel incontinence—that is, have an accident because you could not make it to the bathroom in time?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX46	How often did you soil or dirty your underwear before getting to a bathroom?...	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX47	How often did you leak stool or soil your underwear?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
		Never	**Rarely**	**Sometimes**	**Often**	**Always**
GISX48	How often did you think you were going to pass gas, but stool or liquid came out instead?.....	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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PROMIS Scale v1.0 – Gastrointestinal Constipation 9a

Gastrointestinal Constipation

Please respond to each question or statement by marking one box.

In the past 7 days…

1 GISX63 How often did you pass very hard or lumpy stools?

- ☐ 1 Never → **If Never, go to #3**
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

2 GISX64 How much did hard or lumpy stools bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

3 GISX65 How often did you strain while trying to have bowel movements?

- ☐ 1 Never → **If Never, go to #6**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

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PROMIS Scale v1.0 – Gastrointestinal Constipation 9a

In the past 7 days…

4 GISX66 How much did you usually strain while trying to have a bowel movement?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

5 GISX67 How much did straining during bowel movements bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

6 GISX68 How often did you feel pain in your rectum or anus while trying to have bowel movements?

- ☐ 1 Never → **If Never, go to #8**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

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PROMIS Scale v1.0 – Gastrointestinal Constipation 9a

In the past 7 days…

7 GISX69 At its worst, how would you rate the pain in your rectum or anus during bowel movements?

- ☐ 1 Not bad at all
- ☐ 2 A little bad
- ☐ 3 Somewhat bad
- ☐ 4 Quite bad
- ☐ 5 Very bad

8 GISX72 How often after a bowel movement did you feel unfinished - that is, that you had not passed all your stool?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

9 GISX74 How often did you use your finger or toilet paper to get out a stool?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

Last Updated: 1 September 2016

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PROMIS Scale v1.0 – Gastrointestinal Diarrhea 6a

Gastrointestinal Diarrhea

Please respond to each question or statement by marking one box.

In the past 7 days…

1 GISX38 How many days did you have loose or watery stools?

- ☐ 1 No days → **If No Days, go to #4**
- ☐ 2 1 day
- ☐ 3 2 days
- ☐ 4 3-5 days
- ☐ 5 6-7 days

2 GISX40 How much did having loose or watery stools interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

3 GISX41 How much did having loose or watery stools bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

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PROMIS Scale v1.0 – Gastrointestinal Diarrhea 6a

In the past 7 days…

4 GISX42 How often did you feel like you needed to empty your bowels right away or else you would have an accident?

- ☐ 1 Never → **If Never, you are finished.**
- ☐ 2 One time during the past 7 days
- ☐ 3 2-6 times during the past 7 days
- ☐ 4 Often once a day
- ☐ 5 More than once a day

5 GISX43 How much did feeling you needed to empty your bowels right away interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

6 GISX44 How much did feeling you needed to empty your bowels right away bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

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PROMIS Scale v1.0 – Gastrointestinal Disrupted Swallowing 7a

Gastrointestinal Disrupted Swallowing

Please respond to each question or statement by marking one box.

	In the past 7 days…	**Never**	**Rarely**	**Sometimes**	**Often**	**Always**
GISX31	How often did food get stuck in your chest when you were eating?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX32	How often did food get stuck in your throat when you were eating?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX33	How often did you feel pain in your chest when swallowing food?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX34	How often did you have difficulty swallowing solid foods like meat, chicken or raw vegetables, even after lots of chewing?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX35	How often did you have difficulty swallowing soft foods like ice cream, apple sauce, or mashed potatoes?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX36	How often did you have difficulty swallowing liquids?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
GISX37	How often did you have difficulty swallowing pills?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

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PROMIS Scale v1.1 – Gastrointestinal Gas and Bloating 13a

Gastrointestinal Gas and Bloating

Please respond to each question or statement by marking one box.

In the past 7 days…

1 GISX94 Did you have swelling in your belly?

- ☐ B No → **If No, go to #5**
- ☐ A Yes

2 GISX95 How bad did the swelling in your belly get?

- ☐ 1 Not bad at all
- ☐ 2 A little bad
- ☐ 3 Somewhat bad
- ☐ 4 Quite bad
- ☐ 5 Very bad

3 GISX96 How much did the swelling in your belly interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

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PROMIS Scale v1.1 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

4 GISX97 How much did having swelling in your belly bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

5 GISX98 How often did you feel bloated?

- ☐ 1 Never → **If Never, go to #12**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

6 GISX99 In general, how severe was your bloating?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

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PROMIS Scale v1.1 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

7 GISX100 At its worst, how severe was your bloating?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

8 GISX101 In general, how severe did your bloating feel?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

9 GISX102 How often did you know that you would feel bloated before it happened?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

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PROMIS Scale v1.1 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

10 GISX103 How much did feeling bloated interfere with your day-to-day activities?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

11 GISX104 How much did feeling bloated bother you?

- ☐ 1 Not at all
- ☐ 2 A little bit
- ☐ 3 Somewhat
- ☐ 4 Quite a bit
- ☐ 5 Very much

12 GISX105 How often did you pass gas?

- ☐ 1 Never
- ☐ 2 Only once or twice a day
- ☐ 3 About every 3-4 hours
- ☐ 4 About every 2 hours
- ☐ 4 About every hour

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PROMIS Scale v1.1 – Gastrointestinal Gas and Bloating 13a

In the past 7 days…

13 GI6X109	How often did you have gurgling or rumbling in your belly when you were not hungry?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

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PROMIS Scale v1.0 – Gastrointestinal Nausea and Vomiting 4a

Gastrointestinal Nausea and Vomiting

Please respond to each question or statement by marking one box.

In the past 7 days…

1 GISX49 How often did you have nausea—that is, a feeling like you could vomit?

- ☐ 1 Never → **If Never, go to #3**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

2 GISX52 How often did you know that you would have nausea before it happened?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

3 GISX55 How often did you have a poor appetite?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

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PROMIS Scale v1.0 – Gastrointestinal Nausea and Vomiting 4a

In the past 7 days…

4 GISX59	How often did you throw up or vomit?

- ☐ 1 Never
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

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Gastroesophageal Reflux

Please respond to each question or statement by marking one box.

1 GISX2 In the past 7 days, how often did you have regurgitation—that is, food or liquid coming back up into your throat or mouth without vomiting??

- ☐ 1 Never → **If Never, go to #3**
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

2 GISX3 In the past 7 days, what was the most food or liquid you had come back up into your mouth at one time?

- ☐ 1 None
- ☐ 2 Enough to fill a little of my mouth
- ☐ 3 Enough to fill some of my mouth
- ☐ 4 Enough to fill most of my mouth
- ☐ 5 So much that it filled my entire mouth

3 GISX9 In the past 7 days, after eating a meal how often did food of liquid come back into your throat without vomiting?

- ☐ 1 Never → **If Never, go to #5**
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

4 GISX10 In the past 7 days, how often did you re-swallow food that came back into your throat?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

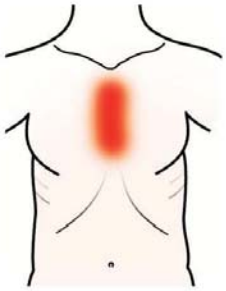
5 GISX11 In the past 7 days, how often did you feel like you were going to burp, but food or liquid came up instead?

- ☐ 1 Never
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

6 GISX12 In the past 7 days, how often did you feel like there was too much saliva in your mouth?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

7 GISX14	Look at the picture below. In the past 7 days, how often did you feel burning in the red area shown in the picture — that is, behind the breastbone?



- ☐ 1 Never
- ☐ 2 One day
- ☐ 3 2-6 days
- ☐ 4 Once a day
- ☐ 5 More than once a day

8 GISX21	In the past 7 days, how often did you feel burning in your throat?

- ☐ 1 Never
- ☐ 2 Rarely
- ☐ 3 Sometimes
- ☐ 4 Often
- ☐ 5 Always

9 GISX22	In the past 7 days, how often did you burp?

☐ 1 Never → **If Never, go to #11**

☐ 2 One day

☐ 3 2-6 days

☐ 4 Once a day

☐ 5 More than once a day

10 GISX24	In the past 7 days, how much did burping bother you?

☐ 1 Not at all

☐ 2 A little bit

☐ 3 Somewhat

☐ 4 Quite a bit

☐ 5 Very much

11 GISX25	In the past 7 days, how often did you have hiccups?

☐ 1 Never

☐ 2 Rarely

☐ 3 Sometimes

☐ 4 Often

☐ 5 Always

12 GISX28	In the past 7 days, how often did you feel like there was a lump in your throat?

☐ 1 Never → **If Never, you are finished.**

☐ 2 Rarely

☐ 3 Sometimes

☐ 4 Often

☐ 5 Always

13 GISX30	In the past 7 days, how much did having a lump in your throat bother you?

☐ 1 Not at all

☐ 2 A little bit

☐ 3 Somewhat

☐ 4 Quite a bit

☐ 5 Very much