TITLE PAGE

PROTOCOL TITLE: A PHASE 2, RANDOMIZED, DOUBLE-BLIND, COMPARATOR-CONTROLLED, MULTICENTER STUDY TO EVALUATE THE SAFETY AND EFFICACY OF CRS3123 COMPARED WITH ORAL VANCOMYCIN IN ADULTS WITH *CLOSTRIDIOIDES DIFFICILE* INFECTION

Protocol Number: 19-0021

Compound Number: CRS3123

Study Phase: 2

Short Title: A Randomized, Double-blind Evaluation of CRS3123 Versus Oral Vancomycin in Adult Patients with *Clostridioides difficile* Infection

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1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A Phase 2, Randomized, Double-Blind, Comparator-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of CRS3123 Compared with Oral Vancomycin in Adults with *Clostridioides difficile* Infection

Short Title: A Randomized, Double-blind Evaluation of CRS3123 Versus Oral Vancomycin in Adult Patients with *Clostridioides difficile* Infection

Rationale:

Clostridioides difficile (*C. difficile)* infection (CDI) is currently classified as an urgent threat by the Centers of Disease control and Prevention (CDC, 2015; CDC, 2019; McDonald et al, 2018), and is the most common cause of healthcare-associated infections in the US (Magill et al, 2014). Over approximately the last decade, the incidence of *C. difficile* in the US has ranged from approximately 200,000 to 500,000 cases per year and mortality rate has ranged from approximately 13,000 to 29,000 deaths per year (Lessa et al, 2015; CDC, 2019). Because *C. difficile* is more common and more severe in older patients (CDC, 2019) as the US population ages, the total numbers of patients may be expected to rise.

CRS3123 is a novel narrow-spectrum small molecule antibiotic that selectively inhibits methionyl-tRNA synthetase of *C. difficile*. As a protein synthesis inhibitor, CRS3123 inhibits *C. difficile* toxin production and spore formation with no cross-resistance to existing antibiotics. CRS3123 exhibited minimal disruption of commensal gastrointestinal microbiota, blocking toxin and spore formation and exhibiting low recurrence rates compared to vancomycin in preclinical studies.

CRS3123 has been evaluated in Phase 1 trials to determine its safety and tolerability in healthy subjects following a single oral dose of 100, 200, 400, 800 and 1200 mg (Division of Microbiology and Infectious Diseases [DMID] 10-0008), or multiple ascending doses of 200, 400 and 600 mg orally twice daily (bid) for 10 days (DMID 10-0009). CRS3123 was generally safe and well tolerated with no significant treatment-emergent adverse events (TEAEs) reported. CRS3123 showed very limited systemic absorption in humans and the majority of the oral dose remained intraluminal. Fecal concentrations reached levels above 1000 µg/g, substantially above the minimum inhibitory concentration necessary for 90% inhibition (MIC_{90}, 1 µg/ml) at all dosages tested, and more than 10-fold above mutation prevention concentration (MPC). CRS3123 exhibited minimal perturbation of normal intestinal microbiota and had no effects against important commensal anaerobes including *Bacteroides*, *Bifidobacteria*, and commensal *Clostridia*. The results demonstrate the differentiating profile of CRS3123 from other agents and support its further development as an oral agent for the treatment of CDI. In this Phase 2, randomized, double-blind, comparator-controlled clinical study, CRS3123

will be compared with oral vancomycin in adult participants experiencing a primary episode or first recurrence of CDI.

Objectives and Endpoints:

This is the first study in which CRS3123 is administered to participants with a primary episode or first recurrence of CDI. The primary objectives of the study are the evaluation of safety and efficacy (rate of clinical cure at the test of cure [TOC] visit in the intent-to-treat [ITT] population). The secondary and exploratory objectives are additional evaluation of efficacy, assessment of plasma concentrations of CRS3123, outcomes of health-related quality of life (HRQoL), the effect of CRS3123 on the microbiology, fecal microbiome, fecal markers of inflammation, and metabolomics; and the assessment of fecal concentration of CRS3123.

The study objectives and endpoints are presented in Table 1. Detailed schedules of study assessments are provided in Section 1.3.

Table 1 Objectives and Endpoints

Objectives	Endpoints
Primary	
To assess the safety and efficacy of CRS3123 administered at 200 mg and 400 mg po bid and vancomycin administered 125 mg po qid in participants with CDI	**Efficacy (Primary Endpoint):** Rate of clinical cure at TOC in the ITT population **Safety**: An overall assessment of safety and tolerability of CRS3123 based on: • Incidence, severity, and relatedness of AEs • Assessment of vital signs, laboratory data, ECG findings, as well as clinical observations and physical examination
Secondary	
To further assess the efficacy of CRS3123 administered at 200 mg and 400 mg po bid and vancomycin administered 125 mg po qid in participants with CDI	**Efficacy (Secondary Endpoints):** • Rate of clinical cure at TOC in the Micro-ITT, PP, and ME populations • Rate of clinical cure at TOC as assessed by the investigator in the ITT, Micro-ITT, PP, and ME populations • Rate of total relief of symptoms of CDI at TOC in the Micro-ITT, PP, and ME populations • Time to resolution of diarrhea through TOC in the Micro-ITT, PP, and ME populations • Rate of early recurrence of CDI through FUV2 in the Micro-ITT and ME populations • Rate of late recurrence of CDI (between FUV2 and FUV3) in the Micro-ITT and ME populations • Rate of recurrence of CDI through FUV3 in the Micro-ITT and ME populations • Time to recurrence of CDI though FUV3 in the Micro-ITT and ME populations • Rate of global cure (clinical cure at TOC and no recurrence through FUV2 in the Micro-ITT, PP, and ME populations
To assess the plasma concentrations of CRS3123 following oral administration at 200 mg and 400 mg po bid	**The following will be assessed in the Plasma-PK population**: • Plasma concentrations of CRS3123 will be measured by sparse PK sampling at selected timepoints. • If feasible, primary parameters will be estimated from concentration versus time data using a population PK approach. PK parameters may include C_{max}, t_{max}, $t_{1/2}$, AUC_{0-24}, and CL/F, and accumulation ratio calculated using the estimated AUC_{0-24} at EOT vs Day 1.
To assess the health-related quality of life (HRQoL) outcomes in participants with CDI receiving CRS3123 administered at 200 mg and 400 mg po bid and vancomycin administered at 125 mg po qid	**HRQoL**: • *Clostridium difficile* Infection-Daily Symptoms (CDI-DaySyms™) change from baseline to each post-baseline visit in domain scores (diarrhea symptoms, abdominal symptoms, and systemic/other symptoms) in the Micro-ITT and ME populations

Objectives	Endpoints
Exploratory	
To assess the effect of CRS3123 on the microbiology, fecal microbiome, and fecal biomarkers of inflammation, to assess microbiological effect, and to evaluate metabolomics	The following parameters will be evaluated in the Micro-ITT and ME populations: • Quantitative *C. difficile* toxin production • Vancomycin-resistant enterococcus (VRE) culture and identification • *C. difficile* spore enumeration • Antimicrobial susceptibility testing • *C. difficile* culture • PCR Ribotyping • Toxin A/Toxin B titer Additional exploratory evaluations will include[a] • Effects of treatment on fecal inflammation biomarkers (calprotectin, lactoferrin, IL-1β, IL-2, IL-8, IL-15, IL-17, and GM-CSF) • Effects of treatment on fecal microbiome • Metabolomics
To assess the fecal concentrations of CRS3123 in the CRS3123 200 mg and 400 mg dose groups	Fecal concentrations of CRS3123 in the PK-Fecal population

Abbreviations: AE = adverse event; AUC = area under the concentration-time curve; bid = twice daily; CDI = *Clostridioides difficile* infection; CL/F = apparent clearance; C_{max} = maximum concentration; ECG = electrocardiogram; EOT = end of treatment; GM-CSF = granulocyte macrophage colony-stimulating factor; ITT = intent-to-treat; FUV = follow-up visit; Micro-ITT = microbiological ITT; ME = microbiologically evaluable; PD = pharmacodynamics; PK = pharmacokinetics; po = oral; PP = per-protocol; qid = 4 times daily; $t_{1/2}$ = terminal half-life; t_{max} = time to maximum concentration; TOC = test of cure visit (occurring at FUV1); VRE = vancomycin-resistant enterococcus

[a.] Population for analysis of the fecal microbiome, fecal biomarkers of inflammation, and metabolomics will be defined separately in a standalone analysis plan(s).

Overall Design:

This Phase 2, randomized, double-blind, comparator-controlled, multicenter study will be conducted to evaluate the primary objectives of safety and efficacy (rate of clinical cure) of 2 dosages of CRS3123 (200 mg and 400 mg) administered orally (po) twice daily (bid) and vancomycin administered 125 mg po 4 times daily (qid) in adults ≥ 18 years of age with a primary episode or first recurrence of CDI. The study will investigate the plasma concentrations and HRQoL outcomes of CRS3123 and additional efficacy and microbiology endpoints as well as the effect of CRS3123 on the fecal microbiome, fecal biomarkers of inflammation, and metabolomics in participants with CDI, and an assessment of fecal concentration of CRS3123 after dosing.

The duration of the treatment period for all study treatment arms is 10 days (40 doses; administration of last dose may occur on Day 11).

The Schedule of Activities is presented in Table 3 (Section 1.3).

Participants must meet the entry criteria, including the diagnosis of CDI by having ≥ 3 diarrheal (Bristol Stool Scale scores 5, 6, or 7) stools/day in 24 hours prior to randomization and in the judgment of the investigator that *C. difficile* is the causative agent for the diarrhea. Stool must be positive for *C. difficile* Toxin A and/or B antigen using an FDA or Health Canada approved/cleared EIA or ELISA laboratory test (e.g., Abbott/Alere QUIK CHEK COMPLETE®, Premier® Toxins A&B, etc.). A non-CLIA certified site may perform an Abbott/Alere QUIK CHEK COMPLETE® stool test provided by TechLabs, and if the test is positive for toxin A and/or B antigen, the subject may randomize while the local standard of care test is pending (i.e., NAAT, PCR, other toxin testing via EIA or ELISA).

Participants will be randomized (1:1:1) to 1 of 3 treatment arms as shown below. The treatment period for each arm will be 10 days. Randomization will be stratified by CDI type (first episode of CDI or first recurrence of CDI) to ensure proper balance among treatment arms.

- Treatment Arm A (n = 30-36): CRS3123 200 mg po bid (i.e., 400 mg/day)
- Treatment Arm B (n = 30-36): CRS3123 400 mg po bid (i.e., 800 mg/day)
- Treatment Arm C (n = 30-36): Vancomycin 125 mg po qid (i.e., 500 mg/day)

Study procedures will be completed at the following visits:

Screening Visit, Day -3 to Day 1: This is the pretreatment screening/baseline visit to determine study eligibility.

Treatment Period: Day 1 through EOT: Participants will receive study treatment qid at approximately 6-hour intervals for a total of 4 doses per day. During the treatment period, in Treatment Arm C, vancomycin comparator will be given as 125 mg capsules po qid; in Treatment Arms A and B, CRS3123 (200 mg and 400 mg, respectively) will be given po bid as doses 1 and 3, with placebos being given as doses 2 and 4. All study treatment (CRS3123, vancomycin, and placebo) will have an identical appearance. Study treatment will be administered for a total of 10 days for a total of 40 doses in each Treatment Arm. The first dose of study treatment may occur on the same calendar day as the screening visit. The participants will be instructed to take all study treatment doses with water. Participants, regardless of treatment arm, should make every attempt to maintain the qid (approximately every 6 hours (q6h) [± 1.5 h]) schedule. If they slip outside of this schedule, the participants should take their dose as soon as possible and should not skip the dose. On the Participant Daily Diary, the participants should record any missed doses.

If participants miss a dose of study treatment, the participants should record which dose of study treatment they missed in the Participant Daily Diary.

The end of treatment (EOT) assessments (described in Section 1.3) will be performed on the last calendar day that the participants receive the 40th dose of study treatment. For participants who prematurely discontinue study treatment, the EOT visit should occur as soon as possible after discontinuation of study treatment; these participants should continue to follow the Schedule of Activities for all subsequent planned visits.

Participants may be either inpatient (at the clinical site) or outpatient (this group may also include patients residing at skilled nursing facilities and rehabilitation facilities) based on investigator's discretion, but regardless of status, all must comply with all study requirements, including visits, follow-up visits, and suspected recurrence visits during the follow-up period.

Telephone contact during the treatment period will occur on Days 3 and 6 as described in Schedule of Activities (Section 1.3) to assess symptoms of clinical response or new or ongoing adverse events (AEs)/serious adverse events (SAEs) and will comprise a scripted interview with a questionnaire capturing the severity of GI symptoms/resolution of symptoms.

Participants will complete the Participant Daily Diary from Day -1 (≤24 hours prior to randomization) through the test of cure /Follow-up Visit 1 (TOC/FUV1, described below), as well as for episodes of recurrence during the follow-up period, as specified in the Schedule of Activities (Section 1.3). The Participant Daily Diary will include the date, time, and the Bristol Stool Scale score of each bowel movement and concomitant medications through TOC/FUV1 and at suspected recurrence, and adherence to study treatment through the EOT visit. The Participant Daily Diary will also include the patient-reported outcome (PRO) questionnaire (CDI-DaySyms), which will be used to assess CDI symptoms as well as quality of life parameters from screening through TOC/FUV1 (Kleinman et al, 2018; Talbot et al, 2019). The Daily Diary must be reconciled by study staff at every visit to ensure it is congruent with the eligibility criteria, the Investigator's assessment of clinical response, or diagnosis of recurrence.

Safety assessments will be performed at each visit from screening to FUV2 as indicated in the Schedule of Activities (Section 1.3). Safety will be assessed by Aes, physical examinations, vital signs, laboratory evaluations (hematology, chemistry, and/or urinalysis at most visits and coagulation at screening and EOT visit only), and electrocardiogram (ECG) parameters. Plasma concentrations will be assessed by collecting blood samples on Day 1 and the EOT visit (Section 1.3). Exploratory pharmacokinetic (PK) fecal concentration analysis will be assessed by collecting fecal samples at the Day 1 and EOT visit.

Follow-up Period: There will be a total of 3 Follow-up Visits (FUVs). The TOC/FUV1 will be at least 2 full days after last dose, FUV2 (early recurrence visit), and FUV3 (late recurrence visit). The TOC/FUV1 and FUV2 follow-up visits will be conducted as direct

site visits and include stool and blood sample submission as well as clinical assessments and ascertainment for recurrence of CDI. Telephone contact, using a scripted interview, will occur on Day 20 (± 3 days), Day 27 (± 3 days), and FUV3 (± 3 days) of the follow-up period to assess symptoms of clinical recurrence and new or ongoing Aes/SAEs, and capture the severity of gastrointestinal (GI) symptoms/resolution of symptoms.

If signs and symptoms of CDI recurrence are suspected, participants must come in for an unscheduled Suspected Recurrence Visit earlier than the next planned visit, as applicable. Following the Suspected Recurrence Visit, participants will be treated per standard of care and continue to follow the Schedule of Activities for all subsequent planned visits and telephone calls. A test for *C. difficile* Toxin A and/or B antigen using an FDA or Health Canada approved/cleared EIA or ELISA laboratory test must be performed at the unscheduled Recurrence Visit.

The study participant is expected to complete all study visits unless the participant dies or withdraws consent.

A Data Safety Monitoring Board (DSMB) will be established and managed in this study by The National Institute of Allergy and Infectious Diseases (NIAID) to analyze aEs and other safety data.

Number of Participants:

This protocol plans to enroll approximately 90 to 108 participants who meet study entry criteria, who will be randomly assigned to treatment in 1 of 3 treatment arms in a 1:1:1 ratio as presented in Table 2. This study will provide an initial assessment of safety and efficacy data to inform the future development of CRS3123. This study is not powered to detect a difference between the treatment groups or for non-inferiority testing.

If 36 participants are enrolled in each CRS3123 treatment arm and the true rate of clinical cure at TOC is 0.83 in each group, then the exact 95% confidence interval for the treatment of cure rate is (0.67, 0.94). Alternatively, if 30 participants are enrolled in each CRS3123 treatment arm and true rate of clinical cure at TOC is 0.83, then the 95% CI for the rate is (0.65, 0.94).

Number of Sites and Duration of Study:

Up to 50 sites will be used to recruit participants, based on published CDI enrollment rate of 2.4 participants per site per year for fidaxomicin Phase 3 study (Cornely et al, 2012).

The study will be conducted in United States and Canada.

The duration of study will be approximately 36 months.

Treatment Arms and Duration:

The estimated duration of study participation is approximately 70 days from the screening visit to FUV3; see Table 3 Schedule of Activities footnote 'a'.

The screening period will be up to 72 hours in duration before treatment, followed by a treatment period of 10 days, with an EOT visit on the day of last dose or as soon as possible after early study treatment discontinuation, and 3 follow-up visits (FUV1, FUV2, and FUV3). Additionally, phone calls during the treatment period (on Days 3 and 6) and during the follow-up period (Day 20, Day 27, and FUV3) will be conducted, as described above and shown in Figure 1.

The 3 treatment arms are presented in Table 2.

Table 2 Treatment Arms

Treatment Arm	Study Treatment, Dose, Duration	Study Treatment Administration	Approximate Planned Number of Participants
A	CRS3123, 200 mg po bid (i.e., 400 mg/day) (daily doses 1 and 3) and placebo bid (daily doses 1 to 4) given approximately q6h (± 1.5 h) for 10 days	CRS3123 (1 capsule of 200 mg + 1 capsule of placebo) at daily doses 1 and 3 1 capsule of placebo at daily doses 2 and 4)	30-36
B	CRS3123, 400 mg po bid (i.e., 800 mg/day) (daily doses 1 and 3) and placebo bid (daily doses 2 and 4) given approximately q6h (± 1.5 h) for 10 days	CRS3123 (2 capsules of 200 mg) at daily doses 1 and 3 1 capsule of placebo at daily doses 2 and 4	30-36
C	Vancomycin, 125 mg po qid (i.e., 500 mg/day) given approximately q6h (± 1.5 h) for 10 days	Vancomycin (1 capsule of 125 mg + 1 capsule of placebo) at daily doses 1 and 3 Vancomycin (1 capsule of 125 mg) at daily doses 2 and 4	30-36

Abbreviations: bid = twice daily; h = hours; qid = 4 times daily, q6h = every 6 hours
Note: Vancomycin will be over-encapsulated to match the appearance of CRS3123 and placebo capsules.

Summary of Statistical Methods

Baseline data including demographic information (age, sex, race), medical history, and baseline signs and symptoms will be summarized by treatment arm using descriptive statistics.

Safety will be assessed in the Safety population. The number and percentage of participants with TEAEs will be tabulated by system organ class, preferred term, and treatment arm. TEAEs will also be summarized by relationship to study treatment, system

organ class, and preferred term, and also by maximum severity, system organ class, and preferred term. Serious adverse events will be summarized similarly. Clinical laboratory test results, ECG parameters, and vital signs will be summarized by visit as will changes from baseline for each treatment arm.

For the following efficacy endpoints, rates will be summarized by treatment arm and exact confidence intervals will be created to compare the difference in rates in the each of the CRS3123 groups versus the vancomycin group:

- Clinical cure at TOC (primary endpoint in ITT population)
- Clinical cure at TOC (secondary endpoints in Micro-ITT, PP, and ME populations)
- Clinical cure at TOC as assessed by the investigator
- Total relief of symptoms of CDI at TOC
- Early recurrence of CDI through FUV2
- Late recurrence of CDI through FUV3
- Recurrence of CDI though FUV3
- Global cure (clinical cure at TOC) and no recurrence through FUV2

Kaplan-Meier analyses of time to resolution of diarrhea through TOC as well as time to recurrence through FUV3 will be provided by treatment arm.

For the HRQoL CDI-DaySyms analysis, domain scores will be summarized by visit and treatment arm using summary statistics. Changes from baseline in domain scores to each post-baseline visit will also be summarized by visit and treatment arm.

If feasible, primary parameters will be estimated from concentration versus time data using a population PK approach (plasma PK only). Plasma PK parameters may include maximum concentration (C_{max}), time to maximum concentration (t_{max}), terminal half-life ($t_{1/2}$), area under the concentration-time curve from 0 to 24 hours (AUC_{0-24}) and apparent clearance (CL/F), and accumulation ratio calculated using the estimated AUC_{0-24} at EOT vs Day 1.

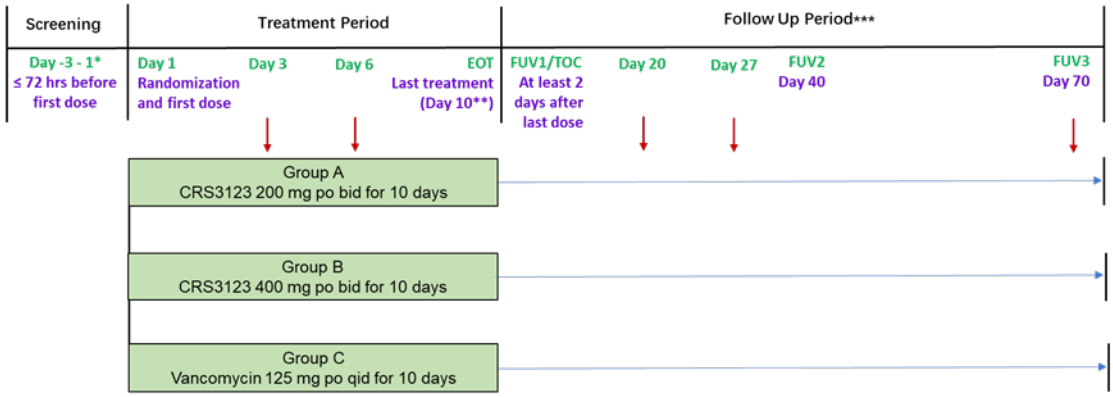
All data collected during the study will be analysed and presented in the clinical study report. If possible, subjects determined to be immunosuppressed under Protocol Amendment 6 will be subject to a sensitivity analysis.

The Statistical Analysis Plan will be developed and finalized before the database is locked and will provide additional details regarding statistical procedures.

1.2 Schema

An overview of the study design is presented in Figure 1.

Figure 1: Overview of Study Design



Abbreviations: bid = twice daily; CDI = *Clostridioides difficile* infection; EOT = end of treatment; FUV = Follow-up Visit; po = oral, qid = 4 times daily; TOC = test of cure
Note: Red arrows indicate follow-up phone contact: Days 3 and 6 during the treatment period, and Day 20, Day 27, and FUV3 during the follow-up period. Screening/Day 1, EOT, TOC/FUV1, and FUV2 are in-person visits.
* Screening and randomization may both occur on Day 1.
** The EOT assessments will be performed on the last calendar day that the participants receive the 40th dose of study treatment. TOC assessments will be performed on FUV1. TOC/FUV1 should occur at least 2 days after the last dose of study drug. For participants who discontinue study treatment prematurely, EOT should occur as soon as possible after study treatment discontinuation; these participants should continue to follow the Schedule of Activities for all subsequent planned visits and telephone follow-up calls, including TOC1/FUV1 through FUV3.
*** If signs and symptoms of CDI recur at any point in the study between FUV1 and FUV3 following clinical cure, participants must come in for an unscheduled Suspected Recurrence Visit.

1.3 Schedule of Activities

A Schedule of Activities is presented in Table 3.

Table 3 Schedule of Activities

		Treatment Period (Days 1-10)										Follow-up Period (Days 11-70)				Suspected Recurrence Visit (Unscheduled)
Study Visit	**Screening**										**EOT**	**TOC/FUV1**	**Phone Contact**	**FUV2**	**FUV3 (Phone Contact)**	
Study Day	**D-3 to D1[a]**	**D1[a,b]**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10[b,c]**	**(Last dose +2[b,c])**	**D20+ D27[d]**	**D40[b,e]**	**D70[d]**	**Post-TOC to FUV3[b,d,e]**
Study Windows (days)	--	--	--	(±1)	--	--	(±1)	--	--	--	(+2)	(+3)	(±3)	(±3)	(±3)	
Informed consent[f]	X															
Fecal sample for *C. difficile* Toxin A or B[e,f] antigen	X	X														X
Inclusion/exclusion criteria[f]	X	X														
Medical history, demographics	X															
Height and weight[g]	X											X				
Physical examination[g]	X											X		X		X
Vital signs[h]	X	X									X	X		X		X
12-lead ECG (supine)[i]	X										X					
Local lab for screening eligibility[j]	X															
Pregnancy test (urine)[k]	X													X		
Randomization[l]		X														
Study treatment[m]		X[m]	X	X	X[m]	X	X	X	X	X	X[m]					
Scripted telephone interview[d]				X			X						X		X	
Concomitant medications[n]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events[o]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

		Treatment Period (Days 1-10)										Follow-up Period (Days 11-70)				
Study Visit	**Screening**										**EOT**	**TOC/FUV1**	**Phone Contact**	**FUV2**	**FUV3 (Phone Contact)**	**Suspected Recurrence Visit (Unscheduled)**
Study Day	**D-3 to D1[a]**	**D1[a,b]**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10[b,c]**	**(Last dose +2[b,c])**	**D20+ D27[d]**	**D40[b,e]**	**D70[d]**	**Post-TOC to FUV3[b,d,e]**
Study Windows (days)	**--**	**--**	**--**	**(±1)**	**--**	**--**	**(±1)**	**--**	**--**	**--**	**(+2)**	**(+3)**	**(±3)**	**(±3)**	**(±3)**	
Safety lab assessments (central lab)[p]		X									X[p]	X[p]		X		X
Participant Daily Diary, CDI-DaySyms, review by investigator[q]	X	X	X[q]	X	X	X[q]	X	X[q]	X	X	X	X				X[q]
Investigator assessment of clinical response[r]											X	X		X		X
Blood and fecal samples for PK[s,v]	X[v]	X[v]									X					
Fecal sample for microbiology[t, v]	X[v]	X[v]									X[w]	X[w]				X[t]
Fecal sample for microbiome and host biomarkers testing[t, v]	X[v]	X[v]										X		X		X[t]
Metabolomics[u, v]	X[v]	X[v]										X				

Abbreviations: aEs = adverse events, ALT = alanine aminotransferase, AST = aspartate aminotransferase, β-HCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CBC = complete blood count; CDI = *C. difficile* infection; CDI-DaySyms = *C. difficile* Infection-Daily Symptoms questionnaire; CPK = creatine phosphokinase; CRP = C-reactive protein; D = Day; ECG = electrocardiogram; EOT = end of treatment; FUV = Follow-up Visit; GDH = glutamate dehydrogenase; h = hours; IRT = interactive response technology; PD = pharmacodynamic; PK = pharmacokinetic; po = orally; PRO = patient-reported outcomes; qid = 4 times daily; SAE = serious adverse events; TOC = test of cure; VRE = vancomycin-resistant enterococcus

a Screening and Day 1: Screening assessments for study eligibility can occur within 72 hours of the administration of the first dose of study treatment. Screening and Day 1 can occur on the same calendar day. For instances when the study run-in period for study eligibility may be delayed (e.g., weekends, holidays, etc.), the screening period can be extended up to 72 hours (all eligibility criteria still apply including the requirement for ≥3 diarrheal (Bristol Stool Scale scores 5, 6, or 7) stools/day within in a 24-hour period prior to randomization).

b Mandatory clinical site visits occur on Day 1, EOT, TOC/FUV1, and FUV2, and at suspected recurrence, as shown in the Table of Activities. For participants who are inpatient at the clinical site, all safety assessments during the treatment period are performed in person daily. Participants will attend EOT, TOC/FUV1, and FUV2 for collection of safety and efficacy data. Fecal samples for PK, microbiology, microbiome, host biomarkers of inflammation, and/or metabolome testing will occur at all in-person visits based on the Schedule of Activities. If signs and symptoms of CDI recur at any point in the study between TOC/FUV1 and FUV3 following clinical cure, participants must come in for an unscheduled Suspected Recurrence Visit.

c EOT assessments will be performed on the last calendar day that the participants receive the 40th dose of study treatment (ostensibly Day 10; with a +2 day window. Record the date and time of the last previous dose associated with the EOT plasma PK sample. TOC/FUV1 assessments will occur at least 2 full days after the day of the last administered study drug. Note: Participants who discontinue study treatment prematurely should come in for an EOT visit as soon as possible after study treatment discontinuation, and then continue to follow the Schedule of Activities for all subsequent planned visits and telephone calls, including TOC/FUV1 through FUV3 (telephone contact scheduled for Days 3 and 6 that fall after early EOT do not need to take place).

d Telephone contact will occur on Days 3 and 6 during the treatment period to assess clinical response as well as on Day 20, Day 27, and FUV3 of the follow-up period to assess symptoms of clinical recurrence, or new or ongoing AEs/SAEs. A scripted interview capturing the severity of gastrointestinal symptoms/resolution of symptoms, including resolution of diarrhea, will be administered during the call and participants will be reminded of any upcoming clinical site visits. If signs and symptoms of CDI recurrence are suspected at any point between FUV1/TOC and FUV3, participants must come in for an unscheduled Suspected Recurrence Visit. Note: In the event a participant is an inpatient at the clinical site at the time of a scheduled Telephone Contact, the Telephone Contact will be conducted as an in-person visit during the treatment period.

e Participants who have a recurrence after TOC/FUV1 should be treated per standard of care and continue to follow the Schedule of Activities for FUV2 and FUV3. *C. difficile* culture as well as a stool evaluation for Toxin A and/or B antigen should be performed at suspected recurrence. A non-CLIA certified site may also perform an Abbott/Alere QUIK CHEK COMPLETE® test according to the package insert with results verified by a second individual and a photograph of the result recorded with the date and time of the result.

f Written informed consent must be obtained before any nonstandard of care screening assessments are performed. All inclusion and exclusion criteria need to be met before randomization on Day 1.

g A complete physical examination evaluation will be performed at screening and include evaluation of the head, eyes, ears, nose, and throat (HEENT), neck, lungs, heart, chest, abdomen, extremities, neurological status, and skin, as well as height, and weight. A focused physical examination and measurement of weight will be performed on subsequent indicated visits and at Suspected Recurrence Visit (if applicable).

h Vital signs include temperature, pulse rate, respiratory rate, and blood pressure.

i 12-lead ECG will be performed in a supine position.

j CBC with differential, chemistry (including sodium, potassium, bicarbonate, chloride, BUN, serum creatinine, glucose, total protein, albumin, alkaline phosphatase, total bilirubin, ALT, AST, lipase, amylase, and CPK), coagulation, and CRP will be performed in the local laboratory at screening with a subset of tests used to evaluate eligibility. It is not necessary for all labs to be resulted prior to randomization except those that would affect eligibility. Results that affect eligibility are confirmation of *C. difficile* toxin A and/or B antigen in stool, pregnancy test. Labs drawn within 48 h prior to screening are acceptable for screening eligibility purposes. However eligible subjects cannot have received anti-CDI therapy for longer than 24 hours. When the turn-around time of laboratory results affecting study eligibility may be delayed (e.g., weekends, holidays, etc.), the screening period is up to 72 hours (all eligibility criteria still apply including the requirement for ≥3 diarrheal (Bristol Stool Scale scores 5, 6, or 7) stools/day in 24 hours prior to randomization).

k Female participants of childbearing potential must have a urine pregnancy test (β-HCG) at screening and FUV2 performed at the local laboratory. Women of non-childbearing potential include women who are postmenopausal as demonstrated by amenorrhea for ≥ 12 months or surgical sterilization (ie., tubal

ligation, bilateral salpingectomy, bilateral oophorectomy, or hysterectomy). Provision of documentation is not required for female sterilization; verbal confirmation is adequate.

l All inclusion and exclusion criteria need to be met before randomization on Day 1. All participants will be centrally assigned to randomized study treatment via IRT.

m Day 1 is the first day for administration of study treatment and subsequent days are calendar days. Blister packs (each containing 20 doses) will be dispensed on Day 1. Participants will receive study treatment on Days 1 through 10, qid, at approximately 6-hour intervals (± 1.5 h), for a total of 4 doses per day. Study treatment comprises vancomycin 125 mg po qid and CRS3123 capsules 200 mg or 400 mg po bid. Identical placebo capsules will be given as the second and final daily dose in participants randomized to CRS3123. On Day 1, the first dose of study treatment should be administered as quickly as possible after eligibility criteria are met. If the participant discontinues study treatment prematurely, an EOT visit should occur as soon as possible after study treatment discontinuation. NOTE: All doses should be taken with water. Participants, regardless of treatment arm, should make every attempt to maintain the qid (approximately every 6 hours (q6h) [± 1.5 h]) schedule. If they slip outside of this schedule, the participants should take their dose as soon as possible and should not skip the dose. On the Participant Daily Diary, the participants should record any missed doses. Dose 1 will be administered on site to facilitate plasma PK sample collection.

n All medications taken within 30-days prior to screening as well as medications taken during the study period (randomization through FUV3) should be recorded.

o All adverse events, including serious adverse events, will be collected from randomization through FUV3 at the timepoints indicated in the Schedule of Activities, above.

p Safety laboratory assessments performed by the central laboratory will include CBC with differential, chemistry, CRP, coagulation parameters, and urinalysis at the indicated timepoints. Coagulation will be assessed only at screening (local laboratory) and EOT visit (central laboratory).

q The Participant Daily Diary will be completed from D-1 (≤24h prior to randomization) daily through TOC/FUV1 and at suspected recurrence and reviewed by study staff at each visit. The Participant Daily Diary includes details for each bowel movement and concomitant medications through TOC/FUV1 and at suspected recurrence; any missed doses of study treatment through EOT, and the CDI-DaySyms PRO questionnaire through TOC/FUV1. For participants treated as outpatients, the Participant Daily Diary will be reviewed during visits at the clinical site and phone call days during the treatment period (i.e., Days 3 and 6). For participants treated in the hospital, Participant Daily Diary review will occur daily.

r Patients that are clinical failures at TOC or at any subsequent visit do not need to complete further investigator's assessments beyond that visit; however, patients will continue visits per the Schedule of Activities for all other assessments.

s Blood and fecal samples for assessment of plasma and fecal concentrations will be collected at the clinical site and provided to the bioanalytical laboratory for CRS3123 concentration analysis. The date and time of each study treatment dose for each respective PK sampling, and the date and time of each sample collection (not processing), must be captured in the clinic.

t Fecal samples for microbiology, microbiome, and host biomarkers of inflammation testing must be collected at the clinical site prior to first dose of study treatment (as described in footnote "v") and at subsequent post-Day 1 visits shown in the Schedule of Activities, including at time of suspected recurrence if applicable. Of note, the culturing of toxigenic *C. difficile* and microbial susceptibility testing will be performed only from the initial, TOC, and recurrence episode stool samples (including early discontinuation due to clinical failure).

u A portion of the collected fecal sample will also be used for metabolomics analysis prior to first dose of study treatment (as described in footnote "v") and subsequent post-Day 1 timepoints indicated in the Schedule of Activities.

v Fecal sample collection: Fecal samples of ≥ 6 g will be collected at all timepoints requiring a fecal assessment. A fecal sample for PK, microbiology, microbiome, host biomarkers testing, and metabolomics will be collected at the clinical site prior to first dose of study treatment. This sample can be collected at Screening or on Day 1 (predose). For fecal PK analysis only, an additional fecal sample will be taken on Day 1 postdose (first bowel movement after dose). Fecal samples collected on subsequent visits will be used only for the analyses planned for that specific visit as indicated in the Schedule of Activities. (Note: The date and time of each study treatment dose and the date and time of each sample collection (not processing) must be captured in the clinic. Fecal samples will be processed by the central laboratory and portions of each sample will be provided to the relevant designated laboratories for analysis. Stool samples may be collected as early as 24-hours prior to each study visit and up to 24-hours after each study visit. Stool samples collected within 24-hours of the desired collection day will not constitute a protocol deviation.

w Microbiology samples should be collected at both the EOT visit and TOC/FUV1 visit; however, the EOT visit sample will be analyzed if the TOC/FUV1 sample is missing or if the participant discontinues study treatment prematurely.