VA Cooperative Studies Program #596

Optimal Treatment for Recurrent *Clostridium difficile* Infection (OpTION)

STUDY PROTOCOL

Version 10.1 NCT02667418

August 15, 2025

Principal Proponents' Office:

Stuart Johnson, MD, Principal Proponent

Dale N. Gerding, MD, Co-Principal Proponent

Curtis J. Donskey, MD, Co-Principal Proponent

Neil C. Johnson, MS, National Coordinator

Cooperative Studies Program Coordinating Center, Hines VA:

Hua Feng, PhD, MS, Biostatistician

Michelle Johnson, MPH, PMP, Project Manager

Cooperative Studies Program

Clinical Research Pharmacy Coordinating Center, Albuquerque VA:

Alexa Goldberg (Argyres), PharmD, Clinical Research Pharmacist

TABLE OF CONTENTS

[THIS PAGE LEFT INTENTIONALLY BLANK]

LETTERS OF SUBMITTAL: Principal Proponents

DEPARTMENT OF VETERANS AFFAIRS
Edward Hines Jr. VA Hospital
5000 South 5th Avenue
Hines, IL 60141

October 31, 2014

Timothy J. O'Leary, MD, PhD

Chief Research and Development Officer
VA Central Office

810 Vermont Avenue, NW
Washington, DC 20420

Dear Dr. O'Leary:

We are submitting the revised protocol with inclusion of a pilot phase for VA Cooperative Studies Program #596, "Optimal Treatment for Recurrent *Clostridium difficile* Infection" for review.

In response to specific reviewer comments in the CSSEC Minutes of June 12, 2014, we have made the following protocol revisions:

- Modification of secondary non-inferiority analysis to assess the non-inferiority of vancomycin taper and pulse (VAN-TP/P) to fidaxomicin treatment (FID-TX) if **both** FID-TX and VAN-TP/P are found to be superior to vancomycin treatment (VAN-TX).
- Adverse events (specifically, withdrawal from treatment due to adverse events or severe adverse events) were removed from the composite outcome measures (D-COM and CDI-COM) but will be included in the safety analysis.
- The patient stool diary was simplified to one page.
- Inclusion of a patient-centered outcome assessment that will be initiated and modified during the pilot phase of the study.

Inclusion of a pilot phase in CSP #596 will allow for assessment of enrollment targets as well as allow for developmental work on patient-centered outcome assessments that have not previously been included in clinical trials of CDI but for which may prove useful for future trials. We want to reiterate that the importance of this study is based on the fact that recurrence of CDI is a common and serious event for which there has never been a well-controlled randomized clinical trial. We believe the proposed revised study will be the first to systematically compare the effectiveness of three alternative therapies for the management of first and second episodes of recurrent CDI for which there is currently inadequate comparative data. Sincerely,

Stuart Johnson, M.D.
Co-Principal Proponent

Dale Gerding, M.D.
Co-Principal Proponent

LETTERS OF SUBMITTAL: Principal Proponents

DEPARTMENT OF VETERANS AFFAIRS

Edward Hines Jr. VA Hospital
5000 South 5th Avenue, IL 60141

April 17, 2014

Timothy J. O'Leary, MD, PhD

Acting Chief Research and Development Officer
VA Central Office

810 Vermont Avenue, NW
Washington, DC 20420

Dear Dr. O'Leary:

We are submitting the revised protocol for VA Cooperative Studies Program #596, "Optimal Treatment for Recurrent *Clostridium difficile* Infection" for review by the Cooperative Studies Scientific Evaluation Committee meeting in Washington, DC June 12, 2014.

In response to specific reviewer comments in the CSSEC Minutes of December 12, 2013, we have made the following protocol revisions:

- At reviewer suggestion we have revised the primary outcome to a diarrhea composite outcome measure or D-COM to include diarrhea (with or without documentation of toxigenic *C. difficile* or its toxin in stool) and severe abdominal pain, toxic megacolon, colectomy, death, or withdrawal from treatment due to adverse event.
- At reviewer suggestion the primary endpoint for all treatments is day 59.
- We have clarified that early treatment failures will be carried forward as failures in the sustained clinical response.
- Patient assessments will be based on diary assessments but the frequency of telephone calls to review diary completeness has been increased to weekly throughout the study.
- We share reviewers concerns about the large number of pills taken in the first 10d of the study, but to maintain blinding we are not able to avoid this.
- Given reviewer concerns, the follow-on salvage regimen of extended duration fidaxomicin treatment has been deleted.

- Several CDI treatment and prevention initiatives are currently in phase II and III clinical trials including fecal microbiota transplants (FMT), but none are expected to impact on this trial of first and second recurrences of CDI treatment before this trial is completed.
- Cubist, the manufacturer of fidaxomicin, remains interested in supporting the trial and discussions of the extent of their commitment are actively being pursued.
- To compare the two intervention arms (fidaxomicin and vancomycin taper/pulse) to each other, a statistical test of treatment non-inferiority has been proposed. If either of these treatments is found to be superior to vancomycin, then the non-inferiority of vancomycin taper/pulse to fidaxomicin will be assessed.
- Study stopping rules have been clarified and will apply only to efficacy, not futility.

Recurrent CDI is the most challenging clinical dilemma facing clinicians who treat this disease. As members of the Infectious Diseases Society of America (IDSA) C. difficile Guideline Committee, we are acutely aware of the need for high quality evidence to make recommendations for treating these patients. The importance of this study is based on the fact that recurrence of CDI is a common and serious event for which there has never been a well-controlled randomized clinical trial. We believe the proposed revised study will be the first to systematically compare the effectiveness of three alternative therapies for the management of first and second episodes of recurrent CDI for which there is currently inadequate comparative data.

Sincerely,

Stuart Johnson, M.D.

Co-Principal Proponent

Dale Gerding, M.D.

Co-Principal Proponent

LETTERS OF SUBMITTAL: Principal Proponents

DEPARTMENT OF VETERANS AFFAIRS

Edward Hines Jr. VA Hospital
5000 South 5th Avenue, IL 60141

October 3, 2013

Timothy J. O'Leary, MD, PhD

Acting Chief Research and Development Office Director
VA Central Office

810 Vermont Avenue, NW
Washington, DC 20420

Dear Dr. O'Leary:

We are submitting the protocol for VA Cooperative Studies Program #596, "Optimal Treatment for Recurrent *Clostridium difficile* Infection" for review by the Cooperative Studies Scientific Evaluation Committee meeting in Washington, DC in December 2013. Recurrent CDI is the most challenging clinical dilemma facing clinicians who treat this disease. As members of the Infectious Diseases Society of America (IDSA) *C. difficile* Guideline Committee, we are acutely aware of the need for high quality evidence to make recommendations for treating these patients. The importance of this study is based on the fact that recurrence of CDI is a common and serious event for which there has never been a well-controlled randomized clinical trial. The proposed study will be the first to systematically compare the effectiveness of three alternative therapies for the management of recurrent CDI for which there is currently inadequate comparative data.

Sincerely,

Stuart Johnson, M.D.
Co-Principal Proponent

Dale Gerding, M.D.
Co-Principal Proponent

LETTERS OF SUBMITTAL: Center Director, Hines CSPCC

DEPARTMENT OF VETERANS AFFAIRS

Edward Hines Jr. VA Hospital
5000 South 5th Avenue, IL 60141

April 18, 2014

Timothy J. O'Leary, MD, PhD

Acting Chief Research and Development Officer
VA Central Office

810 Vermont Avenue, NW
Washington, DC 20420

Dear Dr. O'Leary:

We are pleased to resubmit for review the revised protocol for VA Cooperative Studies Program #596, "Optimal Treatment for Recurrent *Clostridium difficile* Infection". I believe we have been responsive to the reviews provided by CSSEC at its December 2013 review of the original study proposal. The key revisions are summarized in the resubmission letter provided by Drs. Johnson and Gerding. We look forward to meeting with CSSEC in June 12, 2014.

Sincerely,

Domenic J. Reda, Ph.D.
Center Director, Hines CSPCC

DEPARTMENT OF VETERANS AFFAIRS

Edward Hines Jr. VA Hospital
5000 South 5th Avenue

Hines, IL 60141

October 8, 2013

Timothy J. O'Leary, MD, PhD

Acting Chief Research and Development Officer
VA Central Office

810 Vermont Avenue, NW
Washington, DC 20420

Dear Dr. O'Leary:

I am pleased to submit for review the protocol for VA Cooperative Studies Program #596, "Optimal Treatment for Recurrent *Clostridium difficile* Infection". We convened an expert planning committee which was very thoughtful in its considerations of the clinical relevance of the study objective, the study design and the feasibility of conducting this trial in the VA. I am confident this study will provide meaningful and practical answers to the question of optimal treatment for early (first and second episode) recurrent CDI, which is one of the most challenging clinical dilemmas facing clinicians who treat this disease. The responses we received from potential study sites indicate high enthusiasm for this study. Furthermore, I am confident that Drs.

Johnson and Gerding have the knowledge, experience and leadership qualities to lead this effort to a successful conclusion.

Sincerely,

Domenic J. Reda, Ph.D.
Center Director, Hines CSPCC

Department of Veterans Affairs
VA Cooperative Studies Program
Clinical Research Pharmacy Coordinating Center (151-I)
2401 Centre Avenue SE
Albuquerque, NM 87106-4180

April 11, 2014

In Reply Refer To: 501/151-I
CSP #95/ #596
File: STD_DOC

Timothy J. O'Leary, M.D., Ph.D.
Acting Chief Research and Development Officer
Department of Veterans Affairs (10P9)
131 M. Street, NE
Washington, DC20002

Dear Dr. O'Leary:

SUBJ: Request to Continue Planning of CSP# 596, "Optimal Treatment for Recurrent Clostridium Difficile Infection"

The following Pharmacy Coordinating Center (PCC) related issues have been identified:

Drug Supply

General

The PCC will over-encapsulate (OE) commercial vancomycin 125 mg capsules and fidaxomicin 200 mg tablets, along with a matching placebo capsule manufactured by the Pharmacy Coordinating Center. The current PCC budget is based on 26 sites and 531 participants. A small change in the number of participants (15-20) will not significantly impact on the PCC budget.

Study medication

Fidaxomicin is now marketed by Cubist Pharmaceuticals. The study chairs have been in contact with Cubist, but currently the company is non-committal regarding drug donation or a specific level of support they may be willing to provide; therefore, the PCC budget assumes all clinical trial materials will be purchased or manufactured by the PCC. The current VA contract price for fidaxomicin is $90.02, and is a major cost driver of the PCC budget. Vancomycin is comparatively inexpensive with a current unit cost of $8.72/capsule. The placebo capsules are very low cost, but will require some tooling equipment and raw material purchases.

Current VA Catalog	Total estimated cost (drug only)	Price per unit	Contract Type
Vancomycin HCl 125 mg capsules	$208,771	$8.72	FSS
Fidaxomicin 200 mg tablet	$427,227	$90.08	NAT
Placebo capsule	$1750	$0.11	N/A

RJR/rkr

Timothy J. O'Leary, M.D., Ph.D.

Study medication will be dispensed at scheduled clinic visits to minimize drug loss should a participant drop out from the study or not tolerate the intervention.

Timing of Study Initiation

The PCC budget currently includes a 9 month startup period to account for initiating and completing the contracting processes, procuring tooling and manufacturing supplies, and subsequent manufacturing of study medication. Most contracting and procurement activities require funding to be in place prior to initiating the work.

IND Requirement

The study is likely to require an IND, and CSP CRPCC plans to file one, or alternatively request a "Letter of Determination" from the FDA should CSSEC approve the study. While both active treatments are approved for treating C. Difficile associated diarrhea, CSP#596 is specifically looking at recurrence and it is unclear if an IND will be necessary for this study population. Whether or not the trial will require an IND will depend on the FDA's determination regarding "significant increase in risk" as outlined in 21 CFR 312.2 (b). The FDA will either accept or reject the IND and issue the relevant documents. The VA CIRB is highly likely to request copies documenting the FDA's decision.

Good Clinical Practices Training and Auditing/Monitoring Plan

The personnel and travel costs for kick-off meetings, training, and for monitoring/auditing of sites are in the Site Monitoring, Auditing and Resource Team (SMART) portion of the PCC budget. SMART has assigned CSP#596 a "Category II" risk level. This level is designated for Drug/Device Studies with IND/IDE, but not intended for NDA/PMA Submission to FDA.

Please contact me at (505) 248-3200 if there are any questions concerning these issues.

ROBERT J. RINGER, Pharm.D., BCNP
Assistant Center Director
Pharmaceutical Research and Management

Concur/Non Concur

MIKE R. SATHER, Ph.D., F.A.S.H.P.
Director, VA CSPCRPCC

Date: 4/11/14

RJR/rkr

Department of Veterans Affairs
VA COOPERATIVE STUDIES PROGRAM
CLINICAL RESEARCH PHARMACY COORDINATING CENTER (151-I)
2401 Centre Avenue, SE
Albuquerque, NM 87106-4180

September 30, 2013

In Reply Refer To: 501/151-I
CSP #95/ #596
File: STD_DOC

Timothy J. O'Leary, M.D., Ph.D.
Director, Clinical Service R&D
VA Cooperative Studies Program (10P9CS)
810 Vermont Avenue NW
Washington, DC 20420

SUBJ: Cooperative Studies Program Clinical Research Pharmacy Coordinating Center Issues Concerning CSP #596, "Optimal Treatment for Recurrent Clostridium Difficile Infection"

The following Pharmacy Coordinating Center (PCC) related issues have been identified:

Drug Supply

General

The PCC will over-encapsulate (OE) commercial vancomycin 125mg capsules and fidaxomicin 200mg tablets, along with a matching placebo capsule manufactured by the Pharmacy Coordinating Center. The current PCC budget is based on 26 sites and 531 participants.

Study Medication

Fidaxomicin is marketed by Optimer Pharmaceuticals, although our understanding is that Optimer is in the process of being bought by Cubist Pharmaceuticals. The study chair has contacted the Optimer, but they are non-committal about drug donation until the sale of the company is finalized. Vancomycin is comparatively inexpensive with a current unit cost of $8.72/capsule. Fidaxomicin is more expensive at $90.08/tablet at current VA National Contract prices. The placebo capsules are very low cost, but will require some tooling equipment and raw material purchases.

Current VA Catalog	Total estimated cost (drug only)	Price per unit	Contract Type
Vancomycin HCl 125mg capsules	$208,771	$8.72	FSS
Fidaxomicin 200mg tablet	$1,117,278	$90.08	NAT
Placebo capsule	$17,650	$0.11	N/A

Purchase and Blinding of Study Drugs

The CSPCRPCC plans to purchase commercially available generic vancomycin 125mg capsules and Fidaxomicin 200mg tablets through the current VA contracted wholesaler. There are several issues to consider surrounding commercial product.

1. Blinding of active study medications: All blinded studies carry the risk of the treatment assignment being discovered by participants or site personnel. Over-encapsulated study medication has the commercial product inside of a capsule surrounded by excipient filler; therefore, it is possible for someone to disassemble the capsule and discover the inner contents. Vancomycin and fidaxomicin are supplied in a capsule and tablet form, respectively, so the inner contents are visibly different, and the placebo will only have excipient. To mitigate this risk the PCC uses "DB" capsules that are difficult (but not impossible) to separate.

2. Product availability may change during the treatment phase of the trial. While both vancomycin and fidaxomicin are currently available through the VA National Contract, there have been instances of price instability of products from the wholesaler. The prices in the table are current as of this writing. If the preferred agent or supplier(s) should change or becomes unavailable it will be necessary to procure a substitute product. This could require contracting for an alternative source of study medications and possible retooling.

3. The drug cost in the CSPCRPCC budget is based on current VA drug pricing. Both drug pricing and availability is subject to change. Currently vancomycin is listed as an FSS contract type, which is subject to price changes at any time. Fidaxomicin is a NAT, or national contract. NAT tend to be have stable pricing, but given the expected acquisition of Optimer by Cubist it is unknown if a new contract price will be negotiated. Any change in contract status could require additional funds.

Packaging and Distribution of Study Medication and Dosing Schedule

Study medication will be packaged in blister cards for participant safety, convenience, and adherence to the dosing schedule.

Study medication will be dispensed upon initial randomization (10 days treatment card), followed by a blinded 21 day treatment card for the taper/pulse card issued at the day 10 clinic visit. The blinded study medication would be packaged in blister cards in a manner to blind the treatment assignments with regard to dosing schedule. The "Salvage" treatment would be open-label, but still OE to aid adherence to the potentially confusing fidaxomicin taper/pulse treatment dosing schedule and maintain product uniformity. Study medication will be dispensed at scheduled clinic visits to minimize drug loss should a participant drop out from the study or not tolerate the intervention.

MRS/mbg

Timing of Study Initiation

The PCC budget currently includes a nine month startup period to account for initiating and completing the contracting processes, procuring tooling and manufacturing supplies, and subsequent manufacturing of study medication. Most contracting and procurement activities require funding to be in place prior to initiating the work.

IND Requirement

The study is likely to require an IND, and CSPCRPCC plans to file one with the FDA if CSSEC approves the study. While both active treatments are approved for treating C. Difficile associated diarrhea, CSP#596 is specifically looking at recurrence and it is unclear if an IND will be necessary for this study population. Whether or not the trial will require an IND will depend on the FDA's determination regarding "significant increase in risk" as outlined in 21 CFR 312.2 (b). The FDA will either accept or reject the IND and issue the relevant documents. The VA CIRB is highly likely to request copies documenting the FDA's decision.

Good Clinical Practices Training and Auditing/Monitoring Plan

The personnel and travel costs for providing GCP training and for the monitoring/auditing of sites are in the Site Monitoring, Auditing and Resource Team (SMART), portion of the PCC budget. SMART has assigned CSP#596 a "Category II" risk level. This level is designated for Drug/Device Studies with IND/IDE, but not intended for NDA/PMA Submission to FDA.

Please contact me at (505) 248-3200 if there are any questions concerning these issues.

ROBERT J. RINGER, Pharm.D., BCNP
Assistant Center Director
Pharmaceutical Research and Management

Concur/Non Concur

Date: 9/30/13

MIKE R. SATHER, Ph.D., F.A.S.H.P.
Director, VA Cooperative Studies Program
Clinical Research Pharmacy Coordinating Center

MRS/mbg

[THIS PAGE LEFT INTENTIONALLY BLANK]

EXECUTIVE SUMMARY

Clostridium difficile is the most common cause of healthcare-associated infectious diarrhea among adults in industrialized countries. In addition to diarrhea, *C. difficile* infection (CDI) may also result in serious complications such as shock, toxic megacolon, colectomy, and death. The CDC has estimated *C. difficile* results in 250,000 hospital infections, 14,000 deaths, and $1 billion in excess costs annually. From 2000 to 2009, the number of hospitalized patients with any CDI discharge diagnosis in the U.S. increased from 139,000 to 336,600. *C. difficile* now rivals methicillin-resistant *Staphylococcus aureus* (MRSA) as the most prevalent cause of healthcare-associated infections in the U.S. CDI trends in VA hospitals have mirrored trends in non-VA hospitals.

Recurrent CDI is the most challenging clinical dilemma facing clinicians who treat this disease. An estimated 30% of patients who respond to initial treatment with either vancomycin or metronidazole develop recurrent CDI, usually within 1-4 weeks of completing treatment. Many patients develop repeated episodes that continue to occur over a period of months or years.

Management of recurrent CDI is poorly studied with no randomized trials of sufficient power to provide a good clinical basis for treatment recommendations. Current guidelines for CDI treatment give recommendations for recurrent CDI that are based on limited evidence, but include (1) treatment of the first recurrence with the same agent used initially; (2) avoiding prolonged or repeated courses of metronidazole because of the risk for neurotoxicity; and (3) treatment of multiple recurrences with vancomycin using a taper and pulsed regimen.

Vancomycin taper and pulsed regimens are commonly used for treatment of recurrent CDI and investigators from the largest published study concluded that tapered and pulsed dosing of vancomycin resulted in better CDI cure rates than traditional vancomycin dosing. However, the conclusions are very limited because the study was not randomized and the sample size was small (7 participants received pulsed doses with 1 recurrence [14%]; 29 received tapered courses with 9 recurrences [31%]). Moreover, tapered and pulsed regimens only had significantly fewer recurrences in comparison to a 1g/day dose of vancomycin, but not less than doses of 500mg/day or 2g/day while 500 mg/day is the dose recommended in current guidelines.

Fidaxomicin was recently approved by FDA for treatment of initial CDI. Two multi-center, randomized phase 3 trials of a 10-day course of fidaxomicin (200 mg twice daily) versus vancomycin (125 mg four times daily) for CDI showed fidaxomicin to be non-inferior to

vancomycin for initial cure and superior for sustained clinical response (defined as symptom resolution or cure without recurrence) at 28 days following treatment completion. In a subsequent publication, a subgroup analysis of 128 participants from these trials who had experienced one prior episode of CDI within 3 months of enrollment demonstrated a lower subsequent recurrence rate in the fidaxomicin arm compared to the vancomycin arm (13/66, 20% versus 22/62, 36%). However, statistical significance was marginal (p=0.045) and the analysis was compromised by failure to include dropouts in the analysis.

The primary objective of this study is to determine whether 1) standard fidaxomicin treatment and 2) standard vancomycin treatment followed by taper and pulse vancomycin treatment are superior to standard vancomycin treatment alone for sustained clinical response at day 59 for all treatments, for participants with either their first or second recurrence of CDI.

Veterans presenting with a first or second CDI recurrence will be screened, consented and randomly assigned in a double-blind manner to one of three treatment groups: 1) a 10-day course of oral vancomycin (VAN-TX), 2) a 10-day course of fidaxomicin (FID-TX) or 3) a 31-day course of vancomycin which includes a taper and pulse following daily treatment (VAN-TP/P). Participants will have 5 clinic visits (days 0, 10, 31, 59, 90 after randomization) and 10 follow up. phone calls (days 5, 17, 24, 38, 45, 52, 66, 73, 80, 87). The primary outcome of sustained clinical response will be evaluated at day 59 for all treatments.

Sustained clinical response is a diarrhea composite outcome measure (D-COM) defined as symptom resolution during treatment without recurrence of diarrhea or occurrence of other clinically important outcomes at any time through study day 59. Although recurrent diarrhea will likely be the most common failure outcome for D-COM, severe abdominal pain, toxic megacolon, colectomy, or death will also be included. Symptom resolution is defined as an improvement or resolution of diarrhea ($\leq$3 unformed bowel movements over 24 hours) for 48 consecutive hours compared to the participant's baseline. Recurrence is defined as having diarrhea (>3 loose or semi-formed stools over 24 hours for 48 consecutive hours). Those participants failing to meet the criteria of symptom resolution by the end of fully active treatment (day 10 for all groups), or who experience a recurrence during the 49-day period following the end of fully active treatment (day 59 for all groups), will be considered treatment failures. Symptom resolution and recurrence will be assessed at all follow-up contacts using the participant's Stool Diaries. CDI Composite Outcome Measure (CDI-COM) will be a secondary

outcome and will include the same criteria as D-COM with the exception that patients who have diarrhea will also be required to have a positive stool assay for *C. difficile* or its toxins.

Prior to initiation of the full study, a pilot phase was conducted in the same manner as the full study with inclusion of the following additional objectives. The goals of the pilot study were to: 1) evaluate compliance with and efficiency of the primary data collection tool, the simplified daily participant stool diary; 2) develop a patient-centered outcome questionnaire; and 3) assess the recruitment rate of the study. The pilot phase included 6 sites and a target of 42 participants over one year as an original plan and then extended for 6 additional months with an overall goal of 60 participants. Following a feasibility assessment and approval by VA Central Office (CO), CSP #596 enrolled into the full study with enrollment from 24 units (26 sites) (including sites from pilot phase) and an overall target of 549 participants (including subjects enrolled from the pilot phase) as its initial target, and a recruitment goal of **6** participants (on average) per site per year for a site that primarily recruits from the main hospital and **9** participants (on average) per site per year for a site that partners with independent VAMCs close in distance to allow a shared site coordinator. Due to the suspension of recruitment by the COVID-19 pandemic in mid-March 2020 and its significant impact on the slow recruitment after the sites resumed recruitment activities, as well as the termination of underperformed sites and the startup of replacement sites, the sample size of the study is reduced to 459, and a new recruitment goal of 4 participants (on average) per site per year for a site that primarily recruits from the main hospital and 6 participants (on average) per site per year for a site that partners with independent VAMCs close in distance to allow a shared site coordinator. The primary analysis will be based on modified intention to treat (mITT) population that consists of all participants who are randomized and received at least one dose of study treatment medication and met study entry criteria. to yield 444 participants included in the mITT analysis. The recruitment timeline is extended as well with an intention of funding till August 31, 2024. The reduced sample size will provide 85% global power to detect a 16% absolute difference (expected proportion of 31% in the VAN-TX group) in sustained clinical response (D-COM) proportion for at least one comparison (VAN-TP/P vs. VAN-TX, FID-TX vs. VAN-TX) at the family wised error rate (FWER) 0.05 level. The marginal probability (disjunctive power) of detecting 16% absolute difference in each comparison is 60% if the trial stops if any treatment arm (VAN-TP/P or FID-TX) crosses efficacy boundary at either interim looks (i.e., 40% and 70% subjects are recruited). If the trial continues with the treatments which have not yet crossed the efficacy boundary, this marginal probability is increased to 73%. The expected withdrawal rate

prior to day 59 (prior outcome assessment) is estimated to be 10%. If both FID-TX and VAN-TP/P are found to be superior to VAN-TX, then the non-inferiority of VAN-TP/P to FID-TX will be assessed. The primary analysis will be based on modified intention to treat (mITT) population that consists of all participants who are randomized and received at least one dose of study treatment medication and met study entry criteria.

Recurrence of CDI is a common and serious event for which there have been few randomized clinical trials. The proposed study will be the first to systematically compare the effectiveness of three alternative therapies for the management of recurrent CDI for which there is currently inadequate comparative data.

Planning Committee Members

Principal Proponent
Stuart Johnson, M.D.
ACOS/Research
Hines VA Hospital
5000 South 5th Avenue
Hines, IL 60141

Principal Proponent
Dale N. Gerding, MD
Hines VA Hospital
5000 South 5th Avenue
Hines, IL 60141

Domenic Reda, Ph.D.
Director
Cooperative Studies Program Coordinating Center (151K)
5000 South 5th Avenue
Hines, IL 60141

Derrick Kaufman, PhD*
Biostatistician
Cooperative Studies Program Coordinating Center (151K)
5000 South 5th Avenue
Hines, IL 60141

Robert Ringer, Pharm.D.
Assistant Center Director
Cooperative Studies Program Clinical Research Pharmacy Coordinating Center
2401 Centre Ave SE
Albuquerque, NM 87106

Matt Goetz, M.D.
Chief of Infectious Diseases
VA Greater Los Angeles Healthcare System (111-F)
11301 Wilshire Blvd
Los Angeles, CA 90073

Fred Gordin, M.D.
Chief of Infectious Diseases
VAMC Washington D.C.
50 Irving St, NW
Washington, DC 20422

Michael Climo, M.D.
Infectious Disease
VAMC Richmond
1201 Broad Rock Blvd.
Richmond, VA 23249

Curtis Donskey, M.D.
Infectious Disease
VAMC Cleveland
10701 East Blvd.
Cleveland, OH 44106

Tom Sindowski, BA
Project Manager
Cooperative Studies Program Coordinating Center (151K)
5000 South 5th Avenue
Hines, IL 60141

Dave Hunt, MS
Pharmacy Project Manager
Cooperative Studies Program Clinical Research Pharmacy Coordinating Center
2401 Centre Ave SE
Albuquerque, NM 87106

Consultant to the Planning Committee
Melinda Soriano, PharmD
University of Illinois at Chicago
Department of Pharmacy Practice (MC 886)
833 S. Wood Street, 164 PHARM
Chicago, IL 60612

✶ Former Member

[THIS PAGE LEFT INTENTIONALLY BLANK]

I. INTRODUCTION AND BACKGROUND

Introduction

Clostridium difficile is an anaerobic spore-forming bacillus that is the most common cause of healthcare-associated diarrhea among adults in industrialized countries. The Centers for Disease Control has just published a report entitled 'Antibiotic Resistant Threats in the United States, 2013' (http://www.cdc.gov/drugresistance/threat-report-2013/). In this report, *C. difficile* has been prioritized as one of 3 organisms in the category of urgent concern resulting in 250,000 infections, 14,000 deaths, and $1 billion in excess costs annually. Recurrent CDI is a major contributor to the morbidity and cost of this infection.

During the past decade, there have been large outbreaks of *C. difficile* infection (CDI) in North America and Europe, and an increase in the severity of disease [1-3]. From 2000 to 2009, the number of hospitalized patients with any CDI discharge diagnosis in the U.S. more than doubled, from approximately 139,000 to 336,600 [4]. Increases have occurred in all age groups, but the elderly have been disproportionally affected [2, 4]. Mortality attributable to CDI also increased dramatically. These marked changes in the CDI incidence and mortality have coincided with the emergence and spread of an epidemic strain of *C. difficile* designated BI/NAP1/027 [1, 2]. *C. difficile* now rivals methicillin-resistant *Staphylococcus aureus* (MRSA) as the most prevalent cause of healthcare-associated infections in the U.S. [5].

CDI has been a particularly vexing problem in VA hospitals as the risk factors for CDI are also the characteristics of Veteran patients who are often elderly, who have multiple comorbid problems and prolonged hospital stays, and who are frequently exposed to antibiotics. CDI trends in VA hospitals have mirrored trends in non-VA hospitals.

As shown in figure 1, CDI incidence in VA hospitals nationwide increased slowly from 1994 to 2000 and then more than doubled from 2001 to 2005, with the elderly being particularly affected [6]. Although the incidence decreased somewhat in VA facilities from 2006 to 2009, rates remain much higher than those documented in the mid-1990s and the rates appear to be increasing again since 2010. The estimated incidence of CDI among Veterans, based on ICD- 9CM codes from 2010 was approximately 10.4 cases/1,000 discharges with 2% of patients over

Figure 1.CDI incidence within VA Hospitals, National Data

Figure 1. Rates of *C. difficile* infection (CDI) in VA Hospitals between 1994 and 2012* by number of cases (orange bars) and rates per 1000 patient discharges (red line) as identified by ICD-9 discharge coding. Courtesy of Stephen M. Kralovic, MD and the Dept. of Veterans Affairs Multidrug-Resistant Organism Program office. *2012 rates are preliminary.

age 65 carrying the diagnosis during that year [6]. Based upon an average length of stay of 4-5 days, it has been estimated that the VA incidence may be as high as 20 cases/10,000 patient-days [7]. This compares to a reported incidence of 6.4 to 7.9 cases/10,000 patient-days in Ohio non- VA hospitals and nursing homes in 2006 [8].

Recurrent CDI is perhaps the most challenging clinical dilemma facing clinicians who treat this disease. Although effective treatments are available for CDI, 20 to 30% of patients who respond to initial courses of treatment develop recurrent CDI, usually within 1-4 weeks of completing treatment with either vancomycin or metronidazole [9]. The risk of recurrent CDI is even higher in patients who have already had one or more recurrences, and many patients develop repeated

episodes that may continue to occur over a period of months or years [9-11]. Recurrent CDI has a significant impact on healthcare systems due to need for multiple courses of treatment, increased length of hospitalization, and increased hospitalization costs [10, 11]. In addition, hospitalized patients with CDI have a 62% increase in odds of being discharged to a long-term care facility rather than to their home [12]. Recurrent episodes of CDI can also be associated with adverse outcomes. For example, in a recent study conducted in the setting of an outbreak associated with the BI/NAP1/027 strain, 11% of participants with a first recurrence of CDI had serious complications, including shock, toxic megacolon, colectomy, and death [13]. Moreover, recurrent CDI often has a significant negative impact on the wellbeing of patients and their families. Common complaints of patients include loss of independence and inability to travel or enjoy normal activities due to fear of uncontrolled episodes of fecal incontinence or diarrhea.

Two primary mechanisms have been proposed to explain the pathogenesis of recurrent CDI. First, the absence of an adequate immune response to *C. difficile* toxin is associated with recurrence [14, 15]. In a prospective study, participants who developed a systemic anamnestic antibody response to toxin A, as evidenced by increased serum levels of IgG antibody against toxin A, became asymptomatic carriers of *C. difficile*, whereas those who did not have such a response developed CDI [14]. In a subsequent study, the development of increased levels of toxin-A specific IgM and IgG antibodies were associated with a significantly reduced risk of recurrence in participants with an initial episode of CDI [15]. The association between increasing age and CDI recurrence may be in part due to immunological senescence accompanying ageing [16]. Second, persistent disruption of the normal colonic microbiota after completion of CDI treatment predisposes to recurrence [17-19]. Chang et al. demonstrated that participants with recurrent CDI had highly variable bacterial composition and markedly decreased diversity in comparison to control participants with an initial episode of CDI [17].

Others have demonstrated that marked disruption of the microbiota commonly persists for 2-3 weeks after completion of CDI treatment with oral vancomycin or metronidazole [18, 19].

A number of risk factors for recurrence of CDI have been identified in clinical studies. In 2009, Garey et al. published a meta-analysis of 12 publications involving 1382 participants that assessed risk factors for recurrence [20]. Continued use of non-CDI antibiotics after diagnosis of CDI, concomitant receipt of antacid medications, and older age were significantly associated with

increased risk for recurrence. Subsequently, Hu et al. derived and validated a clinical prediction rule for recurrent CDI that included age >65 years, severe or fulminant disease (by Horn index), and additional antibiotic use after diagnosis of CDI [21]. More recently, D'Augustino et al, using the phase 3 fidaxomicin vs. vancomycin data bases, developed a prediction rule for recurrent CDI that includes the following four independent risk factors: age (<75 vs. ≥75years), number of unformed bowel movements during previous 24 hours (<10 vs. ≥10), serum creatinine levels (<1.2 mg/dL, ≥1.2 mg/dL) and prior episode of CDI (yes vs. no) [22].

Important and consistently reported risk factors for recurrent CDI are shown in Table 1. With the exception of anti-acid medication use and continuation of non-CDI antibiotics, these risk factors are not modifiable. It has been demonstrated in a VA setting that non-CDI antibiotics continued or newly prescribed during CDI treatment are often unnecessary [23]. In fact, 26% of patients with recent CDI received only unnecessary and therefore potentially avoidable antibiotics. These data suggest that stewardship interventions focused on CDI patients might be helpful as a strategy to prevent some recurrences.

Table 1: Important risk factors for the development of recurrent *C. difficile* infection

- Advanced age
- Long hospital stays
- Concomitant receipt of antacid medications
- Persistent disruption of the colonic microbiota
- Use of any non-*C. difficile* antimicrobial therapy following a first episode of CDI
- Inadequate antitoxin antibody response
- Elevated serum creatinine
- High number (>10) stools per day at diagnosis
- Prior episode of CDI

CDI: *Clostridium difficile* infection.

Study Rationale

Management of recurrent CDI is poorly studied with no randomized studies of sufficient power to provide a good clinical basis for treatment recommendations. Current SHEA/IDSA clinical practice guidelines for CDI give recommendations for recurrent CDI that are based on relatively poor quality of evidence, but include (1) treatment of the first recurrence with the same agent used

initially, stratified by disease severity with the understanding that antimicrobial resistance to metronidazole and vancomycin has not been shown to be clinically relevant; (2) avoiding prolonged or repeated courses of metronidazole because of the risk for neurotoxicity; and (3) treatment of multiple recurrences with vancomycin using a taper and pulsed regimen [24]. The evidence supporting use of vancomycin tapers and several other potential treatments is outlined below. Currently available options for management of recurrent CDI are summarized in Table 2.

Table 2. Treatment options in clinical use for recurrent CDI and evidence for benefit

Type of therapy	Currently available treatments	Evidence for benefit
Antimicrobials	Vancomycin (standard dosing) Vancomycin (taper/pulse) Fidaxomicin Nitazoxanide Rifaximin Metronidazole	-Effective at suppressing CDI, but recurrence after stopping is predictable -Frequently used, but no comparison data available -Maybe less recurrence than vancomycin, but only studied in first CDI recurrence -Recurrence rate similar to vancomycin & metronidazole -Used in a post-vancomycin 'chaser' strategy, but resistance increasing -Toxicity with repeated use; not recommended for recurrent CDI
Traditional probiotics	*Saccharomyces boulardii*	-Studied as adjunctive therapy, but randomized trial showed no benefit
Biotherapeutics	Fecal microbiota transplantation	-Randomized controlled trial (RCT) showed superiority over vancomycin; Participant selection criteria and donor screening process not well defined
Immune-based therapies	Intravenous immunoglobulin (IVIG)	-Anecdotal evidence only; No RCTs, expensive & limited resource

Vancomycin taper and pulsed regimens are commonly used for treatment of recurrent CDI, but only one published study has evaluated the effectiveness of this approach. McFarland et al. retrospectively analyzed data from 163 participants with one or more recurrences of CDI who had participated in the placebo arm in two clinical trials evaluating efficacy of *Saccharomyces boulardii* in combination with vancomycin or metronidazole to prevent further recurrences [25]. In one study, the dose and duration of treatment regimens were determined by physicians. In the other, treatment was limited to low or high dose vancomycin (500 or 2,000 mg/d for 10 days) or

metronidazole 1 g daily for 10 days, followed by treatments chosen by physicians if there was no response. Participants with recurrent disease after these regimens then received further treatments chosen by their physicians. The investigators concluded that tapered and pulsed dosing schedules of vancomycin resulted in better CDI cure rates than traditional vancomycin dosing. However, as shown in figure 2, the conclusions are very limited due to the small number of participants in each arm of the study (7 participants received pulsed doses with 1 recurrence [14%]; 29 received tapered courses with 9 recurrences [31%]). Moreover, tapered and pulsed regimens only had significantly less recurrences in comparison to the medium dose of vancomycin (1 g/day) which is not the dose recommended in current guidelines (500 mg/day) [25].

Figure 2. CDI recurrence by vancomycin treatment group

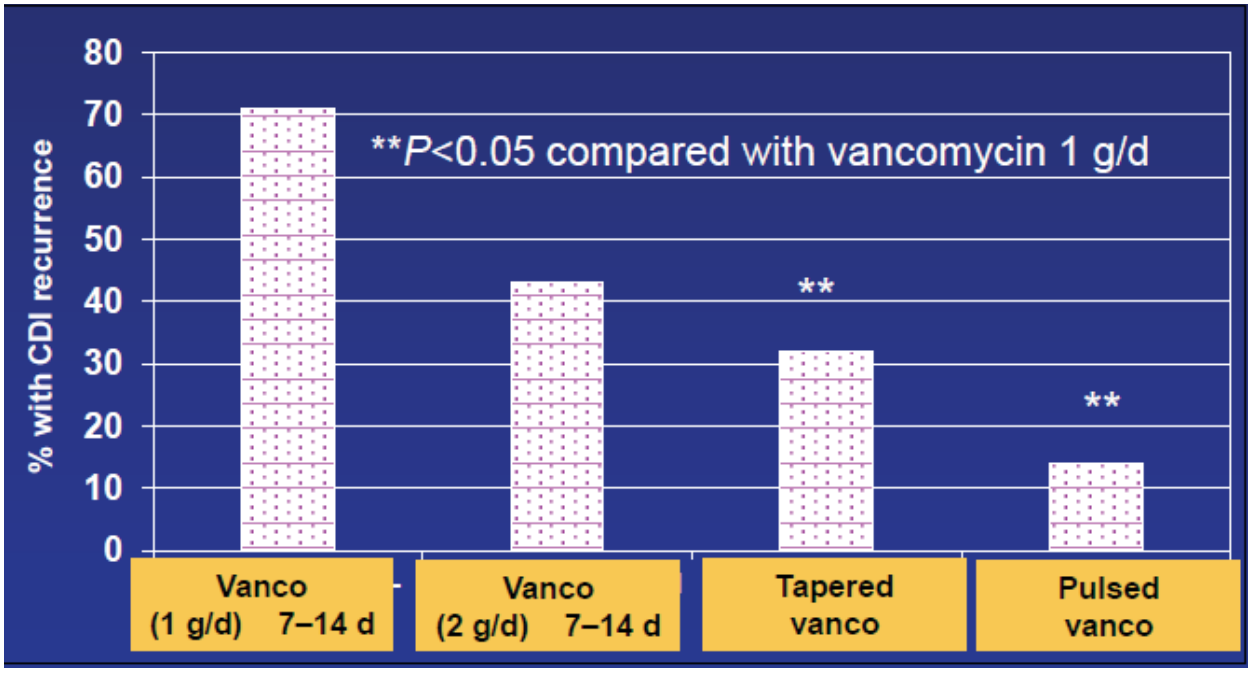


Figure 2. Vancomycin (Vanco) regimens used for treating recurrent CDI from a post-hoc analysis of two clinical trials of *S. boulardii* as adjunctive therapy for CDI (n= 163) [25].

One additional unpublished study provides some support for use of vancomycin tapers for recurrent CDI. In a retrospective analysis, Lieu et al. found that participants with a previous history of CDI were significantly less likely to have a subsequent recurrence if they received a 6- week vancomycin taper versus a standard course of metronidazole (7/76, 9% versus 5/19, 26%; P =0.04) [26]. There was also a non-significant trend toward reduced recurrences when the 6-week vancomycin taper was compared to a standard course of vancomycin (7/76, 9% versus 7/39,

18%; *P* =.23).

The mechanism by which tapering and pulsed vancomycin regimens may reduce recurrences is unclear. As noted previously, standard vancomycin regimens cause marked disruption of the intestinal microbiota that inhibit colonization by *C. difficile* in healthy individuals [18, 19]. For example, vancomycin achieves very high levels in stool (500 to >1,000 µg/gm) resulting in suppression of *Bacteroides* spp. that would ordinarily be considered resistant to vancomycin (typical minimum inhibitory concentration, >128 µg/mL) [18, 19]. It is presumed that tapering and pulsing of vancomycin may result in reduced colonic levels that allow re-population of the normal microbiota; however, this has never been demonstrated in patients. Another potential explanation for the reduction in recurrences with tapered and pulsed vancomycin is that prolonged courses of treatment may allow more time for clearance of *C. difficile* from the colon.

Figure 3. Effect on Bacteroides/Prevotella between fidaxomicin and vancomycin treatment

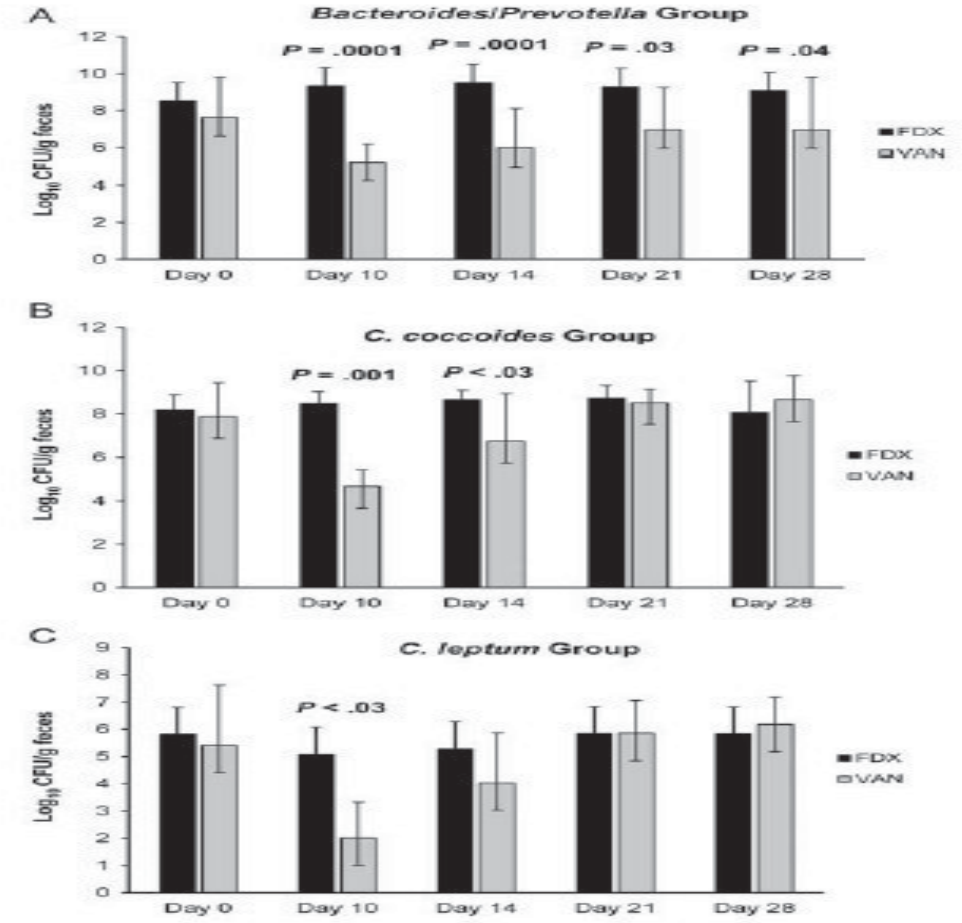


Figure 3. Relative levels of *Bacteroides/Prevotella*, *Clostridium coccoides*, and *Clostridium*

leptum in feces of participants during and following treatment with fidaxomicin (FDX) or vancomycin (VAN) [18].

Fidaxomicin, a macrocycle antibiotic recently approved by the FDA for CDI treatment, has also been studied for treatment of recurrent CDI. Two recently completed multi-center, randomized phase 3 trials of a 10-day course of fidaxomicin (200 mg twice daily) versus vancomycin (125 mg four times daily) for CDI showed fidaxomicin to be non-inferior to vancomycin for initial cure and superior to vancomycin for sustained response at 25 days following treatment completion [27, 28]. Fidaxomicin is presumed to be associated with fewer recurrences than vancomycin because it causes minimal disruption of the microbiota that inhibit *C. difficile*. As shown in figure 3, vancomycin causes persistent disruption of anaerobic organisms in stool during and after treatment, whereas fidaxomicin does not suppress these organisms and in fact significant recovery occurs during treatment [18].

In a subsequent publication, a subgroup analysis was performed for 128 participants from the trials who had experienced one prior episode of CDI within 3 months of enrollment [29]. Analysis of this subgroup with recurrent CDI demonstrated a lower subsequent recurrent CDI rate in participants in the fidaxomicin arm compared to that in the vancomycin arm (13/66, 20% versus 22/62, 36%; P=0.045). This marginal statistical significance was further compromised by failure to include dropout participants in the analysis as treatment failures. This difference in subsequent CDI episodes was seen most convincingly in the first 2 weeks following treatment. These data suggest that fidaxomicin may be preferred over vancomycin for first recurrences of CDI because it is associated with fewer subsequent recurrences.

Additional agents and strategies available for the treatment of recurrent CDI include rifaximin, nitazoxanide, IVIG, *S. boulardii*, and fecal microbiota transplant (FMT) (Table 2). Only FMT has randomized data supporting its use in recurrent CDI, but FMT has traditionally been reserved for a select group of patients with multiple CDI recurrences, e.g., $\geq$3 recurrences. We reported our experience using rifaximin as a post-vancomycin treatment in 14 consecutive participants with multiple CDI recurrences [30, 31]. Treatment was not randomized, but included participants with multiple recurrences (mean number of previous episodes= 6) who had failed multiple other treatments for recurrent CDI. Eleven of the 14 participants (79%) had no further episodes after a course of rifaximin (400 mg twice daily for 2 weeks) given immediately after the last course of

vancomycin. Although rifaximin chasers appear promising based upon the results reported to date, enthusiasm for this approach is tempered by concerns about the emergence of resistance. In the initial report of this strategy, 2 of 3 *C. difficile* isolates recovered from participants who failed rifaximin tapers were highly resistant to rifaximin in vitro (MIC $\geq$256 μg/ml). Other recent reports of *C. difficile* isolates with high MICs to rifamycins (including rifaximin) have raised additional concern that resistance to rifaximin will limit this approach for treating CDI and recurrent CDI [32, 33].

Nitazoxanide is a 5-nitrothiazole compound reported to have some success in treatment of small numbers of patients who failed to respond to metronidazole [34]. In a small randomized trial, nitazoxanide was reported to be at least as effective as metronidazole [35]. Nitazoxanide was also purported to be as effective as vancomycin in another randomized trial, but the small sample size precluded making any conclusions about noninferiority to vancomycin [36]. These studies suggest that nitazoxanide might have a role in CDI treatment, but additional studies would be required. Nitazoxanide has not been specifically evaluated for treatment of recurrent CDI.

Because failure to mount an immune response to toxin is associated with recurrence of CDI, strategies involving enhancement of passive or active immunity to toxin are an alternative approach to prevention of recurrences. Several case reports and case series have reported use of intravenous immunoglobulin (IVIG) as an adjunctive therapy for participants with multiple recurrences of CDI [37]. Overall, 35 of 41 (85%) participants receiving IVIG had no recurrence after treatment. However, no randomized trials of IVIG have been reported and the expense and lack of availability of IVIG makes this approach impractical.

Administration of probiotic microorganisms is also a theoretically attractive strategy to prevent initial and recurrent episodes of CDI through restoration of intestinal bacteria that may compete with *C. difficile*. The organisms most often studied include preparations of species such as lactobacilli and bifidobacteria and the non-pathogenic yeast *Saccharyomyces boulardii*. Results in primary prevention have been mixed, with some studies demonstrating a reduction in CDI and others showing no benefit [38, 39]. *S. boulardii* has been studied as adjunctive treatment in a placebo-controlled, randomized study of participants with CDI (i.e., secondary prophylaxis), but only showed a marginal effect in a post-hoc, subset analysis of participants receiving high dose

vancomycin [40]. Thus, based upon the currently available evidence, it is not likely that use of currently available probiotics will provide an effective strategy to minimize recurrence of CDI.

For many years, fecal microbiota transplantation has been recognized as a highly effective means to prevent recurrences of CDI through restoration of the intestinal microbiota. However, fecal microbiota transplantation has not been widely used, primarily due to the unappealing nature of the treatment. In a recent randomized trial, van Nood et al. reported that duodenal infusion of donor feces was much more effective than vancomycin for treatment of multiple recurrent (median 3 recurrences, range 1-9) CDI episodes (31% for vancomycin versus 81% for first fecal transplant infusion) [41]. These results demonstrate the crucial role that the intestinal microbiota plays in prevention of recurrence of CDI. Although fecal transplantation is becoming a more widely used procedure, it is likely that this approach will continue to be reserved for patients with multiple recurrences. Factors such as the expense of the screening tests for donors and the potential for complications including unintended transmission of infection will limit use to patients with three or more recurrences. Future development of defined mixtures of bacteria that inhibit colonization by *C. difficile* may ultimately make this approach much more acceptable [42].

A number of new agents and strategies are under development for prevention of recurrent CDI (Table 3). Some of these approaches involve antibacterial treatment agents and some are primary or secondary prevention approaches.

Table 3. Treatment options under development for CDI and recurrent CDI

Type of therapy	**Potential future treatments**	**Stage of development**
Antimicrobials	Surotomycin Cadazolid	-Both in Phase 3 trials
Traditional probiotics	*None currently proposed for treatment*	
Biotherapeutics	Nontoxigenic *C. difficile* Defined collections of bacteria derived from intestinal microbiota	-Completed Phase 2 trial -Pre-clinical
Immune-based therapies	Systemic monoclonal antibodies Active vaccines Intraluminal toxin-binding agents	-In Phase 3 trials -One in Phase 3, others in Phase 1 -Tolevamer was not effective as primary treatment; Potential use adjunctive

Based upon the success of fidaxomicin, several companies are pursuing development of antimicrobials that are narrow in spectrum and may selectively eliminate C. difficile while preserving the normal intestinal microbiota. Two of these agents (surotomycin and cadazolid) are currently in Phase 3 trials. A novel biotherapeutic approach involves colonization with a nontoxigenic C. difficile strain that confers protection against colonization by toxigenic strains in antibiotic-treated hamsters. A study using a specific nontoxigenic C. difficile strain has now completed Phase 1 testing in healthy adults and Phase 2 trial in subjects with CDI and successfully reduced recurrent CDI to 11% compared to 30% with placebo ($p<0.01$) [43, 44].

Lowy et al. have reported findings of a randomized, double-blind, placebo-controlled Phase 2 trial of human monoclonal antibodies against toxins A and B showing a significant reduction in subsequent recurrence of CDI, including a subgroup with recurrent CDI at study outset [45]. Monoclonal antibodies are currently undergoing phase 3 trials for prevention of CDI. Three companies are currently developing vaccines for *C. difficile*, including an injectable *C. difficile* toxoid vaccine in Phase 3 trials. Vaccination may be a promising strategy for the future, but a number of questions remain unresolved, including the ability of vaccines to provide an immune response in elderly populations, the magnitude and duration of vaccine protection, and the selection of appropriate at-risk populations for vaccination. Finally, while some of these agents appear to be promising, some are likely to be exceedingly expensive and none of these options are likely to be available in the near future. Thus, there is an important unmet need for high quality studies to compare the effectiveness of currently available therapies for recurrent CDI.

Other than vancomycin and fidaxomicin, none of the available agents show enough promise for recurrent CDI to warrant further rigorous study. This current proposal will determine the optimal treatment of first or second recurrence of CDI using vancomycin or fidaxomicin to prevent participants from developing multiple recurrences (e.g., ≥3 recurrences) and the need to consider desperate measures such as FMT.

Summary

Recurrence of infection remains one of the major challenges in the management of CDI. Many of the risk factors for recurrence are common in VA patient populations and are not modifiable. The proposed study will be the first to systematically compare the effectiveness of three alternative

therapies for the management of recurrent CDI for which there is currently inadequate comparative data. The data collected from this study will answer important questions concerning treatment options for recurrent CDI: 1) Is there a difference in treatment outcomes between fidaxomicin and vancomycin therapy and 2) Will a prolonged taper/pulse regimen of vancomycin reduce the risk of subsequent recurrences compared to a shorter (10 day) course.

II. STUDY OBJECTIVES AND OUTCOME MEASURES

Overall Hypothesis

Recurrence of CDI is a common and serious event for which there has never been a well-controlled randomized clinical trial. The standard for treatment is a 10-day course of oral vancomycin at a dose of 125 mg 4 times a day. We hypothesize that either vancomycin in a tapering and pulse dosing regimen, and/or fidaxomicin 200mg given twice daily for 10 days, will be superior to standard oral vancomycin dosing administered for 10 days.

Primary Outcome: Diarrhea Composite Outcome Measure (D-COM)

The Primary outcome will be sustained clinical response as measured at study day 59 for all treatment regimens. Sustained clinical response is a composite outcome that includes symptom resolution during treatment without any of the following (as assessed on day 59):

1. Diarrhea recurrence
2. Other non-fatal clinical events including severe abdominal pain related to current diarrhea illness, toxic megacolon (where diarrhea ceases but is not a beneficial outcome), and colectomy
3. Death

Investigators will assess for symptom resolution and diarrhea recurrence according to the participant's Study Diary entries and from discussion with the participant at each contact point described within the Schedule of Procedures (figure 5). Symptom resolution is defined as an improvement or resolution of diarrhea (≤3 unformed bowel movements over 24 hours over 24 hours) for 48 consecutive hours compared to the participant's baseline. Recurrence will be defined as having diarrhea (>3 loose or semi-formed stools over 24 hours for 48 consecutive hours) following initial resolution.

Justification for Diarrhea Composite Outcome Measure (D-COM)

Based on data from clinical trials of first CDI episodes and clinical experience, over 90% of participants are expected to respond to a 10-day treatment course with either vancomycin or fidaxomicin [27, 28]. We expect most recurrent CDI episodes to occur within the first 2 weeks after discontinuing treatment [27]. The preliminary reports of vancomycin taper suggest decreased recurrence rates compared with a standard vancomycin treatment course, although some participants will 'break through' near the end of their taper/pulse (e.g., during every other or every third day vancomycin) or shortly after the taper [25]. We have defined sustained response as a composite endpoint to include outcomes that are inclusive and meaningful to patients. Past clinical experience indicates that recurrence of diarrhea following initial response to treatment will be the most common reason for not achieving a sustained clinical response. Although we anticipate that these recurrent diarrhea episodes will be due to *C. difficile* in the majority of cases, some recurrent episodes may not be confirmed with a positive stool assay. In addition, we will include other clinically important outcomes such as severe abdominal pain related to current diarrhea illness, toxic megacolon, colectomy, and death.

Primary Objective

The primary objective of this study is to determine whether 1) standard fidaxomicin treatment and 2) standard vancomycin treatment followed by taper and pulse vancomycin treatment are superior to standard vancomycin treatment alone for the composite endpoint (D-COM) of sustained clinical response at day59 for all treatments, for participants with either their first or second recurrence of CDI.

Secondary Outcomes

1. *CDI Composite Outcome Measure (CDI-COM): Sustained clinical response without recurrent CDI (defined as diarrhea plus confirmation of toxigenic C. difficile or its toxins in stool) as measured at study day 59 for all three treatment regimens. Sustained response will be defined using the same composite endpoint criteria as were used in the D-COM outcome but with confirmation of recurrent CDI by a positive C. difficile stool assay on or before day 59.*

2. *Sustained clinical response (D-COM) and sustained clinical response without recurrent CDI (CDI-COM) at **28 days post end of therapy** for all three treatment regimens.* Sustained clinical response will be defined using the same criteria as previously stated except that the endpoint will be 28 days after the last dose of treatment drug for each treatment arm (day 38 for vancomycin and fidaxomicin, and day 59 for vancomycin taper/pulse).

3. *Sustained clinical response (D-COM) and sustained clinical response without CDI (CDI-COM) at **day 90** for all three study treatment arms*. Sustained clinical response will be defined using the same criteria as were used in the D-COM except that the endpoint will be study day 90 (instead of day 59) for all three arms.

4. *Rate of symptom resolution among all study participants.* Days to symptom resolution will be determined while on full treatment doses (measured from day 0 up to day 10 for all study regimens).

5. *Diarrhea recurrence and diarrhea recurrence with confirmation of recurrent CDI following initial symptom resolution*. Diarrhea (>3 loose or semi-formed stools over 24 hours) over 48 consecutive hours in participants who achieved initial symptom resolution will be recorded separately from sustained clinical response as will confirmed CDI recurrence.

6. *Sustained clinical response at day 59 will be determined for subgroups of participants who meet criteria for diarrhea (D-COM) and with confirmation of recurrent CDI (CDI-COM):*

a. Infection with the BI/NAP1/027 strain (yes, no) on study enrollment (baseline).

b. Number of previous CDI episodes (1 or 2) within 6 months of enrollment at baseline.

c. Receipt of concomitant antibiotics at any time during study (yes, no).

d. Sustained clinical response correlated to Horn's and ATLAS severity scores.

7. *Change in patient reported C.diff Health Related Quality of Life (HRQOL) from baseline (day 0) to day 10 (end of treatment of oral Vancomycin and Fidaxomicin) and to day 59 (primary outcome assessment) for all three treatment arms.* The HRQOL is measured with patient self-reported 32-item questionnaire [68]. The summary measure, CDiff32-QOL, is the total score that sums over 32 individual items with each item rated on a 5-point Likert scale and then transformed to a 100-point scale with higher score indicating better C.diff Health Related QOL in general. The overall summary measure consists of 3 sub-scales: physical QOL (P-QOL), emotional/psychological QOL (E-QOL) and social QOL (S-QOL) that measure, respectively, participants' physical, mental, and social aspect of their QOL. Each sub-scale is also transformed to a 100-point scale with higher score indicating better QOL in that function domain.

8. Safety monitoring

a. We will obtain the following laboratory parameters on day 0, day 10, and day 31: CBC and serum chemistry panel to include Cr, AST, ALT, alkaline phosphatase, albumin, total bilirubin.

b. Adverse Events (AEs) related to study treatment of the participant from when signed informed consent is obtained through day 59 (Primary Outcome) of the study will be recorded. If necessary, follow up AE or SAE reports may be collected until end of study (i.e. day 90)

c. Treatment discontinuation due to any AE will also be documented.

d. All serious AEs that result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; result in persistent or significant disability or incapacity; a congenital anomaly/birth defect; or any other condition that, based upon medical judgment, may jeopardize the participant and require medical or surgical treatment to prevent one of the above outcomes. These will be collected from the time of signed informed consent through day 59 (Primary Outcome) of the study. If necessary, follow up AE or SAE reports may be collected until end of study (i.e. day 90)

Justification for Secondary Outcome Measures

The first secondary endpoint is defined using the same composite endpoint criteria as were used in the D-COM outcome but with confirmation of recurrent CDI by a positive *C. difficile* stool assay on or before day 59. We feel this CDI-COM secondary endpoint is required to provide a comparable outcome to similar previous CDI treatment studies in the published literature which use a definition of recurrent CDI based on diarrhea symptoms and a positive stool assay for toxigenic *C. difficile* or its toxins. We expect most recurrent CDI episodes to occur within the first 2 weeks following treatment discontinuation and sustained response at 28 days is a standard time period to assess for response based on prior clinical trials. To allow a minimum 28-day post treatment observation period for all three arms, we will assess sustained clinical response for all regimens at day 59 (28 days post end of vancomycin taper/pulse and 49 days post end of fidaxomicin and vancomycin). Because the post-treatment observation period is longer for fidaxomicin and vancomycin, we will also include a secondary outcome of sustained clinical response 28 days post last treatment dose on day 38 for fidaxomicin and vancomycin vs. day 59 for vancomycin taper/pulse. There are some participants who will experience very late recurrences

(beyond day 59) and we will extend our period of observation to day 90 to document these late recurrences.

Sustained clinical response (primary endpoint), is defined by two criteria (1) symptom resolution (2) without subsequent diarrhea recurrence, but it is important to consider both of these criteria individually and each will be included as secondary endpoints. As we previously demonstrated [46], sustained clinical response can highlight meaningful differences between two agents with similar clinical response rates (e.g., vancomycin and fidaxomicin). However, sustained clinical response in the tolevamer and metronidazole treatment arms of the Phase III trials of the toxin-binding agent, tolevamer would have looked similar, despite the finding that the symptom response rate of tolevamer was clearly inferior to both metronidazole and vancomycin [46, 47]. This agent was not considered efficacious in the treatment of CDI despite the low recurrence rate among those who responded to tolevamer [46, 47]. Diarrhea recurrence is also a useful endpoint, particularly if a stool specimen for *C. difficile* testing is not obtained or re-treatment is initiated before a specimen is sent. This endpoint (which is the primary endpoint of this study) was previously used to show a difference between treatment arms in a pilot study of a rifaximin chaser strategy for CDI [48].

Several factors have been identified that potentially affect treatment outcomes in CDI, both the initial cure rate during treatment as well as subsequent CDI recurrence after initial treatment resolution. A subgroup analysis will compare the primary endpoint among participants infected with different *C. difficile* strains, different number of prior CDI episodes, different antibiotic exposures, and different underlying disease and CDI severity. One consistent observation since 2001 has been the increased rates of CDI and severe CDI that has paralleled the emergence of the BI/NAP1/027 strain of *C. difficile* [1, 2]. Severity of CDI and mortality has also been correlated with infection with this strain [49]. In a combined analysis of the two phase 3 trials of fidaxomicin versus vancomycin, infection with the BI strain was a significant risk factor for CDI recurrence by multivariate analysis (OR, 1.57; 95% CI, 1.01–2.45; P = .046) [50]. The number of prior CDI episodes may also influence the primary outcome. In general, the recurrence rate after the initial CDI episode is in the range of 20 to 30% [24]. Following a first CDI recurrence the rate of a subsequent recurrence is in the range of 33 to 65% [13, 25]. Some patients have multiple CDI recurrences that last for months and years [30]. Antibiotic use is almost a universal risk factor for the initial CDI episode, but also highly influences recurrence rates. In the combined analysis of the

two phase 3 fidaxomicin trials, sustained cure (initial cure without recurrence) was significantly lower among participants who receive additional antibiotics at any time during the study compared to those who did not receive additional antibiotics (66% versus 75%, P= .005) [51]. Finally, severity may also influence outcomes. Severe CDI has been shown to be associated with inferior cure rates with metronidazole treatment, but the influence of severity on recurrence is unknown. However, severity may influence sustained response rates by affecting the cure rate [52].

Both oral vancomycin and fidaxomicin have been studied extensively and have very good safety profiles. Both drugs show limited systemic absorption and achieve high concentration only in the stool. Despite this safety record, some potential concern for nephrotoxicity with oral vancomycin was raised in the recent analysis of data from the randomized trial of tolevamer versus vancomycin and metronidazole [53]. 12 participants (4.6%) treated with vancomycin reported nephrotoxicity events (e.g. renal failure, renal impairment, blood creatinine increased). The median day of onset was Day 16 (range: 3-35 days) and 8 of 12 participants were >65 years of age. Although the relationship to the treatment agent was not determined, these findings led to a change in the prescribing information for vancomycin [54]. Therefore, the safety endpoints include a significant change in basic laboratory chemistries and hematologic parameters over baseline. An absolute increase of $\geq$0.3 of serum creatinine or a > 1.5 times increase over the level recorded at baseline will be considered significant [55]. Additional safety endpoints will include any AE that leads to treatment discontinuation and the serious AEs.

Secondary Objectives

1. Compare sustained clinical response without recurrent CDI at day 59. [Objective for CDI-COM and analogous to Primary Objective for D-COM]

2. Compare sustained clinical response (D-COM) and sustained clinical response without recurrent CDI (CDI-COM) at 28 days post end of therapy for each regimen (day 38 for vancomycin and fidaxomicin, and day 59 for vancomycin taper/pulse)

3. Compare sustained clinical response (D-COM) and sustained clinical response without recurrent CDI (CDI-COM) at day 90 for all three regimens.

4. Compare rate of symptom resolution while on full treatment doses (from day 0 up to day 10 for all study regimens).

5. Compare rates of recurrent diarrhea and recurrent CDI in participants with acute symptom resolution.

6. Compare sustained clinical response rates (D-COM) and sustained clinical response rates without recurrent CDI (CDI-COM) on day 59 in participants:

   a) infected with the BI/NAP1/027 strain of *C. difficile* and those infected with non-BI strains

   b) with 2 previous CDI episodes and participants with 1 previous CDI episode (within 8 weeks of enrollment)

   c) receiving concomitant antibiotics (during CDI treatment and the 28 day follow up period) and those without concomitant antibiotics

   d) by correlating sustained clinical response rates with Horn's Severity of Illness and ATLAS severity scores

7. Compare the change in C.diff HRQOL from baseline to day 10 (end of treatment of oral Vancomycin and Fidaxomicin) and to day 59 (primary outcome assessment).
8. Evaluate the safety of the vancomycin, fidaxomicin, and vancomycin taper/pulse.
9. If the primary analysis indicates that both fidaxomicin and vancomycin taper/pulse are superior to vancomycin, then the non-inferiority of vancomycin tamper/pulse to fidaxomicin will be assessed.

III. SUMMARY OF STUDY DESIGN AND METHODS

This is a prospective, multi-center, double-blind, randomized controlled study comparing the efficacy of three active treatment groups for participants diagnosed with recurrent *Clostridium difficile* infection (CDI). Participants presenting with a first or second CDI recurrence will be screened, consented and upon randomization will be placed in one of three treatment groups. Approximately 459 participants will be enrolled and randomized to receive either a 10-day course of oral vancomycin (VAN-TX), a 10-day course of fidaxomicin (FID-TX) or a 31-day course of vancomycin which includes a taper and pulse following treatment (VAN-TP/P).

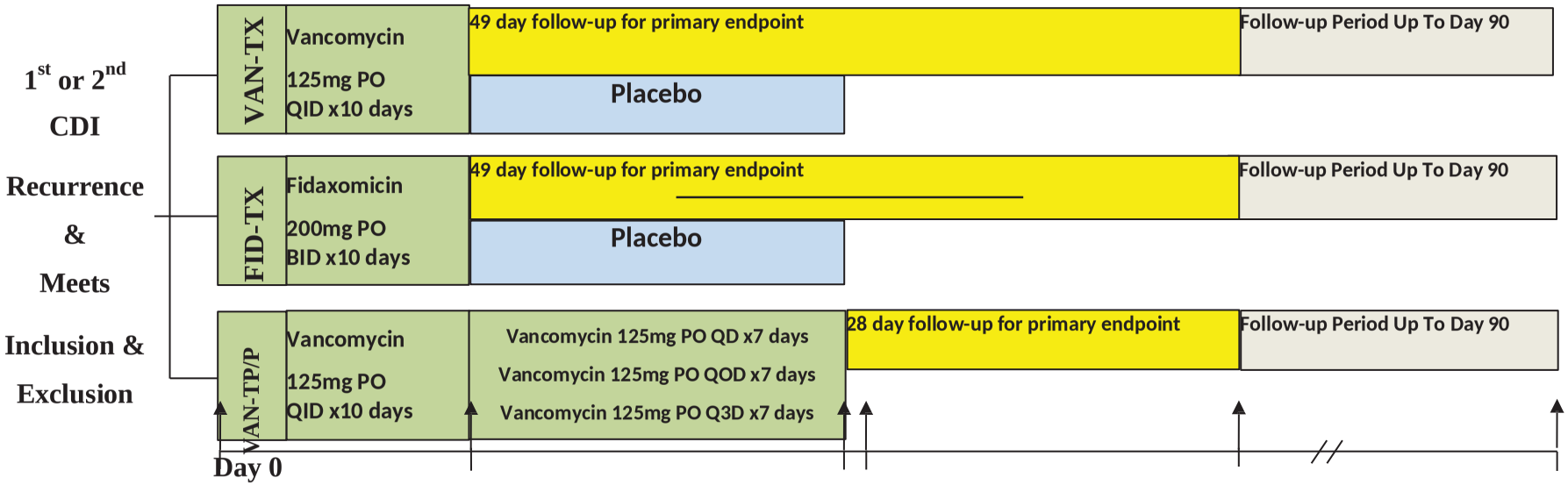
Participants will be prospectively followed from study entry and will have 5 clinic visits (days 0, 10, 31, 59, 90) and 10 follow up phone calls (days 5, 17, 24, 38, 45, 52, 66, 73, 80, 87). These visits and follow-up calls will assess the participants for treatment efficacy, diarrhea recurrence and CDI recurrence. Adverse event monitoring will be assessed through day 59. If necessary, follow up AE or SAE reports may be collected until end of study (i.e. day 90). As this is a double-blind study, multiple follow-up visits/calls will be scheduled for all participants; however, the primary endpoint will be evaluated based on findings on day 59 with a secondary endpoint on

day 90 of the study.

Secondary endpoint analysis will also occur at 28 days following treatment completion (day 38 for VAN-TX and FID-TX, and day 59 for VAN-TP/P).

A diagram of the study design is provided in figure 4.

Figure 4. Study Design



IV. PARTICIPANT POPULATION

Participant Screening and Recruitment

Potential subjects will be identified by site coordinator review of the hospital microbiology laboratory log of stool testing for C. difficile. Charts (CPRS) of those patients with positive tests for C. difficile will then be reviewed by the site coordinator to make sure that there are no obvious exclusions for the study. Patients who are having a first or second recurrence of C. difficile infection will then be approached by study personnel (site coordinator and/or local site investigator) if they are in the hospital. If the patients had submitted stool specimens during a visit to the emergency room or clinic and are no longer physically at the hospital or clinic, they will be contacted by phone. Patients will be informed of potential phone contact by distribution of one-page flyers handed to the patients by the prescribing physician at the time stool testing is ordered in the emergency room and the clinics. The nature of the study will be described in the flyer which will also include a telephone number that can be used to verify that the study constitutes VA research. The flyer will state that the patient may be called regarding participation in the trial. Because the study involves enrollment of patients who have just been identified as having recurrent C. difficile infection (CDI) it is imperative that they are contacted in a timely manner so they can be offered the study medications within 72 hours of treatment (most patients will receive treatment for CDI as soon as the test is reported positive and in some cases, empirically before the results of the test are back). During the phone contact, they will be informed of the study and asked to return to the clinic if they are willing to consider participation. During the phone contact the patient will be asked if they have already been contacted by their physician about the positive C.diff test result. If they have been contacted an apology for the second call will be given and the patient will be asked if they are interested in learning more about the study. After face-to-face screening by the study personnel, patients who meet the inclusion criteria will be asked if they are willing to participate and if willing will be asked to sign informed consent and HIPAA authorization.

A second recruiting strategy will be for the site coordinator to identify patients with first episode CDI in the same manner as described above (review of laboratory log of stool tests for C. difficile and review of patient charts in CPRS). Although patients with a first episode of CDI are not eligible for the study, ~ 25% of these patients will have a recurrent episode usually within 2-4 weeks of discontinuing treatment for the primary episode. Therefore, a letter will be sent to those patients informing them of the study and telling them that they may be contacted over the next 2

months to see if they are willing to participate. The letter will include a telephone number that the patient can call to opt out the study.

A third strategy will involve identifying patients with first episode CDI as described above, but rather than passive follow-up, patients will be actively followed. After verbal consent from the patient is obtained, periodic contact will be made during and after treatment of their first CDI episode. The intent is to establish a relationship between the patient and the study team while monitoring for a possible recurrent CDI episode. The periodic contact will be brief, but focused on the patient's symptoms and will allow for questions by the patient regarding their CDI treatment and expectations for recovery. The frequency of contact will be left to the patient's wishes and to the discretion of the study team, but no more than weekly and up to 3 months after completion of treatment for the first episode. Agreement to active follow-up does not obligate the patient to participation in the study if they develop a recurrent CDI episode. If the patient is willing to participate at that point, a written informed consent will be required. Patients that decline active follow-up will be followed passively as described above.

If the patients identified by either strategy are willing to participate, their primary care physicians will be contacted by phone or page to inform them of the study and of their patient's willingness to participate. If the patient declines to participate or agrees and subsequently declines the primary care physician will be informed so that the provider can deliver the usual standard of care. In this way, duplicate calls to the patient will be avoided.

In the case where it is possible for a site to recruit participants by traveling to nearby VA facilities, sites will document and receive approvals for all off-site locations where study personnel will travel to conduct recruitment.

Potential participants will be identified using the same strategies of identification and referral for outside VA facilities as the primary VA facilities previously described. All Clinics and Labs will be provided with the approved flyers and educational materials to provide to potential participants using study standard methods. In person follow-up visits, will be conducted either at the recruiting outside VA facility or the primary VA facility per the participant's preference. All study visits will be conducted by approved study personnel only.

Inclusion Criteria

1. Informed consent obtained and signed

2. Veteran with age ≥ 18

3. If female, participant must not be pregnant or nursing

   - Negative pregnancy test required for females <61 years of age or without prior hysterectomy unless they were documented as post-menopausal

4. Confirmed current diagnosis of CDI, determined by having

   - >3 loose or semi-formed stools for participants over 24 hours AND
   - Positive stool assay for *C. difficile*
     - EIA positive for toxin A/B; or
     - Cytotoxin assay; or
     - Nucleic Acid Amplification Test (NAAT, PCR or LAMP) based detection of toxigenic *C. difficile*

5. Current episode represents the first recurrent episode of CDI within 3 months of completion of treatment for the primary CDI episode in a patient who has not had CDI in the 3 months prior to the primary episode OR a second recurrent CDI episode occurring within 3 months of completion of treatment for the first recurrent episode, as defined above.

   - At least one of the previous CDI episodes must have been confirmed by a stool assay for *C. difficile* (see previous bullet point)

Justifications for Inclusion Criteria

Only veteran adult participants with a first or second CDI recurrence are eligible for enrollment as this study will focus on the efficacy of therapy within a predominately adult veteran population. Only participants with a documented history of a previous CDI episode and demonstrating signs and symptoms of disease that is diagnostically confirmed will be enrolled. Participants with multiple recurrences (≥3) may represent a separate population that is sometimes confounded by underlying gastrointestinal disorders triggered or precipitated by CDI. These individuals may have already received some or several of the proposed study treatment regimens and subsequently require different treatment modalities. Despite data indicating minimal absorption of both agents, there are limited data available for fidaxomicin use in pregnancy.

Exclusion Criteria

1. Inability to provide informed consent
2. Inability to take oral capsules
3. Receipt of >72 hours of antibiotics considered effective in the treatment of CDI including

vancomycin, fidaxomicin, metronidazole, rifaximin, or nitazoxanide

4. Prior infusion of bezlotoxumab within the previous 6 months

5. Known presence of fulminant CDI, including hypotension, severe ileus or GI obstruction or incipient toxic megacolon

6. Receipt of more than one treatment course of oral vancomycin, more than one treatment course of vancomycin followed by a taper/pulse, and more than one treatment course of fidaxomicin, since the primary episode of CDI as defined above (i.e., one course of any of the above 3 treatment options is allowable)

7. Known allergy to vancomycin or fidaxomicin

8. Acute or chronic diarrhea due to inflammatory bowel disease or other cause that would confound evaluation of response to CDI treatment

9. Anticipation of need for long term systemic antibiotic treatment (beyond 7 days)

10. Patients with an active diagnosis of COVID-19 will be excluded from the study, but patients who have recovered (per current CDC guidance on discontinuation of transmission-based precautions) can be included in the study.

Justifications for Exclusion Criteria

Exclusion of participants who are unable to take oral capsules is necessary because fidaxomicin, vancomycin, and the placebo medications will be over-encapsulated to maintain blinding. In order to determine the efficacy of the treatments for CDI, the use of other antimicrobials considered effective for *C. difficile* will not be permitted beyond a time period that may influence the response to the study medication (i.e., greater than 72 hours). Infusion of the monoclonal anti-toxin B antibody, bezlotoxumab, also influences risk of recurrent CDI and due to its long half-life, use of this agent in the prior 6 months is a necessary exclusion. Additionally, systemic non-CDI antimicrobial exposure, in general places participants at risk for recurrent CDI infection and prolonged antimicrobial exposure may affect resolution of the disease. Those participants previously receiving more than one treatment course of vancomycin, more than one treatment course of vancomycin followed by a taper/pulse, or more than one treatment course of fidaxomicin may be inherently less likely to respond to the study regimen if they are randomized to the same

treatment they received prior to enrollment and may need special management considerations. Participants with severe/fulminant CDI usually require more aggressive therapies, and may have outcomes influenced by additional factors, rather than factors relating to the efficacy of the treatment arms. Those participants with other underlying diseases that manifest as diarrhea will be excluded in order to evaluate the efficacy of the study regimens.

Eligibility Requirements for Participants Enrolled in Long-Term Follow-Up Phase Interventional Studies

Potential patients that meet CSP #596 eligibility criteria and are otherwise participating in a long-term follow-up phase of another interventional study (drug or device) will be eligible to enroll in CSP #596 study. Enrollment of these potential patients will not occur while participating in the active phase (intervention and safety monitoring) of the other interventional study. Since CSP #596 has a short study duration, CSP #596 participants will not enroll in another interventional study while participating in #596 study (up to 3 months). Principal Investigators (s) and Pharmacist(s) (if applicable) of both studies will have a thorough review of both studies' inclusion/exclusion criteria, intervention and safety events follow-up period to ensure there will be no overlap of the studies' active safety monitoring periods thereby eliminating the risk of confounding the attribution for safety events. Principal Investigators from both studies must agree to the conditions under which patients can be enrolled in both studies before any patient in a long-term follow-up phase of another interventional trial can participate in CSP #596.

V. HUMAN RIGHTS ISSUES AND INFORMED CONSENT

Informed Consent

Participants who may meet the inclusion/exclusion criteria will be identified by the site coordinator or site investigator at each site. After each participant has met the pre-screening criteria (through chart review) for the trial, informed consent will be sought for all participants who successfully meet the pre-screening criteria. The site coordinator at each site will introduce and explain the study to the participant and present him/her with the detailed informed consent form and supplementary material to read and review.

The general purpose of the study will be delineated and the treatment comparisons will be clearly described. The process of randomization will be discussed and a clear explanation of what will be

expected of the participant will also be described. The risks associated with treatments and procedures will also be addressed. The importance of participant confidentiality will be emphasized, including a description of the process for maintaining participant confidentiality. Informed consent will be given in a manner compliant with VHA Handbook 1200.05. Study personnel will ensure that the participant understands every aspect of the trial, including its risks and benefits, prior to signing the informed consent.

If the participant agrees, his/her consent to participate in the study will be documented on the Agreement to Participate in Research form (VA form 10-1086). The original is kept on site in the clinical research record. Copies of the consent form will be provided to the participant and to the Hines CSPCC at the time of enrollment in the study.

Informed consent requires that the participant understand the details of the study and agree, without coercion, to participate in the study. To obtain informed consent, the following information shall be provided to each participant:

1. Name of the study
2. Name of the Principal or Site Investigator(s)
3. Explanation that the study involves research
4. Explanation of the purpose of the study
5. Explanation of the treatment procedures
6. Description of randomization
7. Description of the risks and benefits of participation in the study
8. Description of alternatives to participation in the study
9. Explanation that all records will be kept confidential, but that records may be examined by representatives of the VA and/or the FDA
10. Whom to contact for questions about the research and about participants' rights
11. Whom to contact in the event of a research-related injury
12. A statement that participation in the study is voluntary and that a decision not to participate or to withdraw from the study after initially agreeing involves no penalty, loss of benefits, or reduction in access to medical care
13. A statement that the treatments provided as part of this study are free

Merely obtaining signed consent from the participant does not constitute informed consent. However, the use of a standardized consent form aids in assuring that participants receive adequate and consistent information about the trial and that they have consented to participate.

In conjunction with the informed consent procedure, participants will review and be asked to sign the Authorization for Release of Protected Health Information From as required by HIPAA.

VI. EVALUATION PROCEDURES

The following evaluations and laboratory tests will be performed according to the Schedule of Procedures (figure 5).

Figure 5. Schedule of Procedures

Visit No.	1	2	3	4	5	6	7,8, 9	10	11,12,13,14	15	98
Day	0	5	10	17	24	31	38, 45, 52	59	66, 73, 80, 87	90	
	Day 0 Visit	Call 1	Day 10 Visit	Call 2	Call 3	Day 31 Visit	Calls 4, 5, 6	Day 59 Visit	Calls 7, 8, 9, 10	Day 90 Visit	Unscheduled Visit for Recurrence
Inclusion Exclusion & Randomization	X										
Informed Consent	X										
Demographics	X										
Past Medical History	X										
Medication Use[a]	X	X	X	X	X	X	X	X	X	X	X
Targeted Physical Exam	X		X***			X*		X*		X*	X
Laboratory Assessments (collected, processed and stored)	X		X***			X***					
Severity/Horn's Assessment	X										
Stool Sample	X										X
Pregnancy Test	X										

Reinforce Documentation into Stool Diaries[a]	X	X	X	X	X	X	X	X	X	X	X

Record daily unformed bowel movements from the Study Diary[a]	X	X	X	X	X	X	X	X	X	X	X
Collection of Study Diary			X			X		X		X	X
Patient Centered Outcome Questionnaire[a]	X		X					X			
Adverse Events[a]	X	X	X	X	X	X	X	X			(AE-SAE follow up forms closed out)
Assessment of Treatment Failure & Symptom Resolution			X								
Assessment of Recurrence & Sustained Clinical Response**, [a]							X (Day 38)	X		X	X
Drug Dispensed	X		X								

* Physical exam will only be performed on days 31, 59, and 90 if the participant history indicates need for confirmation/corroboration of a specific complaint (e.g., abdominal pain or rash).

**Assessment of sustained clinical response will be performed on day 38 (visit #7) as well as day 59 (visit #10), day 90 (visit #15) and unscheduled visit for recurrence.

*** The day 10 physical exam and the day 10 and day 31 blood draw can be waived in circumstances when the visit would result in increased risk of harm to the participant; and if there is no need for confirmation/ corroboration of a specific complaint. Risk is defined as circumstances where the participant would likely be exposed to conditions that may cause further illness, injury, or discomfort. Reasons for waiving the visit must be documented.

[a] In a situation that a participant cannot come to the clinic for a physical exam and blood draw, some assessments can still be conducted through phone interview with participants along with electronic health record review (i.e., CPRS review of pharmacy profile or medication list, stool lab test result for CDI). This includes the review of concomitant medications, treatment failure, recurrence, and patient centered outcome questionnaire.

Daily Unformed Bowel Movements

The number of daily unformed bowel movements will be recorded from the day prior to randomization up to the end of the study, or CDI recurrence if this event occurs first. Participants will be given a "Study Diary" to describe loose or watery stools up to day 90, according to the Bristol stool chart provided to them. Investigators will reinforce the use of these diaries when contacting the participants (via visit or phone call) and ask them if they are having difficulty filling out the diary (and if so, why?). At each follow-up contact, the study coordinator or site investigators will record the stool data (refer to the Schedule of Procedures, figure 5) and will assess the participant for symptom resolution, recurrence, and sustained clinical response. Investigators will collect each participant's Study Diary on days 10, 31, 59, 90, and confirm the participant's reported stool information. Entry of stool diaries will be emphasized at each call and visit. The completed stool diaries will be kept in the site coordinator's office in a locked cabinet (inside a locked room) and new diary given for the subsequent days. Data from the stool diary will be transcribed onto a form by the site coordinator and transmitted in a secure fashion (DataFax) to the Hines CSP Coordinating Center.

Assessment of Treatment Failure

A treatment failure will be defined as a worsening of CDI after 3 days of treatment which may include progression of CDI into fulminant disease (i.e. toxic megacolon), or an increase in daily unformed bowel movements compared to the participant's baseline. Based on prior clinical trial history and anecdotal experience, we don't anticipate true failures during the treatment phase with either vancomycin or fidaxomicin, However, participants may take several days to respond to standard CDI treatment. Investigators may consider discontinuation of treatment in participants who have a worsening of CDI after 3 days, or lack of clinical improvement after 7 days of treatment. The local study team will need to document each case into a case report form.

Assessment of Symptom Resolution

Investigators will assess the participant for resolution of disease symptoms from randomization up to day 10 as this day indicates the end of full treatment doses for all groups (i.e. vancomycin 125mg PO Q6hrs for VAN-TX and VAN-TP/P or fidaxomicin 200mg BID for FID-TX).

Symptom resolution is defined as an improvement or resolution of diarrhea (≤3 unformed bowel movements over 24 hours) for 48 consecutive hours compared to the participant's baseline. Investigators will assess for the onset of symptom resolution according to the participant's Study Diary entries and from discussion with the participant at each contact point described within the Schedule of Procedures (figure 5).

Assessment of Recurrence

Participants who meet the criteria of symptom resolution by day 10 (for all groups) will be evaluated for recurrence at all points of contact (beyond day 10) according to the Schedule of Procedures. Recurrent diarrhea will be defined as having >3 loose or semi-formed stools over 24 hours for 48 consecutive hours. Recurrent CDI will be defined as recurrent diarrhea with confirmation of toxigenic *C. difficile* or its toxin by stool testing. At randomization, participants will be provided with a stool collection kit and instructed on the proper collection, handling and storage of the samples. Participants who are suspected of experiencing a recurrence will be asked to return to clinic to submit a stool sample for testing of *C. difficile.* Determination of CDI recurrence will be based on the same test used by the laboratory for diagnosis of initial episode.

Assessment of Sustained Clinical Response

The primary outcome of sustained clinical response (D-COM) will be evaluated at study day 59 and secondary outcomes at day 90 and at 28 days after completion of treatment (day 38 for vancomycin treatment and fidaxomicin treatment, day 59 for vancomycin taper/pulse).

Sustained clinical response is a composite endpoint defined as symptom resolution during treatment without diarrhea recurrence, mortality or other important clinical outcomes at any time during the follow-up period. Those participants failing to meet the criteria of symptom resolution (diarrhea resolution) by the end of the active treatment (day 10 for all groups), or who experience a recurrence during the follow-up period, will be considered study treatment failures. Symptom resolution and recurrence will be assessed at all follow-up contacts using the participant's Stool Diaries. Diarrhea resolution is defined as an improvement or resolution of diarrhea (≤3 unformed bowel movements over 24 hours for 48 consecutive hours). Recurrent CDI will be defined as having diarrhea (>3 loose or semi-formed stools over 24 hours for 48

consecutive hours) and a positive stool assay for *C. difficile*. Determination of recurrence will be based on the same test used by the laboratory for diagnosis of initial episode.

Patient-centered Assessment

Incorporation of the pilot phase into CSP #596 will allow us to explore potential outcomes meaningful to patients with CDI and gauge the patient perspective on their illness. To be consistent with previous clinical trials of CDI, the definition of response to therapy includes quantification of stool frequency. However, it is possible that symptoms other than achieving ≤3 loose stools per day may be as meaningful or more meaningful to the patient. In order to solicit information on the participant's perspective on their illness and recovery, we administered a short questionnaire that was administered by the site coordinator at the time of enrollment and after completion of their treatment phase (day 10) and at the time of the primary end point (day 59). The questionnaire started out with general, 4 open-ended (semi-structured) questions and responses to the questionnaire were reviewed periodically during the pilot phase. During the mid of pilot phase, a validated C *difficile* health related quality of life questionnaire with 32 questions on a 5-point Likert scale was published, which included three constructs: emotional/psychological, social and physical. This published questionnaire when reviewed, addressed most of the participant issues that were identified during examination of our open-ended questionnaire using NVIVO 11 plus software from the pilot study. This published and validated questionnaire with 3 added financial impact questions (participant concern regarding economic impact of the disease) has now replaced our open-ended questionnaire. The 3 added financial questions will be scored separately from the validated 32 health related QOL questions.

Safety Laboratory Assessments

Each participant will be asked to provide one-10mL blood sample during their study visits in order to assess for potential systemic toxicities on days 0, 10 and 31. A complete blood panel will be ordered to assess for CBC, serum creatinine, albumin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase. Any abnormal findings will be documented and reported as described within section XI (Adverse Events and Serious Adverse Events). If these tests have already been ordered for the participant at the time of the study visits (e.g., day 0), we will use the data from the electronic medical record and not repeat

the tests. The day 10 physical exam and the day 10 and day 31 blood draw can be waived in circumstances when the visit would result in increased risk of harm to the participant; and if there is no need for confirmation/ corroboration of a specific complaint. Risk is defined as circumstances where the participant would likely be exposed to conditions that may cause further illness, injury, or discomfort. Reasons for waiving the visit must be documented.

Central Laboratory

We will obtain stool samples from all participants at the time of randomization and at the time of CDI recurrence for those participants that experience recurrence. Participating facilities will freeze and store stool samples locally for quarterly batching and mailing (frozen on dry ice) to the Central Laboratory. Central Laboratory will receive coded samples labeled with the Participant ID number and date of sample which will be cultured and the recovered *C. difficile* isolates processed for typing analysis. The samples will be cultured anaerobically for *C. difficile* on selective taurocholate-cycloserine-cefoxitin-fructose (TCCFA) media. Stool specimens that are culture-negative by selective agar culture will undergo alcohol shock to enhance isolation of spores on TCCFA and to increase the recovery rate. Strain type will be determined using restriction endonuclease analysis (REA). Although the Central laboratory will retrospectively confirm the presence of *C. difficile* in the stool samples, no intervention or change to the management of participants will occur on the basis of this analysis. Typing information will be used to determine the effect of the epidemic BI/NAP1/027 strain on disease presentation and therapy outcome. Further microbiome analysis of stool specimens will also be performed using a combination of deep 16S rRNA sequencing and quantitative real-time PCR (qPCR). For deep sequencing analysis, the V6 hypervariable region of 16S rRNA will be PCR-amplified from extracted bacterial DNA and sequencing will be performed to determine the relative abundance of taxa from phylum to genus levels qPCR of extracted DNA will be performed to quantify specific bacterial groups and total bacteria using the methods and primers of Louie et al. [61] and Jump et al. [62].

VII. BASELINE PROCEDURES

The baseline visit will consist of obtaining informed consent, completing all baseline procedures described in this section and determining whether the patient qualifies for randomization from a review of the inclusion/exclusion criteria.

Informed Consent

Prior to randomization, the investigators will discuss the details of the study and participants will provide voluntary, written informed consent using the provided informed consent form (ICF). HIPAA authorization will also be obtained. The participant will receive a copy of the signed ICF and HIPAA authorization, and the originals will be filed in the Master ICF binder provided by SMART during study start-up. Hines, CSPCC will also receive a copy of the signed ICF and HIPAA authorization as per CSP Policy.

Data to be Collected

1. Demographics: age, gender, race and ethnicity
2. Contact information: home, work, cell phone number of participant and an additional contact person.
3. Specific clinical stool assay for *C. difficile* used for confirmation of current diagnosis of CDI fulfilling inclusion criteria at time of study enrollment and potentially for confirmation of recurrent CDI if diarrhea symptoms develop after study day 10. Data will be collected centrally at the Hines CSP Coordinating Center using Joint Legacy Viewer (JLV).
4. Inpatient or outpatient status upon randomization
5. Past Medical History

a. Comorbid Diseases

b. Surgical History

c. Previous CDI history

-Number of previous CDI episodes and dates

-Therapies used for prior episodes and outcomes

-Complications, if any

d. Medication Use: over the last 3 months, particularly antibiotics (including OTC)

Information to be collected from Participant Interview

1. Contact information and release of information for non-VA primary care or specialty physicians or hospitals that may also provide care to the participant
2. Medication Use: over the last 3 months (including OTC)

3. Confirm medical/surgical history

Physical Exam

1. A focused physical exam will be performed on day 0, 10, and at the time of recurrence if applicable by qualified study personnel. In the event qualified study personnel are not available to conduct a physical exam in a timely manner, an appropriate physical exam conducted for reasons of routine care by an accredited examiner within 5 days, may be substituted for the study physical exam, provided the LSI reviews and approves of the exam, and study personnel follow-up the findings. Physical exam will only be performed on days 31, 59, and 90 if the participant history indicates need for confirmation/corroboration of a specific complaint (e.g., abdominal pain or rash). The day 10 physical exam and the day 10 and day 31 blood draw can be waived in circumstances when the visit would result in increased risk of harm to the participant; and if there is no need for confirmation/ corroboration of a specific complaint. Risk is defined as circumstances where the participant would likely be exposed to conditions that may cause further illness, injury, or discomfort. Reasons for waiving the visit must be documented. The exam will include:

    - Vital signs: weight, blood pressure, heart rate, respiratory rate, and temperature
    - Cardiac, pulmonary, abdomen, and extremity exam

Laboratory Specimens

1. Stool specimen
2. Blood Sample

    -Complete Blood Count (CBC)

    -Serum Creatinine (SCr)

    -Albumin

    -Liver Function Tests (LFTs)

        -alanine aminotransferase (ALT)
        -aspartate aminotransferase (AST)
        -alkaline phosphatase (Alk Phos)

-total bilirubin

Modified Horn's Index [56]

The investigators will assess the severity of each participant's underlying comorbid conditions using a modified Horn's index. Underlying disease severity is rated on a score of 1-4 according to the investigator's clinical judgment.

-Low (single mild illness)

-Moderate (more severe disease but uncomplicated recovery)

-Major (major complications or multiple conditions requiring treatment)

-Extreme (catastrophic illness leading to death)

CDI Severity Assessment [57]

The ATLAS scoring system will be used to define CDI disease severity for each participant at baseline. This scoring system has been recently validated using the data from the two phase III fidaxomicin versus vancomycin clinical trials. The score was found to predict the treatment response for either therapy and may potential influence our primary endpoint, sustained clinical response.

Table 4. ATLAS scoring system categories

Parameter	0 points	1 point	2 points
Age (years)	<60	60-79	≥80
Treatment with systemic antibiotics during CDI therapy (≥1 day)	No	--	Yes
Leukocyte count (total)	< 16,000	16,000-25,000	> 25,000
Albumin (serum, g/L)	>35	26-35	≤25
Serum creatinine (μmol/L)	≤120	121-179	≥180

VIII. STRATIFICATION AND RANDOMIZATION

The treatment allocation ratio for the three treatment regimens will be 1:1:1 using a random permuted block scheme with random block size. The randomization will be stratified by study site. Within each site subjects will be randomized in an equal ratio into one of three treatment

groups. The random treatment scheme will be generated by the Hines CSPCC. Site Coordinators will use an Interactive Touch Tone Randomization System (ITTRS) to obtain randomization codes. CSPCC will assign each site a unique 3-digit password that the staff can use to access the ITTRS system. The Site Coordinator will call the ITTRS, enter participant information required to randomize and obtain the randomization code. This procedure will be tested and validated before enrollment begins. The Hines CSPCC must receive a copy of the signed consent form and HIPAA authorization within 24 hours of each randomization.

The Albuquerque CSPCRPCC will be responsible for supplying the drugs. This procedure will be tested and validated before enrollment begins.

The Hines CSPCC will review the overall randomizations at least weekly during the enrollment phase of the study and will be monitoring the randomization assignments on a daily basis. The unique study ID number will be linked in the randomization file to the treatment assignment for each randomized participant. The randomization file data will remain separate from the rest of the study data on the central database.

Randomization will occur on the same day that the participant has completed the necessary portions of the Screening and Baseline assessments is judged eligible for randomization, and the informed consent process is completed. When a new participant has been randomized, his/her electronic medical record will be updated indicating participation in the study. Source documentation for eligibility criteria and randomization will be kept at the site with the participant's study folder.

Blinding of the Randomization Scheme

The CSPCRPCC will purchase commercially available vancomycin HCl 125mg capsules and fidaxomicin 200mg tablets through the VA national contract wholesaler. Preliminary testing by the CSPCRPCC has demonstrated the both products can be over-encapsulated (OE) to blind the treatment assignment. The CSPCRPCC will also manufacture a matching placebo capsule to correspond to the dosing schedule of the 10-day treatments and to the vancomycin taper/pulse regimen. OE is an accepted method for blinding treatment assignment in drug trials; however, it does have some limitations. There is a risk that study personnel or a participant may discover the treatment assignment if they tamper with the OE capsule and discover its contents. The CSPCRPCC plans to use "Double Blinded (DB) capsules" that are more difficult to separate to

minimize this risk. It is also possible participants may compare their study medications with one another, but may only discover that they are "different" and not become aware of their treatment assignment.

Study medication will be dispensed upon initial randomization (10-day treatment card), followed by a blinded 21-day treatment card for the taper/pulse card issued at the Day 10 clinic visit. The blinded study medication are packaged in blister cards in a manner to blind the treatment assignments with regard to dosing schedule.

Note: The study medication cards SHOULD NOT be altered in any way. The cards are designed to blind the study medication, and account for the differences in dosing frequency. It is important that the participant take the study medication as prescribed on the card.

Blinded 10-day Treatment Card (Day 1-10)			
10 day	Vancomycin 125mg capsules 4 times daily x 10 days	Vancomycin 125mg capsules 4 times daily x 10 days	Fidaxomicin 200mg capsules + Placebo capsules 4 times daily x 10 days (alternated)
Blinded 21 Day Taper/Pulse Card (day 11-31)			
21 day	Vancomycin 125mg Taper/Pulse 1 time daily	Placebo Taper/Pulse 1 time daily	Placebo Taper/Pulse 1 time daily

IX. TREATMENT REGIMENS

Comparator Regimens

The primary objective of CSP 596 is to determine which of the three treatment arms leads to higher rates of sustained clinical response (D-COM). Sustained clinical response is a composite endpoint incorporating clinical cure and the absence of recurrence at day 59 for all treatment arms, with a secondary endpoint of a 28-day follow-up period following the end of active treatment in each arm (day 38 for fidaxomicin and vancomycin, day 59 for vancomycin taper/pulse) and a secondary endpoint at day 90 for all three treatment arms. Eligible participants will be randomized in a 1:1:1 ratio to a 10-day course of vancomycin, a 10-day course of fidaxomicin, or a 31-day vancomycin taper/pulse regimen.

Both vancomycin arms will receive 125mg capsules given four times daily for the first 10 days of therapy to reflect standard, FDA recommended dosing of the antibiotic for the treatment of *Clostridium difficile* infections. Those participants randomized to the vancomycin taper/pulse

regimen will then receive 125mg capsules given daily for 7 days, 125mg capsules given every other day for 7 days, and 125mg capsules given every third day for 7 days. Participants within the fidaxomicin arm will receive 10 days of 200mg tablets given twice daily. All participants will receive 2 blister packs containing 31 days of over-encapsulated medications containing either active treatment or a combination of active and placebo treatment in order to maintain blinding.

Dosage Adjustment

As both vancomycin and fidaxomicin are minimally absorbed antibiotics that work within the gastrointestinal tract, no dosage adjustments are recommended by the manufacturers for either product. Although a recent warning of potential renal toxicity has been added to the Vancocin (brand name vancomycin) package insert as a result of isolated observations seen in a previous multicenter study, safety analysis from the two phase III fidaxomicin studies were unable to confirm a similar finding [27, 28, 47, 54].

Discontinuation of Treatment

1. Decision to discontinue study drug treatment by the participant

Participants will be informed of their right to discontinue study drug at any time without affecting their medical care. Participants will not be required to provide a reason for discontinuation; however, investigators will make an effort to determine if the cause for discontinuation is due to an adverse event (AE). Participants who discontinue study drug will be asked to continue with follow-up safety assessments until day59 per the protocol. If participants elect to be removed completely from the study, all follow-up will cease.

2. Decision to discontinue study drug treatment

Participants found to have a medical condition (i.e. AE that are possibly related to study drug) that is negatively affected by the continuation of study drug can be removed from active treatment by the local study investigators while in collaboration with the participant's primary providers. Participants who discontinue study drug will be asked to continue with safety assessments until the end of the study (day 59, or day 90 for follow up if necessary).

Medications that may confound the study

All non-study medications that have activity against *C. difficile* will be discouraged during the period of the study (day 0 to day 90). These include metronidazole, oral vancomycin,

fidaxomicin, nitazoxanide, rifaximin, rifampin, fusidic acid, and oral bacitracin and luminal toxin binders such as cholestyramine. If any of these medications are incidentally prescribed and taken (e.g., prescribed by an outside practitioner), the dosage, time and duration will be recorded.

Participants will be informed to avoid the use of medications that can affect diarrheal symptoms and/or impair clinical evaluation of CDI disease symptoms. Use of these medications will not preclude participants from randomization or study participation. Investigators will be required to document the frequency of use of anti-diarrheas (loperamide, diphenoxylate/atropine) and all opiates.

The use of systemic antimicrobials beyond 7 days is discouraged and should be discontinued if possible. Investigators will document systemic antimicrobial use throughout the duration of the study.

X. FOLLOW-UP ASSESSMENT

All participant contact and assessments will be made according to the Schedule of Procedures (figure 5). A participant develops COVID-19 infection while enrolled in the study, may continue participation only if the participant is willing to continue his/her involvement with the study, and the LSI/SC determine that the participant's involvement will not increase risk or harm to the participant or study staff, and that participant's remaining participation can be completed safely. Participant withdrawal due to COVID-19 infection must be documented on the withdrawal form.

Scheduled follow-up calls

Participants will have 10 phone contacts during the study period to assess for potential recurrence or adverse events. There will be weekly contact (by phone or clinic visit) with the participant up to day 59 and continuing weekly until a final clinical visit at day 90.

Concurrent non-study medication use will be documented during these calls. Investigators will also document the participant's daily stool habits, including stool frequency and characteristics

as reported by the participant according to their Study Diary. Based on the participant's reported symptoms, investigators will assess for treatment failure, symptom resolution, sustained clinical

response and/or recurrence as defined within section VI (Evaluation Procedures) of the protocol. Investigators will be responsible for documenting the timing of these events.

Participants suspected of experiencing a treatment failure or recurrence of diarrhea will be asked to return to the clinic for an unscheduled visit in order to submit a stool sample to evaluate for recurrence of *C. difficile*.

Scheduled follow-up visits

Four follow-up clinic visits are scheduled at Day 10, Day 31, Day 59, and Day 90. Both end of active study treatment visits (Day 10 and Day 31) are scheduled in order to maintain blinding for the end of study visits. If participants are suspected of having a recurrence of diarrhea, they will be asked to return to clinic for an unscheduled visit. During the Day 10, Day 31, Day 59, and Day 90 visits and unscheduled visits for recurrence, investigators will document the stool frequency and characteristics from the participant's Study Diary to assess for symptom resolution, sustained clinical response and/or recurrence as defined within section VI (Evaluation Procedures) of the protocol. Participant's Study Diaries will be collected in order to confirm their reported symptoms from prior contacts to those documented within the diaries. Participants will be asked to provide a stool sample if investigators suspect CDI recurrence. Concurrent non-study medication use will be documented at this visit. A targeted physical exam will be done in order to assess potential adverse events or toxicity from study therapy. One-10mL blood sample will be taken on Day 0, 10 and Day 31 Visits. These samples will be sent to the local clinical laboratory for chemistry and hematology evaluation of safety. The day 10 physical exam and the day 10 and day 31 blood draw can be waived in circumstances when the visit would result in increased risk of harm to the participant; and if there is no need for confirmation/ corroboration of a specific complaint. Risk is defined as circumstances where the participant would likely be exposed to conditions that may cause further illness, injury, or discomfort. Reasons for waiving the visit must be documented. Approved staff will be permitted to travel to the participant to conduct follow-up visits in situations where it would be prohibitive for the participant to travel, provided the participant consents to the off-site visit.

Transition from study to standard care

Study participants who fail to respond in any of the 3 treatment arms or have subsequent diarrhea recurrence or experienced complications (including toxic megacolon, colectomy, or

severe abdominal pain related to current diarrhea illness that requires re-treatment for CDI), in the follow up period will be transitioned to the care of their primary care physicians (after obtaining stool specimen test result for CDI. e.g., up to 8 days) with consultation from the LSI or from one of the Co-proponents of CSP# 596.

XI. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

Both vancomycin and fidaxomicin were found to have similar AE profiles as reported by the combined safety analysis from the two phase III studies of fidaxomicin vs. vancomycin for CDI. Additionally, no Serious Adverse Events (SAEs) were found to be attributed to either drug.

Definitions

Adverse events (AE) and serious adverse events (SAE) will be collected per the ICH for Clinical Safety Data Management (ICH-E2A) and CSP Global SOP 3.6 definitions.

Adverse Event (AE)

An AE is any untoward physical or psychological occurrence in a human participating in research. An AE can be any unfavorable and unintended event, including an abnormal laboratory finding, symptom, or disease associated with the research or the use of a medical investigational test article. An AE does not necessarily have to have a causal relationship with the research.

Serious Adverse Events (SAEs)

SAEs are a subset of adverse events. SAEs collected for CSP #596 are those defined by the ICH for Clinical Safety Data Management and CSP Global SOP 3.6, as untoward medical occurrence that results in

- Death;
- A life-threatening experience;
- Inpatient hospitalization or prolongation of existing hospitalization;
- Persistent or significant disability or incapacity;
- Congenital anomaly/birth defect; or Any other condition that, based upon medical judgment, may jeopardize the participant and require medical, surgical, behavioral, social or other intervention to prevent one of the above outcomes.

Relatedness:

Relatedness involves an assessment of the degree of causality (attributability) between the *study intervention* and the event. Site investigators will be asked to provide an assessment of relatedness of the AE/SAE to the study intervention. The assessment provided by the site investigator is part of the information used by the sponsor (CSP) to determine if the AE/SAE represents an alteration in the safety profile of the study intervention. All AE/SAEs with a reasonable causal relationship to the investigative treatment should be considered "related". A definite relationship does not need to be established.

Anticipated:

The Sponsor (Cooperative Studies Program) will assess whether an adverse event is anticipated or unanticipated (sometimes referred to as expected/unexpected, respectively), and is evaluated in terms of nature, severity, or frequency given the drug profile and research plan described in the protocol documents (e.g. informed consent, study population characteristics; and related or possibly related to participation in research, or there is evidence that the research places subjects at a greater risk of harm (including physical or psychological) than was previously recognized.

Monitoring and Reporting of Adverse Events and Serious Adverse Events

The Site Research Team is responsible for the following:

1. Closely monitoring all participants for adverse events (AEs) and/or serious adverse events (SAEs);
2. Reviewing accuracy and completeness of all AEs and/or SAEs reports.
3. Complying with Cooperative Studies Program policies for reporting AEs and/or SAEs.
4. Complying with local Research & Development Committee (R&DC) policies for reporting AEs and/or Serious AEs.
5. Complying with:
    a. VA Central IRB (http://www.research.va.gov/vacentralirb/) requirements.
    b. VHA Handbook 1058.01 Research Reporting Compliance Requirements
    c. VHA Handbook 1108.04 Investigational Drug and Supplies
6. Notifying VA Central or local IRB and local R&DC of safety issues reported to the investigator by the CSP.
7. Managing and reporting adverse events or serious adverse events, and responding when questions arise, in consultation with the LSI, Clinical Research Pharmacist,

and/or Study Chair(s).

8. The study investigator will assess and record all AEs according to severity, seriousness, relatedness to study drug and action taken.

Reporting of AEs and SAEs in CSP#596

All study intervention **related** (or attributable) events meeting the definition of an AE will be reported by the LSI for CSP#596. An assessment of relatedness to study intervention must be documented in the source documentation, but only related AE's need to be reported via the study reporting system.

All events meeting the definition of an SAE, regardless of relatedness must be reported by the site investigator via the study reporting system.

Monitoring of AEs and SAEs begins when the participant signs the informed consent.

Participants will be monitored at each clinic visit and telephone contact for AEs and SAEs through day 59 of the study. If necessary, follow up AE or SAE reports may be collected until end of study (i.e. day 90). All AEs and SAEs will be recorded on the appropriate adverse event form(s). AEs and SAEs will be followed up no less than every 30 days until the event resolves, or no change is expected. If necessary, follow up AE or SAE reports may be collected until end of study (i.e. day 90). In cases where an AE or SAE persists past the end of study, or the participant experiences a new event, the event may be marked as "ongoing" and closed out

Expedited Reporting of Serious Adverse Events

All SAEs will be reported by submission of the event into the CSP #596 reporting system within 72 hours of the site investigator/site coordinator being informed of the event.

The reporting system will immediately inform the study biostatistician, Clinical Research Pharmacists and Study Chairs. All SAEs found to be attributable or related to study drug administration will be documented as "possibly related" or "related" within the Serious Adverse Event Form.

Reporting of Serious Related and Unexpected Events

All events will be assessed by the study Sponsor to determine if an event is anticipated or unanticipated. The source of information that will be utilized in the determination of expectedness will include the CSP #596 Drug Information Report, the CSP #596 Informed

Consent, published literature, and the FDA Adverse Event Reporting System (AERS). SAEs found by the Sponsor (CSP) to be both related to the investigative treatment *and unexpected* will be reported to the VA Chief Research and Development Officer, the FDA, and site investigators *after* review by the Study Chairs, the CSPCRPCC Director and the Hines CSPCC Director.

Reporting of Adverse and Serious Adverse Events to the DMC

The CSP #596 Biostatistician will tabulate and summarize all study intervention related AEs and all SAEs (intervention related or not related) for the DMC on a schedule set by the DMC, but no less than annually. The DMC will also determine when the committee should be unblinded to treatment assignment in reviewing AE/SAE data. The DMC will advise the CSP Director whether the study should continue or be stopped for safety reasons.

Reporting Requirements of the VA Central IRB

Sites are additionally responsible for following the VA Central IRB policy in submitting safety data and protocol deviations. The VA Central IRBs' most recent policy including a Table of Reporting Requirements, instructions, and forms can be found at Table of Reporting Requirements to the VA Central IRB.

Note: In reporting to the CIRB, the LSI must also evaluate whether an adverse event is unanticipated based on CIRB definition, and if the adverse event is related to the study intervention. This evaluation by the LSI is distinct from the Sponsor's assessment of relatedness.

XII. QUALITY CONTROL PROCEDURES

Standardization/Validation of Measurements

1. Training for Study Personnel

All local study personnel will receive training on diagnosis and assessment of the study endpoints at the study start-up meeting, and will receive support of these procedures throughout the study as listed below. The Study Chair's office will be responsible for the development and implementation of study clinical and research procedural trainings. The Study Chair's office

and Hines CSPCC will be jointly responsible for overseeing the development and implementation of training for data collection activities.

2. Start-Up Meeting

Once all site requirements for study initiation have been met an orientation and training meeting will be scheduled prior to the initiation of participant enrollment. The CSP Study Co-Proponents, members of the Executive Committee and staff from the CSP program sites will be in attendance. Two representatives from the local study sites will be required to attend, the local site investigator (LSI) and the site coordinator (SC).

Local site investigators will be given an in-depth presentation of the study rationale, methodology and procedures of the research protocol. An Operations Manual will be provided to the site investigators and site coordinators to guide the operation and management of the study. Over the course of two days, CSP# 596 investigators will address the study aims and protocol; issues in the protection of human participants and CIRB processes; organizational structure of the study ; clinical treatment and research procedures including fidelity to the protocol and safety procedures; the program management and monitoring system; research procedures including understanding reliability and validity, data collection and transmission and other data integrity procedures; administration of study screening, baseline and outcome assessments; and administrative procedures and problem solving. Additionally, the CSP#596 study team members will recommend methods to facilitate recruitment and retention and key contacts will be provided to address and triage any study related issues.

-SC and SI will be provided educational material and interactive training on evaluating participants for the presence of disease, and assessing symptom resolution and sustained clinical response. They will be educated on the collection of proper data points during clinic visits and follow-up phone calls, specifically the recording and interpretation of the participant's Study Diary that will be used to assess symptom resolution and sustained clinical response.

-Training with the SC on data management topics will be led by the CSPCC study team and will include the use of the web-based communications system, general data entry and management procedures, correcting data, coding missing data, and running reports. The Study Co-Proponents and the National Study Coordinator will train the SC on the identification of eligible participants.

-The SI will be trained on the study aims and protocol, including treatment arms, pharmacology, side effects, drug interactions and recommended treatment procedures. An overview and interpretation of clinical measures, including measures to assess for symptom resolution, side effects, and measures to determine adjustment of treatment will be reviewed, as well as risks and benefits to participants for study participation and reporting of Serious Adverse Events. Simulated case vignettes will be used to address common questions related to the study protocol.

3. Ongoing Teleconferences and Web-Based Live Meetings

Training updates in clinical and research procedures and special topics will be reviewed on an ongoing basis during scheduled and ad hoc teleconferences. Initially, there will be monthly teleconferences and quarterly web-based live meetings (as needed) for the SIs and the SCs. These calls will be used to reinforce enrollment procedures, and will keep local investigators well informed on clinical and research methods. They will also maintain a line of communication between the local sites and the CSP#596 Management in order to address concerns between the two parties. These include the need for additional training session in order to ensure the integrity of the protocol or recruitment.

Protocol Deviations

Documentation will be required for any protocol deviation or breach of protocol. Protocol deviations must be reported to the Hines CSPCC on the appropriate case report form and VA Central IRB following appropriate reporting rules to ensure immediate hazard to the participant did not occur. Any medical center with repeated protocol violations may be recommended to the DMC for termination. If a participating LSI feels that adherence to the protocol will in any way be detrimental to a particular participant's health or well-being, the interest of the participant must take precedence. The Site Monitoring, Auditing and Resource Team (SMART), Hines CSPCC, Albuquerque CSPCRPCC, Executive Committee, and DMC will monitor protocol adherence. The Executive Committee will consider recommending a site visit for any participating center with repeated protocol violations.

Site Performance Monitoring

By agreeing to participate in this study, the medical center delegates the responsibility for global monitoring of the ongoing study to the DMC, the Cooperative Studies Scientific Evaluation Committee, and the Hines CSPCC. However, the Research and Development

(R&D) Committee and the Human Studies Subcommittee/Institutional Review Board (IRB) at each participating medical center may require the local investigators to submit annual reports concerning the status of the study at the medical center for local monitoring purposes.

Data quality and completeness of data retrieval will be closely monitored on an ongoing basis by the CSPCC. The Study Biostatistician will present interim monitoring reports (overall and by site) to the Executive Committee and DMC that will include: recruitment of participants, characteristics of the population, completeness of data retrieval and data quality. If a site is identified as an outlier in terms of data quality, a site conference call or site visit will be initiated to assess the reasons that problems are occurring and how they can be corrected. If the problems are not corrected, the site may be placed on probation or terminated from the study.

Site Visits

SMART will conduct site monitoring visits fand routine or for cause audits as described in section XIV (Good Clinical Practices).

Hines CSPCC Quality Assurance Section

The Quality Assurance Section, Hines CSPCC will provide central monitoring of study sites to ensure compliance with Good Clinical Practice. Monitoring may include but not be limited to the informed consent process, data validation, source verification, and safety reporting. Additional site-specific monitoring may be conducted if triggered by study performance metrics. Site performance findings may result in on-site visits by a CSPCC QA Nurse Specialist or other CSPCC central monitoring personnel to evaluate the need for additional site training to remedy compliance concerns.

Probation/Termination of Participating Centers

The Study Chairpersons and the Study Biostatistician will monitor the participant intake rate and operational aspects of the study. Participating medical centers will continue in the study only if adequate participant intake is maintained. The Executive Committee may take action leading to the discontinuation of participant enrollment at a center only with the concurrence of the DMC and CSP Director.

If recruitment is not proceeding at an appropriate rate, the Study Chairpersons and the Study Biostatistician will scrutinize the reasons for participant exclusions. Based on this information,

the Executive Committee may choose, with the approval of the DMC and CSP Director, to drop centers or add additional centers, or to make modifications to the inclusion/exclusion criteria.

Medical centers will only be allowed to continue in the study if adequate participant intake is maintained. With the understanding that there is a ramp-up in recruitment early on, participating sites that do not enroll at least 1 participant during their first three months and sites that are unable to meet the goal will participate in one on one conference call(s) by Study Chairpersons to discuss site issues and ways to enhance recruitment. Sites that consistently demonstrate low recruitment will be considered for probation. If a medical center is placed on probation, the Study Chairpersons and Study Biostatistician will confer with the site personnel and visit the site, if necessary, to help improve the rate of recruitment. If there is no improvement in accrual after the probation period, the site may be subject to reduced funding or termination as a study site. The Executive Committee will only take actions leading to discontinuation of a center with the concurrence of the DMC or the CSP Director. The CSPCC Director has the authority to act independently with the concurrence of the CSP Director to take action leading to the termination of a study site. If a center is terminated from the trial, resources will be reallocated to other sites that may be exceeding the recruitment goals or be used to start up a backup site.

XIII. DATA COLLECTION AND MANAGEMENT

Data Management

The Hines CSPCC will be responsible for the management and the quality control of the data. After the study is approved, data forms will be finalized and field tested. An Operations Manual will be provided to the investigators to guide the operation and management of the study. A training session at the kickoff meeting is planned prior to the initiation of participant enrollment for all study personnel to assure uniformity in participant management and data collection procedures, and to train all study personnel in study procedures. Training will also be held on an as-needed basis to ensure uniformity in participant management and data collection procedures (e.g., new study personnel who will submit study data). The Study Coordinator at each medical center will complete data forms daily and transmit them to the Hines CSPCC.

DataFax, a clinical trial data management system (by DF/Net Research), will be used for data collection and management. DataFax allows for data collection via paper case report forms (CRFs) as well as electronic data capture (EDC) via iDataFax (current version 2016). CSP 596

is using paper CRFs during its pilot phase and will switch to EDC as its primary data collection method with paper CRF data collection as the backup data collection method. During pilot phase, paper CRFs are used, and the Site Coordinators from the sites will complete case report forms (CRFs) on a daily basis and fax them directly to the DataFax computer server, where data images of the CRFs are stored as files. The original forms will be kept in the investigator's study files. The DataFax system uses an optical character recognition (OCR) paradigm to automatically process and store the information from the image as data into the study database. The original fax image is also stored. Data management staff at CSPCC will review each CRF by comparing the faxed image with the OCR data and ensure that the two matches. During the expansion phase, electronic CRFs (i.e., EDC) are used, the Site Coordinators will log into the DataFax system (iDataFax) and enter study data directly, rather than completing and sending a paper CRF. The use of paper CRFs may be reserved as a backup process.

Data management staff at CSPCC will review CRFs for protocol adherence data consistency, and add data queries to items that fail these checks. Checks will be performed manually and programmatically. On a regular basis, data management staff will produce site-specific Quality Control reports that list all unresolved data queries. Data management staff will make the reports available to each site and work with the Site Coordinators to help them resolve queries. Queries will be resolved when the appropriate corrections to the CRF are made and data resent, or when an explanation is provided that allows for data management staff to resolve the query. All corrections and changes to the data will be reviewed by data management staff. In addition to the Quality Control report, CSPCC may generate and distribute targeted data edit reports on an as needed basis.

The Study Co-Chairs, the Site Investigators and Site Coordinators will receive periodic reports regarding the quality and quantity of data submitted to the CSPCC. Other quality control measures include periodic reports containing participant recruitment information and relevant medical data for review by the Study Co-Chairs. The CSPCC will also prepare summary reports for the Study Co-Chairs, the Data Monitoring Committee, and other monitoring groups to track progress, and conduct final analyses of the study data.

Study reports will be generated using DataFax, SAS, and other tools (e.g., Microsoft Excel and Access). SAS and other statistical software packages will be used to conduct data analysis for

the study. The CSPCC is using SAS Version 9.4 in February 2016 and will upgrade to newer versions once they are purchased and validated.

Data Security

The DataFax system is fully compliant with US Federal regulations regarding electronic data capture systems established by the Food and Drug Administration under 21 CFR 11. Data entered directly into the database provides the official clinical record for data collection. Source documentation is handled in the same manner as a paper-based system. All paper-based records will be kept in locked file cabinets at the sites and Hines CSPCC. The servers housing the study databases will be located at a secure VA facility and housed behind the VA firewall on VA -owned and -maintained servers. The system will be monitored to ensure that all applicable VA regulations and directives are strictly followed.

Access to the study data is restricted by the CSPCC to properly-credentialed research staff who have completed required VA security trainings. Only CSP-approved individuals (such as: staff at the study site, CSPCC, Site Monitoring, Auditing and Resource Team (SMART) and CSP Clinical Research Pharmacy Coordinating Center (CSPCRPCC) will have access to the personal health information (PHI) of study participants.

Research data will only be stored on secure VA servers within the VA firewall (and not on desktops or on University affiliate servers). The data will be coded with a unique study identifier for each participant and stored using that study identifier. Identifiable information will be collected for participant tracking and safety purposes, and to collect health care usage data. Coded clinical data will be stored separately from the participant's name, contact information, and real SSN. The analytical database will not contain information that can directly identify the study participant (such as name, address, etc.); however, it will not be a completely de-identified database since age and study visit dates will be collected. The Hines CSPCC requires that a copy of the signed consent form and a participant contact sheet be on file at the Hines CSPCC. The consent form is required by CSP policy for the coordinating center to independently certify that all study participants have been consented. Because the Hines CSPCC is the final data repository for the study, participant contact sheets are collected in the event study participants need to be contacted (such as for safety notices) after study sites have completed the study. Consent forms and participant contact sheets are stored separately from

study data. Access to the cross-walk file linking the participant's identifiers and their study data will be restricted to the clinical site and to the study staff at the CSPCC.

In case of improper use or disclosure of study data, the facility's ISO and Privacy Officer, and the individual's direct supervisor will be notified immediately per VA Directive and Handbook 6500. Research data will be stored for a minimum of 5 years after the end of the study. Records will be destroyed in accordance with the VHA Records Control Schedule and VHA Handbook 1200.05.

Quality control checks and clinical monitoring will enable the CSPCC to examine the database and the clinical sites to ensure data have not been improperly used or accessed. Audit trails and access logs compliant with 21 CFR part 11 will be checked routinely, and clinical monitors will provide continuing education on GCP and check clinical site operations for violations of data security policies and best practices.

Proposed Data Collection Forms

Copies of the proposed data collection forms can be found in Appendix E. Forms will be finalized and field tested prior to the start of recruitment.

XIV. GOOD CLINICAL PRACTICES AND SITE REVIEW PLANS

This trial will be conducted in compliance with Good Clinical Practices (GCP) and Cooperative Studies Program Guidelines. The purpose of GCP is to safeguard subjects' welfare and ensure the validity of data resulting from the clinical research. VACSP will assist study personnel in complying with GCP requirements through its Site Monitoring, Auditing and Resource Team (SMART) based in Albuquerque, NM. SMART serves as the Quality Assurance arm of CSP for GCP compliance. SMART will provide training and guidance materials to assist study personnel in organizing study files and will be available throughout the trial to advise and assist Local Site Investigators regarding GCP issues.

Summary of Monitoring and Auditing Plans:

Monitoring Visits –

(1) Initiation visits will occur as soon as possible following the randomization of between the first and fourth participant at each site.

(2) Additional monitoring visits may be conducted as deemed necessary by study leadership or SMART based on an assessment of risk.

Audits

(1) Routine audits – independent site visits to one or more sites per year as determined by SMART.

(2) For-Cause audits –independent audit of a site as requested by study leadership or CSP Central Office.

Note: Audits may be announced or unannounced.

XV. BIOSTATISTICAL CONSIDERATIONS

Hypothesized Event Rates and Treatment Effect

For clarity, we define two outcome measures, with the singular difference underlined in bold, as follows:

Diarrhea Composite Outcome Measure (D-COM) – Primary Outcome Measure:

Symptom resolution (as defined in section II.B – Study Objectives and Outcome Measures) within the first 10 days of treatment (all groups) without any of the following (as assessed through day 59)

1. Diarrhea recurrence **(positive stool assay for *C. difficile not required***)
2. Other non-fatal clinical events including severe abdominal pain related to current diarrhea illness, toxic megacolon (where diarrhea ceases but is not a beneficial outcome), and colectomy
3. Death

CDI Composite Outcome Measure (CDI-COM): A Secondary Outcome Measure

Symptom resolution (as defined in section II.B – Study Objectives and Outcome Measures) within the first 10 days of treatment (all groups) without any of the following (as assessed through day 59)

1. Diarrhea recurrence (return of diarrhea **with a positive stool assay for toxigenic *C. difficile* *or its toxins***)
2. Other non-fatal clinical events including severe abdominal pain related to current diarrhea illness, toxic megacolon (where diarrhea ceases but is not a beneficial outcome), and colectomy
3. Death

As previously stated, D-COM is the primary outcome measure for this trial. See section II.C (Study Objectives and Outcome Measures) for justification of this choice. The assumed D-COM rates are 31% in the vancomycin group and 47% in both the fidaxomicin and vancomycin-taper groups. In order to arrive at these estimates, we first calculate CDI-COM rates using data from Cornely et al and McFarland et al [25, 29]. Then we convert the CDI-COM rates to D-COM rates using data from Garey et al [48].

A.1 CDI-COM Rate Estimates

The first publication by Cornely *et. al.*, consists of an analysis of a subgroup of participants from 2 phase III double-blind, randomized, controlled trials [29]. This subgroup of 178 patients (15% of the entire sample) consisted of those who had experienced a single prior episode within 3 months of the current CDI episode and were randomized in a 1:1 ratio to receive oral fidaxomicin (200 mg twice daily) or vancomycin (125 mg 4 times daily) for 10 days. From the information provided in the paper we determined the CDI-COM rates to be 44.4% in the vancomycin group and 60.2% in the fidaxomicin group (difference of 16% between groups) according to conservative intention-to-treat (ITT) principles (treat withdrawals as failures). In addition, using the same information but this time assuming that exclusions provide no information on response rate, we arrived at CDI-COM rates of 59.1% for the vancomycin group and 75.2% for the fidaxomicin group (difference of 16%).

The second publication from which our CDI-COM rate estimates for the vancomycin-taper and vancomycin groups are based is McFarland *et al* [25]. The paper describes an analysis of 163 participants combined from two double-blind placebo-controlled trials with recurrent *C. diff*. assigned various treatments. This publication provided information for estimates of CDI-COM rates for a vancomycin-taper regimen of 69.0% and also vancomycin group with the same regimen as proposed in CSP#596 of 45.8%.

In both trials presented, the primary outcome measure was clinical cure defined as:
"the resolution of diarrhea (i.e., three or fewer unformed stools for 2 consecutive days), with maintenance of resolution for the duration of therapy and no further requirement (in the investigator's opinion) for therapy for *C. difficile* infection as of the second day after the end of the course of therapy." [28]

While the primary outcome measure reported was clinical cure, CDI-COM rates can be

calculated from the figures reported in the paper. In a substratum of the entire sample, 178 participants who had experienced a single prior episode within 3 months of the current CDI episode were randomized to receive one of two treatments: 90 received vancomycin 125 mg QID and 88 received at least one dose of fidaxomicin 200 mg BID for a 10-day treatment period [29].

A.1.1 Estimation of the CDI-COM Rate in the Vancomycin-Placebo Group

We estimate the expected range for the CDI-COM rate in the vancomycin group for CSP#596 to be 44.4% to 59.1% according to Cornely *et al* [29]. The higher rate was obtained by not including clinical treatment failures and drop outs as failures. [29] If we count these as CDI-COM failures, the estimated CDI-COM rate in the vancomycin group is 44.4%. In the vancomycin group, there were 21 excluded from the analysis, 22 recurrences and 7 clinical failures for a total of 50 failures out of 90 participants leaving the proportion of globally cured 40/90 (44.4%).

If we assume the reason for exclusion does not depend on failure, we can estimate CDI-COM rate for the vancomycin group from the clinical cure probability and probability of recurrent CDI conditional on clinical cure. The estimated CDI-COM rate for the vancomycin group is 59.1%. Of the 90 participants that received vancomycin, 7 were excluded before the treatment ended leaving 83 eligible for clinical failure. Of the 83 eligible, 7 experienced clinical failures. Of the 76 remaining, 14 were excluded. Of the 62 remaining, 22 experienced recurrences. In the following equation, SCR is sustained clinical response, SR is symptom resolution and R is CDI recurrence.

$$(SCR) = (SR) - (R|SR) \times (SR)$$
$$= 76/83 - 22/62 \times (76/83) = 0.591$$

A.1.2 Estimation of the CDI-COM Rate in the Fidaxomicin group

We estimate the expected range for the CDI-COM rate in the fidaxomicin group for CSP#596 to be 60.2% to 75.2% according to Cornely *et al*. [29].

If we count exclusions and clinical failures as CDI-confirmed sustained clinical response failures, the estimated CDI-COM rate for the fidaxomicin group is 60.2%. Among 88 participants in the fidaxomicin group, there were 22 excluded from the analysis and 13

recurrences for a total of 35 failures. That leaves 53 responders. Thus, the CDI-COM rate in the fidaxomicin group is 53/88 (60.2%).

If we assume the reason for exclusion does not depend on the outcome we estimate the CDI-COM rate of the fidaxomicin group to be 75.2%. Of the 88 participants that received fidaxomicin, a total of 9 were excluded before the treatment ended leaving 79 eligible for clinical failure. Of the 79 eligible, 5 experienced clinical failures. Of the 74 remaining, 8 were excluded leaving 66. Of those 66 remaining 13 experienced recurrences.

$$(SCR) = (SR) - (R|SR) \times (SR)$$
$$= 74/79 - 13/66 \times (74/79) = 0.752$$

A.1.3 Estimation of the CDI-COM Rate in the Vancomycin-Taper Group

From McFarland *et al* [25], we estimate CDI-COM rate of the vancomycin-taper group to be 69.0%. Participants were classified as recurrent or cured if they did not experience a recurrence in the 2 months after treatment with antibiotics. Of the 163 participants, 29 received vancomycin followed by varying doses and lengths of vancomycin taper/pulse (mean days of taper 25.4 ± 13.3). Of those 29 participants, 9 had recurrence (31.0%) or a 69.0% CDI-COM rate. In addition, of 169 participants, 48 received low-dose vancomycin (125 mg four times daily) for 10 days and the CDI-COM rate was 22/48 (45.8%), further supporting our assumed 44% CDI-COM rate for our vancomycin group.

The fidaxomicin CDI-COM rate is estimated at 60.2%. This absolute difference is 15.8%. As previously mentioned, the CDI-COM rate for vancomycin-taper was 69.0% and low- dose vancomycin was 45.8% as reported by Mcfarland *et. al* [25], an absolute difference of 23.2%. Therefore, an absolute difference between both fidaxomicin and vancomycin- taper vs. vancomycin control of at least 16% is supported by the current evidence.

Additionally, the FDA reports that in antibiotic trials a 10-15% absolute difference non-inferiority margin is commonly used. Therefore, a difference of at least 16%, exceeding the non- inferiority margin, would likely be viewed by clinicians as a clinically significant difference.
(http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ UCM202140.pdf).

A.2 D-COM Rate Difference Estimate

We anticipate that recurrent diarrhea cases will be more frequent than CDI-confirmed cases. Garey et al. [48] reports the results of a pilot study on 73 participants comparing the rates of recurrent diarrhea in participants with CDI given rifaximin versus placebo immediately after standard therapy. Participants were followed for three months and assessed for recurrent diarrhea that included CDI recurrence (return of diarrhea with a positive toxin test) and patient self- reported return of non-CDI diarrhea after clinical cure. Failure of sustained clinical response with or without confirmation of CDI occurred in 19 of 37 (51%) participants given placebo and 10 of 36 (28%) participants given rifaximin, a difference of 23%. Failure of sustained clinical response with confirmation of CDI occurred in 13 of 37 (35%) participants given placebo and 8 of 36 (22%) participants given rifaximin, a difference of 10%. Five participants in the study failed to resolve within 14 days of standard antibiotics (rifaximin n=3; placebo n=2) were counted as failures in the above estimates, which are different from those reported in the paper.

For sample size estimation, we choose **47%** as our D-COM rate for both the fidaxomicin and vancomycin-taper treatment groups. The CDI-COM rate in the fidaxomicin group of Cornely *et al* [29] (after assuming that all exclusions and clinical failures would count as failures) is 60.2%. We arrive at 47% by averaging the rate ratios of D-COM and CDI-COM rates in both placebo and rifaximin groups reported in Garey et al. [30]. ([0.51/0.35 + 0.28/0.22]/2 = 1.36). We then estimated the recurrent diarrhea rate in the vancomycin control group to be the number of confirmed CDI recurrences increased by one third and the D-COM rate becomes **47%** (1-1.33*0.56=0.468). We assert that the absolute D-COM rate difference between the vancomycin control group and either the fidaxomicin or vancomycin-taper treatment groups will be at least **16%** because the expected difference for CDI-COM rates was estimated at **16%** and from Garey et al. the D-COM rate difference was larger, approximately 23% [48]. Therefore, we view **16%** as a conservative estimate. That leaves us with an estimate of D-COM rate for the vancomycin control group of **31%** (0.47-0.16=0.31).

Sample Size and Power

The hypotheses corresponding to the primary objective of this study are: (1) the sustained day 59 clinical response (D-COM) proportion in the fidaxomicin (FID-TX) group is different

from that of the vancomycin review the Informed Consent form with you in person (VAN-TX) group and (2) the sustained day 59 clinical response (D-COM) proportion in the vancomycin-taper and pulse (VAN-TP/P) group is different from that of the VAN-TX group. The sample size was calculated for testing both hypotheses. Here P_1, P_2, and P_0 are the proportion of day 59 sustained clinical response as defined in section II.B (Study Objectives and Outcome Measures), respectively, for VAN-TP/P, FID-TX and VAN-TX groups.

Hypothesis H_1: H_{10}: $\delta_1=P_1 - P_0 =0$ vs. H_{11}: $\delta_1 \neq 0$ (say $\delta_1=16\%$)

Hypothesis H_2: H_{20}: $\delta_2=P_2 - P_1=0$ vs. H_{21}: $\delta_2 \neq 0$ (say $\delta_2=16\%$)

The study was first designed with 549 participants to obtain 91% global power to detect a 16% absolute difference in composite outcome (D-COM) for at least one comparison (VAN-TP vs. VAN, FID vs. VAN). Due to the suspension of recruitment due to the COVID-19 pandemic and its impact on recruitment after the site resumed recruitment activities, the sample size of the study was reduced to 459, and the global power was reduced by 85%. The following assumptions were used in the determination of sample size for these primary hypotheses:

1. The D-COM rate at day 59 of VAN-TX control group is estimated to be **31**%.
2. The D-COM rate at day 59 of both the FID-TX and VAN-TP/P treatment groups are estimated to be **47%**, a **16%** absolute increase in the D-COM rate compared to the VAN-TX group.
3. The outcomes of toxic megacolon, severe abdominal pain related to current diarrhea illness, and colectomy are assumed to be rare (in the absence of diarrhea) and will not affect the sample size calculation.
4. Mortality may be higher in the control group than in either of the treatment groups. This is supported by Crook *et al*., a similar study of recurrence which reports 7 of 572 (1.2%) participants died in the FID-TX group and 17 of 592 (2.9%) participants died in the VAN-TX group [58].
5. The family-wise error rate (FWER) for both comparisons is controlled at 0.05 level (2-sided), which is the probability of falsely claiming a treatment difference when it is no difference (i.e., reject any H_{i0} where $\delta_i=0$, $i=1, 2$).
6. Global power (i.e., the probability of rejecting at least one H_{i0} (i.e., $\delta_i=0$) given H_{i1}) is

85%.

7. Sample size inflation due to two recommended interim looks (40% and 70% of participants who complete 59-day follow-up) for early efficacy by the DMC. Lan-DeMets O'Brien-Fleming α spending boundary function is used for efficacy boundary.

8. A modified intent-to-treat (mITT) analysis will be performed on all randomized participants according to the group they were randomized and who had at least one dose of treatment. We anticipate up to 3% of randomized participants will be excluded from this population. A corresponding 3% inflation of sample size is assumed.

9. Subjects withdrawal prior day 59 for unknown reasons or reasons unrelated to the study drug will be handled by multiple imputation. Other withdrawals (see section D.2.4 Analysis of Primary Outcome Measures) will be considered as failures to achieve sustained D-COM. Therefore, the sample size will not be further inflated with dropouts.

Recent released EAST (version 6.5) allows sample size and power calculation for a multi-arm multi-stage (MaMs) design that compares multiple active treatments to a common control for a binary outcome [Chapter 38 of East 6.5 user manual] utilizing a generalization of a single-step Dunnett's test [66, 67] with an unpooled variance. Based on EAST module MaMs, 459 participants (153 per group) will yield a 85% global power to detect a 16% absolute difference in sustained day-59 D-COM rate for at least one comparison (VAN-TP/P vs. VAN-TX or FID -TX vs. VAN-TX). The marginal probability of detecting 16% absolute difference in each comparison is 60% (via simulation). The trial stops if any treatment arm (VAN-TP/P or FID-TX) crosses efficacy boundary at either interim looks (i.e., 40% and 70% subjects are recruited). This marginal probability is increased to 73%. If the trial continues with the treatments which have not yet crossed the efficacy boundary. Given 3% subjects randomized will be excluded from the mITT analysis population, 459 subjects (153 per group) will be recruited to the study. The sample size has been adjusted for reasons listed in the preceding paragraphs. Powers at varying control response rate (31% to 43%) and treatment effect (16% to 22%) are given in Table 5.

Table 5. Power at Varying Control Response Rate and Treatment Effect
(2 interim looks when 40% and 70% information is obtained)

FWER (α) 2-sided	Control Group Sustained D-COM Rate	Treatment Group Sustained D-COM Rate by	$\delta_1=\delta_2$	Global Power [a]	N per Group (total for 3 groups)	
					No Inflation	Inflated with 3%
0.05	31%	47%	16%	85.2%	148 (444)	153 (459)
0.05	35%	51%	16%	84.8%	148 (444)	153 (459)
0.05	39%	55%	16%	84.8%	148 (444)	153 (459)
0.05	43%	59%	16%	85.3%	148 (444)	153 (459)
0.05	31%	49%	18%	92.5%	148 (444)	153 (459)
0.05	35%	53%	18%	92.2%	148 (444)	153 (459)
0.05	39%	57%	18%	92.3%	148 (444)	153 (459)
0.05	43%	61%	18%	92.7%	148 (444)	153 (459)
0.05	31%	51%	20%	96.7%	148 (444)	153 (459)
0.05	35%	55%	20%	96.6%	148 (444)	153 (459)
0.05	39%	59%	20%	96.7%	148 (444)	153 (459)
0.05	43%	63%	20%	96.9%	148 (444)	153 (459)
0.05	31%	53%	22%	98.8%	148 (444)	153 (459)
0.05	35%	57%	22%	98.7%	148 (444)	153 (459)
0.05	39%	61%	22%	98.8%	148 (444)	153 (459)
0.05	43%	65%	22%	98.9%	148 (444)	153 (459)

[1] Reject at least one hypothesis (H_{10} or H_{20} or both)

*** The highlighted information identifies the assumptions used for this study.**

Table 6 gives several powers of the study with the proposed efficacy boundary at different alternative hypotheses of treatment effect. Each result is based on 10,000 simulations with 444 subjects (148 per group) per simulation using East v6.5 and assumes two options: a) trial stops if any arm (VAN-TP and/or FID) crosses efficacy boundary, and b) trial continues with the remaining arms that do not enter efficacy region and only terminates the arm that crosses the efficacy boundary. Option b helps to detect a treatment with delayed effect.

For example, for option a, when the day 59 D-COM rates of both active treatments (VAN-TP, FID) are 16% higher than that of VAN control (Scenario 1.4), the study has an 86.5% probability (global power) of declaring any active treatment (VAN-TP, FID) to be efficacious (i.e., rejecting any null hypotheses of no treatment difference), approximately 60% probability of declaring an active treatment arm to be efficacious (P(1), P(2)). When the D-COM rate in one active treatment is 16% higher than VAN control, and the other one has no difference (Scenario 1.2), the study has 73% probability claiming the treatment with 16% difference (treatment 1) to be efficacious and 1.8% chance of falsely declaring treatment 2 different from control. The global power is 74% in this scenario. When both active treatments have no difference from VAN control (Scenario 1.1), the FWER is 5.1%.

For option b, when the day 59 D-COM rates of both active treatments (VAN-TP, FID) are higher than that of VAN control and in similar magnitudes (Scenarios 1.4, 1.6, 1.8), with the same parameter assumptions the probabilities of declaring an active treatment arm and both treatment arms to be efficacious than VAN control are higher than those in option a, but the expected sample sizes are increased. The global powers remain the same (Scenarios 2.4, 2.6, 2.8). For example (scenario 2.4), the study has an 86.5% probability of declaring any active treatment (VAN-TP or FID) to be efficacious (i.e., rejecting any null hypotheses of no treatment difference), approximately 73% probability of declaring an active treatment arm to be efficacious (compared to 60% in scenario 1.4), and a 59% probability of claiming both treatment arms to be efficacious (compared to 34% in scenario 1.4). The expected sample size is increased from 360 (scenario 1.4) to 394 (scenario 2.4). When the D-COM rate in arm 1 is much higher than arm 2 (or vice versa) and are both higher than VAN control (Scenarios 1.3, 1.5, 1.7), option b (Scenarios 2.3, 2.5, 2.7) improves the probability of detecting the active treatment with lower D-COM rate. The probability of detecting the active treatment with higher D-COM rate and the global power remain vastly unchanged.

Table 7 shows the probability of stopping one or both active treatment arms for efficacy at each interim analysis for a range of alternative hypothesis. Each result is based on 10,000 simulations (East v6.5). For example, for option a, when the D-COM rates of both treatments (VAN-TP, FID) are 16% higher than that of VAN control (Scenario 1.4), the chance of declaring one or both treatment arms to be efficacious than VAN is 7.8% at the very first look, 47.8% at the 2nd look and 30.9% at the final look, which yields 86.5% total probability. The marginal probability for each treatment arm (VAN-TP, FID) across efficacy boundary is about

4% at look 1, 33% at look 2 and 23% at final look. The marginal power for each treatment arm is 60-61%. For option b, with the same parameter assumptions (Scenario 1.4), the chance of declaring one or both treatment arms to be efficacious than VAN is similar to option a (i.e., 8% at the very first look, 48.4% at the 2nd look and 30.1% at the final look, which yields 86.5% total probability). The marginal probability for each treatment arm (VAN-TP, FID) across efficacy boundary is about 4.5% at look 1, 35% at look 2 and 33% at the final look.

Table 6. Power of the Study from 10,000 Simulations

(each simulation has 444 subjects, 2 interim looks at 40% and 70% and 1 final look, 2-sided test)

Sce-nario	Sustained D-COM Rate by Day 59 (P_0, P_1, P_2) [a]	P(1) [b]	P(2) [b]	P(1 only) [c]	P(2 only) [c]	P(1&2) [d]	P(none) [e]	Disjunctive Power: P(reject any H_{i0} where $\delta i \neq 0$)	Conjunctive power: P(reject both H_{i0} where $\delta i \neq 0$)	Global Power: P(reject any H_{i0})	FWER= P(reject any H_{i0} where $\delta i = 0$)	Expected Sample Size
a. Trial stops, if any treatment (VAN-TP or FID) crosses efficacy boundary												
1.1	(0.31, 0.31, 0.31)	2.81%	2.71%	2.4%	2.3%	0.41%	94.89%	-	-	5.11%	5.11%	442.6
1.2	(0.31, 0.47, 0.31)	73.05%	1.75%	72.28%	0.98%	0.77%	25.96%	73.05%	73.05%	74.03%	1.75%	386
1.3	(0.31, 0.47, 0.36)	72.32%	4.8%	68.54%	1.02%	3.78%	26.66%	73.34%	3.78%	73.34%	-	385.8
1.4	(0.31, 0.47, 0.47)	60.51%	60.01%	26.47%	25.97%	34.04%	13.52%	86.48%	34.04%	86.48%	-	360.1
1.5	(0.31, 0.51, 0.37)	89.66%	4.4%	85.79%	0.53%	3.87%	9.81%	90.19%	3.87%	90.19%	-	346.3
1.6	(0.31, 0.51, 0.51)	72.45%	72.66%	24.36%	24.57%	48.09%	2.98%	97.02%	48.09%	97.02%	-	319
1.7	(0.31, 0.55, 0.39)	97.36%	5.59%	92.07%	0.3%	5.29%	2.34%	97.66%	5.29%	97.66%	-	309.7
1.8	(0.31, 0.55, 0.55)	77.77%	77.78%	21.78%	21.79%	55.99%	0.44%	99.56%	55.99%	99.56%	-	280.3
b. Trial continues with treatments which have not crossed efficacy boundary												
2.1	(0.31, 0.31, 0.31)	2.65%	2.74%	2.25%	2.34%	0.4%	95.01%	-	-	4.99%	4.99%	443.3
2.2	(0.31, 0.47, 0.31)	73.52%	2.58%	71.97%	1.03%	1.55%	25.45%	73.52%	73.52%	74.55%	2.58%	423.9
2.3	(0.31, 0.47, 0.36)	73.28%	10%	63.91%	0.63%	9.37%	26.09%	73.91%	9.37%	73.91%	-	422
2.4	(0.31, 0.47, 0.47)	72.68%	73.02%	13.43%	13.77%	59.25%	13.55%	86.45%	59.25%	86.45%	-	394.3
2.5	(0.31, 0.51, 0.37)	90.02%	12.46%	77.61%	0.05%	12.41%	9.93%	90.07%	12.41%	90.07%	-	408.4
2.6	(0.31, 0.51, 0.51)	89.85%	89.86%	6.94%	6.95%	82.91%	3.2%	96.8%	82.91%	96.8%	-	357.8
2.7	(0.31, 0.55, 0.39)	97.62%	22.08%	75.6%	0.06%	22.02%	2.32%	97.68%	22.02%	97.68%	-	391.4
2.8	(0.31, 0.55, 0.55)	97.7%	97.58%	2.11%	1.99%	95.59%	0.31%	99.69%	95.59%	99.69%	-	318.5

[a] Sustained day 59 D-COM rate for active control group (VAN-TX) is P_0; for treatment groups (VAN-TP/P or FID-TX) are P_1 and P_2.
[b] P(*i*), *i*=1 or 2, is the probability that treatment *i* (VAN-TP/P or FID-TX) is declared significantly different to control (VAN-TX) (i.e., cross efficacy boundary)
[c] P(*i* only), *i*=1 or 2, is the probability that treatment *i* only is declared significantly different to control (VAN-TX).
[d] P(1&2) is the probability that both treatments (VAN-TP/P, FID-TX) are declared significantly different to control (VAN-TX).
[e] P (none) is the probability that neither VAN-TP/P nor FID-TX are declared significantly different to control (VAN-TX).

Note: The disjunctive power and conjunctive power for scenario 2 are based on the comparison of P_1 vs. P_0 where $\delta_1 \neq 0$. The comparison of P_2 vs. P_0 under null yields type I error rate.

P(1 only) + P(2 only) + P(1&2) + P(none) =100%; and P(1 only) + P(2 only) + P(1&2) = Global power.

*The highlighted information identifies the assumptions used for this study.

Table 7. Probability of Trial/Arm Termination at Each Look from 10,000 Simulations

Scenario	Sustained D-COM Rate by Day 59 (P_0, P_1, P_2) [a]	Look	Info. Fraction (n/n max)	P(1) [b]	P(2) [b]	Probability (Incremental) one or two Active arms Claiming Efficacy
a. Trial stops, if any treatment (VAN-TP or FID) crosses efficacy boundary						
1.1	(0.31, 0.31,0.31)	1	0.40	0.06%	0.08%	0.14%
		2	0.70	0.78%	0.64%	1.37%
		3 (Final)	1	1.97%	1.99%	3.6%
			Total	2.81%	2.71%	5.11%
1.2	(0.31, 0.47, 0.31)	1	0.40	4.56%	0.07%	4.6%
		2	0.70	34.55%	0.82%	34.98%
		3 (Final)	1	33.94%	0.88%	34.47%
			Total	73.05%	1.77%	74.05%
1.3	(0.31, 0.47, 0.36)	1	0.40	4.43%	0.23%	4.55%
		2	0.70	34.39%	2.22%	34.88%
		3 (Final)	1	33.5%	2.35%	33.91%
			Total	72.32%	4.8%	73.34%
1.4	(0.31, 0.47, 0.47)	1	0.40	4.37%	4.5%	7.81%
		2	0.70	32.92%	32.91%	47.76%
		3 (Final)	1	23.22%	22.6%	30.91%
			Total	60.51%	60.01%	86.48%
1.5	(0.31, 0.51, 0.37)	1	0.40	10.62%	0.24%	10.73%
		2	0.70	52.02%	2.67%	52.3%
		3 (Final)	1	27.02%	1.49%	27.16%
			Total	89.66%	4.4%	90.19%
1.6	(0.31, 0.51, 0.51)	1	0.40	10.06%	9.81%	16.5%
		2	0.70	47.12%	47.39%	61.34%
		3 (Final)	1	15.27%	15.46%	19.18%
			Total	72.45%	72.66%	97.02%
1.7	(0.31, 0.55, 0.39)	1	0.40	20.03%	0.44%	20.15%
		2	0.70	60.83%	3.97%	60.98%
		3 (Final)	1	16.5%	1.18%	16.53%
			Total	97.36%	5.59%	97.66%
1.8	(0.31, 0.55, 0.55)	1	0.40	19.72%	20.32%	31.78%
		2	0.70	50.94%	50.28%	59.7%
		3 (Final)	1	7.11%	7.18%	8.08%
			Total	77.77%	77.78%	99.56%
b. Trial continues with treatments which have not crossed efficacy boundary						
2.1	(0.31, 0.31,0.31)	1	0.40	0.01%	0.06%	0.07%
		2	0.70	0.73%	0.72%	1.37%
		3 (Final)	1	1.91%	1.96%	3.55%

			Total	2.65%	2.74%	4.99%
2.2	(0.31, 0.47, 0.31)	1	0.40	4.43%	0.04%	4.46%
		2	0.70	35.64%	0.73%	36.08%
		3 (Final)	1	33.45%	1.81%	34.01%
			Total	73.52%	2.58%	74.55%
2.3	(0.31, 0.47, 0.36)	1	0.40	4.27%	0.19%	4.41%
		2	0.70	35.91%	2.76%	36.36%
		3 (Final)	1	33.1%	7.05%	33.14%
			Total	73.28%	10%	73.91%
2.4	(0.31, 0.47, 0.47)	1	0.40	4.57%	4.49%	7.97%
		2	0.70	34.81%	35.55%	48.35%
		3 (Final)	1	33.3%	32.98%	30.13%
			Total	72.68%	73.02%	86.45%
2.5	(0.31, 0.51, 0.37)	1	0.40	10.21%	0.31%	10.3%
		2	0.70	51.99%	3.62%	52.09%
		3 (Final)	1	27.82%	8.53%	27.68%
			Total	90.02%	12.46%	90.07%
2.6	(0.31, 0.51, 0.51)	1	0.40	10.42%	10.34%	17.33%
		2	0.70	51.76%	52.17%	60.92%
		3 (Final)	1	27.67%	27.35%	18.55%
			Total	89.85%	89.86%	96.8%
2.7	(0.31, 0.55, 0.39)	1	0.40	20.6%	0.53%	20.69%
		2	0.70	61.11%	7.43%	61.18%
		3 (Final)	1	15.91%	14.12%	15.81%
			Total	97.62%	22.08%	97.68%
2.8	(0.31, 0.55, 0.55)	1	0.40	19.8%	20.3%	31.53%
		2	0.70	61.95%	61.87%	60.83%
		3 (Final)	1	15.95%	15.41%	7.33%
			Total	97.7%	97.58%	99.69%

Note: The trial continues with remaining arms if any arm enters efficacy region.

*__The highlighted information identifies the assumptions used for this study.__

Duration of Study and Number of Participating Sites

Study launched its pilot phase in year 2016 with an original goal of projected recruitment rate, 7 participants (on average) per year per site, from six pilot sites for **42** participants over one year of operation. The pilot phase was then extended for 6 additional months to establish more reliable and realistic recruitment rate.

Following a feasibility assessment, the study set a more realistic recruitment goal of **6** participants (on average) per site per year for a site that primarily recruits from the main

hospital, and **9** participants (on average) per site per year for a site that partners with independent VAMCs (Independent VAMCs LSI Applications will be reviewed and approved by Central IRB) close in distance to allow a shared site coordinator (WOC appointed) at an increased funding level. Given revised anticipated recruitment rate and in consideration of sites' variability on start-up dates, the recruitment timeline was set to be 6 years, including 2 years of pilot phase with 6 sites plus transition period from pilot phase to full study, and 4 years of full study with 26 sites (24 units). *Note that given Gainesville and Hines each recruiting from another VA location, we are referencing the dual locations as one unit. e.g., Gainesville and Lake City VA locations are considered one unit*. Due to the suspension of recruitment by the COVID-19 pandemic in mid-March 2020 and its significant impact on the slow recruitment after the sites resumed recruitment activities, as well as the termination of 6 underperformed sites and the startup of replacement sites, the sample size of the study is reduced to 459. The study set a new recruitment goal of 4 participants (on average) per site per year for a site that primarily recruits from the main hospital, and 6 participants (on average) per site per year for a site that partners with independent VAMCs. The recruitment timeline is extended with an intention of funding a full 31-month extension of study recruitment till August 31, 2024.

Statistical Analysis

D.1 Interim Monitoring and Analysis

Interim monitoring will focus on efficacy and safety of the study.

D.1.1 Interim Analysis for Potential Early Study Termination for Efficacy

Two interim looks at the primary endpoint will be proposed to the DMC for making the recommendation about whether or not to continue the trial or to stop one or more of the groups for early efficacy. No futility boundary is proposed for the trial. The first interim analysis already conducted when 1/3 participants (mITT population) completed at least 59 days of follow-up under the original sample size is rescaled accordingly to 40% of the reduced sample size (n=177, Table 8), and the second interim will occur when 70% of participants are randomized (n= 312 subjects in the mITT population) and completed at least 59 days follow-up. Based on the interim analysis results and the recruitment status, DMC will make recommendation if the trial stops or trial continues with remaining arm not crossing efficacy boundary. In the latter situation, the unused sample size due to dropping an arm will not be

reallocated to the remaining arms. In order to preserve Type-I error, proposed O'Brien-Fleming stopping boundary *p*-values and Z-scales are given in Table 8.

Table 8 Stopping Boundary Values at Interim and Final Looks

(P_0 =0.31, P_1= P_2=0.47, global power=0.85, α=0.05, 2-sided)

Look #	Sample Size [a] (n)	Information Fraction	Cumulative α Spent [b]	Efficacy Boundary [c]			Boundary Crossing probability (Incremental)	
				Critical p-value	Z scale			
					Lower	Upper	Under H0	Under H1
1	177	0.40	0.0008	0.0004	-3.546	3.546	0.001	0.054
2	312	0.70	0.015	0.0077	-2.667	2.667	0.014	0.460
3 (Final Analysis)	444	1.00	0.05	0.0242	-2.253	2.253	0.035	0.337
						Total	0.05 (α)	0.85 (Global Power)

[a] Sample size is based on 444 subjects included in the mITT population. The 1st and 2nd interim analysis will be conducted, respectively, when 177 and 312 subjects in the mITT population complete day 59 follow-up. Dropout prior day 59 will be handled using MI method except for scenarios defined in the section of "Analysis of Primary Outcome Measures". Same efficacy boundary will be used when missing outcome is imputed.

[b] α = Family-wise error rate (2-sided).

[c] If the allocation to the three arms is unequal at one or more interim looks, boundary at each look will be recomputed using the average allocation to each arm as an approximation.

D.1.2 Safety Reporting Summaries

Due to slower than anticipated recruitment, and given both study drugs

(vancomycin and fidaxomicin) have been on the commercial market for years, are generally well tolerated, and are being used consistent with FDA approved indications, a safety progress report will be submitted to the Data Monitoring Committee (DMC) annually during the enrollment and participant follow up period, or more frequently if requested by the DMC. SAEs will be summarized by treatment group, frequency, severity, organ system and preferred term (based on MedDRA coding) and relatedness to the assigned study

medications in a closed session meeting. Non-serious AEs that are determined to be intervention related will be summarized in a similar way.

The proportion of participants experiencing an intervention related SAE in each treatment group will be calculated and the proportions will be compared using a chi-square test for the difference in proportions. An SAE rate higher than expected as determined by the DMC may also lead the DMC to recommend that recruitment in the trial, or any one arm of the trial, be stopped, or that the dosing protocol be modified.

In addition to the DMC safety progress reports, Hines CSPCC will produce safety reports regularly (at least every two months) during the enrollment and participants follow-up periods. This safety report will be reviewed by the study Safety Management Team and will include a cumulative count of study drug related AEs and all SAEs (tabulated by severity grade and relatedness to study drug) and a cumulative listing of the events for all treatment groups combined. Any detected safety signals will be reported to the Study Chairs, and if the signals are worthy of the DMC's attention, they will be further communicated to the DMC.

D.2 Final analysis

D.2.1 Analysis Population Modified Intent-to-Treat (mITT) and Per Protocol (PP)

The modified intent-to-treat (mITT) population will consist of all randomized participants who received at least one dose of study treatment medication and met study inclusion criteria. Participants will be analyzed according to their randomized study medication. This population will be considered the primary analysis population for analyses on efficacy outcomes unless otherwise specified. Per protocol (PP) analysis population will consist of subgroup of mITT participants who both (1) completed day 59 follow-up or are transferred to primary care physician prior to day 59 due to fail in achieving sustained day 59 D-COM and (2) took 80% of their assigned drug according to the pill count. This population will be mainly used for secondary analysis of the primary composite outcome as well as the non-inferiority analysis of VAN-TP/P vs. FID-TX. Non-inferiority analysis will also be repeated on mITT population. For all safety outcomes subjects who took at least one dose of study drug will be analyzed based on the actual treatment they received.

All statistical tests will be 2-sided. The only exception will be the 1-sided non-inferiority test on VAN-TP/P vs. FID-TX if both VAN-TP/P and FID-TX are superior to VAN-TX.

D.2.2 Baseline Comparability

The distribution of baseline participant characteristics between the randomization groups will be evaluated using descriptive statistics, confidence intervals and graphical methods.

D.2.3 Description of Participants Screened

For all screened participants, the frequency of reasons that participants with a diagnosed first or second recurrent CDI infection were not eligible, or if eligible, elected not to consent or enroll will be summarized.

D.2.4 Analysis of Primary Outcome Measures

The primary outcome measure of sustained clinical response at day 59 will be analyzed using Z-statistic for equality of proportions for each comparison based on the mITT population.

Hypothesis 1: H_{10}: $P_1 - P_0 = 0$ vs. H_{11}: $P_1 - P_0 \neq 0$
Hypothesis 2: H_{20}: $P_2 - P_0 = 0$ vs. H_{21}: $P_2 - P_0 \neq 0$

Z-statistic comparing the *i*th (*i*=1,2) treatment arm (VAN-TP/P, FID-TX) with the control (VAN-TX) at the *j*th look (*j*=1, 2 and 3):

$$Z_{ij} = \frac{\hat{P}_{ij} - \hat{P}_{0j}}{\sqrt{\frac{\hat{P}_{ij}(1-\hat{P}_{ij})}{n_{ij}} + \frac{\hat{P}_{0j}(1-\hat{P}_{0j})}{n_{0j}}}}$$, where $\hat{P}_{ij}$ and $\hat{P}_{0j}$ are respectively the sample proportions for treatment *i* and control group from data collected up to the *j*th look.

In comparison of proportion of sustained D-COM in the VAN-TP/P group or FID-TX group to that of the VAN-TX group, the observed Z-statistic for each comparison will be compared to the efficacy boundary in Z scale ±3.546, ±2.667, and ±2.253, respectively, at looks 1, 2 and 3 (final look) for equal allocation. The difference in proportions for each comparison and associated repeated 95% confidence interval will be reported at each look.

Repeated 95% for δ_i at look *j* (*i*=1, 2; *j*=1, 2 and 3):

$$\hat{P}_{ij} - \hat{P}_{0j} \pm c_j \sqrt{\frac{\hat{P}_{ij}(1-\hat{P}_{ij})}{n_{ij}} + \frac{\hat{P}_{0j}(1-\hat{P}_{0j})}{n_{0j}}}$$ where δ_i is the treatment difference between treatment *i* (VAN-TP/P, FID-TX) and control (VAN-TX), and c_j is the efficacy boundary on the Z-scale (i.e. c_1=3.546, c_2=2.667, c_3=2.253).

Possible ways the trial or an arm may be stopped early are listed below.

- Both hypotheses (H_{10}, H_{20}) are rejected at look 1 (|Z statistic| > 3.546)

  → **Recommend stopping trial at look 1**

- Both hypotheses (H_{10}, H_{20}) are rejected at look 2 (|Z statistic| > 2.667)

  → **Recommend stopping trial at look 2**

- One hypothesis is rejected at look 1 (|Z statistic| > 3.546) or at look 2 (|Z statistic| > 2.667)

  → **Based on the interim analysis results and the recruitment status, DMC will make recommendation if trial stops or trial continues with remaining arm not crossing efficacy boundary.**

  Since VAN-TP/P and FID-TX are usually more effective than VAN-TX to prevent recurrence of CDI in established literatures, therefore it is unlikely that observed Z statistic from either hypothesis will be less than the lower boundary.

Participants failed to achieve symptom resolution by day 10 or dropouts prior to day 59 due to study-drug related adverse events or because they felt the study drug was ineffective and their symptoms were not improved compared to baseline are considered as failures to achieve sustained day 59 D-COM. Participants who achieved symptom resolution by day 10 but subsequently had CDI symptoms to warrant clinical determination of CDI and were withdrawn from the study for re-treatment for CDI although no 2 consecutive days of ≥4 diarrhea stools are considered as failures for day 59 D-COM. Participants who terminated prior to day 59 for unknown reasons or reasons unrelated to the study treatment (about 10%) will be handled by multiple imputations. Those missing responses at day 59 will have their values imputed from an imputation model which will include baseline covariates that are known risk factors for recurrence of CDI (such as age, prior episode of CDI, baseline CDI severity measured by ATLAS score, baseline comorbid condition measured by modified Horn's index score). Covariates that causes nonconvergence in the MI model will be removed from the imputation model. Multiple imputations will be implemented in SAS PROC MI. We will create n=20 imputed datasets [69] for analysis and combine the results with PROC MIANALYZE.

In addition, a sensitivity analysis will be performed, one assuming all participants with

previously imputed values are non-responders. Sensitivity analysis on completers will also be explored.

D.2.5 Secondary Analyses of Primary Outcome Measure

D.2.5.1 Analysis of Primary Outcome Measure at 28 Days post End of Therapy and at 90 Days post Randomization

Dunnett's test will be performed to compare each of the FID-TX and VAN-TP/P groups to VAN-TX group at FWER=0.05 level. This is consistent with the reporting of primary outcome but without interim looks. This can be handled, for example, using R package 'MCPAN' for multiple comparisons based on normal approximation and extensions (i.e., function 'binomRDci' provides simultaneous CI of proportion differences based on Dunnett adjustment, function 'binomRDtest provides associated p-values).

D.2.5.2 Per Protocol Analysis of Primary Outcome Measure

The same analysis outlined above will be repeated for per protocol (PP) analysis population. Since the PP population includes only the subjects who took 80% of their assigned study drug and also their day 59 D-COM status (yes or no) are known, there will be no missing data involved. The α spent at the interim looks will be based on the information fractions spent at the interim looks. At the final look, the information fraction is set at 1.

D.2.5.3 Vancomycin-Taper vs. Fidaxomicin Non-inferiority

If both the FID-TX and VAN-TP/P vs. VAN-TX comparisons in section D.2.4 are significant (above upper efficacy boundary), we will perform a non-inferiority test with minimal loss of power as per Proschan [60]. The test will be a one-sided Z-test of non-inferiority of proportions of sustained clinical response at day 59 with the hypothesis that VAN-TP/P is non-inferior to FID-TX by a margin of 10% at the 0.05 significance level. The analysis will be performed for both PP and mITT populations. The conclusions from mITT analyses will be considered as the primary one. PP analyses will be additionally as a secondary analysis. Based on 296 subjects whose day 59 D-COM status is known, the calculated power is 53%, assuming both treatments have a 47% response rate and FID-TX and VAN-TP/P continue to the final look (PASS v16). Report the test statistic, *p*-value, cell frequencies, estimated proportion difference and one-sided 95% lower confidence interval (equivalent to a 90% two-sided CI) of the difference in response rates. If the one-sided 95% lower confidence limit for $P_{VAN\text{-}TP/P} - P_{FID\text{-}TX}$ lies within (-10%, + ∞), non-inferiority of VAN-TP/P to FID-TX is achieved based on 5% significance level.

D.2.5.4 Subgroup Analysis of Primary Outcome Measure

Primary outcome will also be analyzed for the subgroups listed below. Those are exploratory analyses and no multiplicity adjustments are considered for multiple subgroups. Also, no interim analysis will be conducted for subgroup analysis.

BI/NAP1/027 strain positive (yes, no)
2 prior CDI recurrences or 1 prior recurrence
Participant received concomitant antibiotics during study (yes, no)

Treatment by subgroup interactions will be assessed using logistic regression model with sustained clinical response as the dependent variable, treatments, subgroup indicator, and treatments by subgroup interactions as independent variables. When subgroup results are reported, for each subgroup, report cell frequencies, estimated proportion difference and 95% confidence interval in primary outcome between each treatment group and VAN-TX under Dunnett's adjustment for multiple comparisons.

Estimate the overall sustained clinical response rate by ATLAS score and group-wise response by ATLAS score, along with cell frequencies. Perform a two-sided Cochran-Armitage test for trend of the 3x11 contingency table with treatment as rows and ATLAS score as columns. Report the cell frequencies, test statistic and *p*-values. If the two-sided test for trend is significant at the 0.05 level, fit a logistic regression model with sustained clinical response as the dependent variable and the following independent variables:

(1)Treatment (Three level class variable: 1=VAN-TX (reference), 2=FID-TX, 3=VAN-TP/P).

(2) ATLAS score (range 0-10) as a continuous variable.
(3) Treatment * ATLAS interaction

Additionally, if the Cochrane-Armitage test for trend is not significant, a logistic regression analysis with ATLAS treated as a categorical class variable will be performed following the procedure outlined above.

Estimate the overall sustained clinical response rate 59 days post randomization by modified Horn's Index and group-wise response by modified Horn's Index, along with cell frequencies.

Perform a two-sided Cochran-Armitage test for trend of the 3x4 contingency table with treatment as rows and modified Horn's Index as columns. Report the cell frequencies, test statistic and *p*-value. If the two-sided test for trend is significant at the 0.05 level, fit a logistic regression model with sustained clinical response as the dependent variable and the following independent variables:

(1)Treatment (Three level class variable: 1=VAN-TX (reference), 2=FID-TX, 3=VAN-TP/P).

(2) Modified Horn's Index (range 0-3) as a continuous variable.

(3) Treatment * modified Horn's Index

Additionally, if the Cochrane-Armitage test for trend is not significant, fit a logistic regression model with modified Horn's Index treated as a categorical class variable will be performed following the procedure outlined above.

D.2.5.5 Assessing the Influence of Predictors with Logistic Regression on Primary Outcome

In order to determine the effect of predictors on sustained clinical response at day 59, fit one logistic regression model with multiple dependent variables. The model will include binary independent variables:

- Treatment (Three level class variable: 1=VAN-TX (reference), 2=FID-TX 3=VAN-TP/P).
- strain (1=BI/NAP1/027 strain positive, 0=otherwise)
- treatment*strain interaction
- prior CDI (1=2 prior CDI recurrences, 0 = otherwise)
- treatment *prior CDI interaction
- concom (1=patient received concomitant antibiotics during study, 0=otherwise)
- treatment *concom

In the first step model, Wald Chi-square tests will be performed on each independent variable and if the tests of the interaction terms are non-significant at the 0.05 level those terms will be dropped from the model and the model will be refitted in the second step. In the second step,

report all for individual predictors and intercept β, SE β, Wald's χ^2, df, unadjusted *p*-value, simulation-adjusted p-values.

Analysis of Secondary Outcome Measures (D.2.6 – D.2.12)

No interim analysis will be conducted for all secondary outcomes.

D.2.6 CDI-COM (28 days post end of the therapy, day 59, day 90)

Response rates in CDI-COM share very similar nature as the corresponding response rates in D-COM. Therefore CDI-COM rates will be analyzed in the same way as D-COM rates outlined in section **D.2.5.1.**

D.2.7 Proportion of Symptom Resolution

Using all study participants, perform Dunnett's test to compare proportions of symptom resolution response as defined in section VI.C between each of the FID-TX and VAN-TP/P groups vs. the VAN-TX group and report the test statistic, *p*-value, cell frequencies, estimated proportion difference and 95% confidence interval.

D.2.8 Proportion of CDI Recurrence Following Symptom Resolution

Including only study participants who experience symptom resolution, perform Dunnett's test to compare proportions of diarrhea recurrence with assay confirmation between each of the FID-TX and VAN-TP/P groups vs. the VAN-TX group and report the p-value and cell frequencies, estimated proportion difference and 95% confidence interval. Additionally, time to CDI recurrence in days will be compared among treatment groups using log-rank test. The survivor function will be estimated using Kaplan-Meier (KM) estimator.

D.2.9 Proportion of Diarrhea Recurrence

Proportion of diarrhea recurrence following symptom resolution will be analyzed in a similar way as CDI recurrence outlined above.

D.2.10. Patient Reported Outcome

Repeated measures model will be used to analyze the changes in the total CDiff32-QOL score and 3 sub-scale scores (physical: P-QOL, emotional/psychological: E-QOL, social: S-QOL) from baseline (day 0) to follow up visits (days 10 and 59), including treatment groups (3-level

class variable: VAN-TX - reference group), time (3-level class variable: day 0- reference category), and interactions of treatment group and time. Interaction terms between treatment and time will be assessed to determine if there are significant differences in mean change scores between VAN-TP/P and VAN-TX, and between FID-TX and VAN-TX. The estimates of the differences in mean changes (days 10 or 59 to day 0) between groups will be obtained by specifying appropriate contrasts for the model. Other potential baseline predictors like age, Horn's Index Score, ATLAS Score and number of prior CDI episode prior enrollment will also be tested in the repeated measures model by likelihood ratio test. Estimated difference in mean changes and 95% simultaneous CI for comparison of VAN-TP/P (or FID-TX) vs. VAN-TX under Dunnett's adjustment for multiplicity will be provided at each follow-up timepoint (days 10 and 59). No multiplicity adjustment for multiple HRQOL measures (CDiff32-QOL, P-QOL, M-QOL, S-QOL) and a HRQOL measure on multiple timepoints (days 10 and 59).

Participants who fail to meet sustained D-COM (i.e., treatment failure within first 10 days, or recurrent diarrhea/complications requiring re-treatment for CDI, or die) prior to day 59 will result in discontinuation of the treatment regimen and/or discontinuation from the study, therefore HRQOL scores are not measurable after patients' discontinuation. To account for informative censoring of longitudinal HRQOLs upon disease progression (non-ignorable dropout), a sensitivity analysis will utilize a shared parameter model [70, 71] that jointly model the longitudinal HRQOLs, and the time from randomization to fail in achieving sustained D-COM that requires re-treatment for CDI (event occurred) or dropout due to other reasons (event censored) whichever comes first. Longitudinal portion of the joint model will include treatment groups (3-level class variable: VAN-TX - reference group), time (3-level class variable: day 0- reference category), interactions of treatment group and time, aforementioned potential baseline predictors, and a random subject intercept effect. Survival portion of the joint model will consider a semi-paramedic cox proportional hazard model. Parametric survival model (Weibull) will also be explored. Covariates included in the survival model are treatment groups, baseline risk factors of CDI recurrence, a random subject intercept effect, and a common association parameter between longitudinal and survival through the random intercept. Model comparison will use likelihood ratio test. Additional sensitivity analysis using Pattern Mixture Model [72] that assumes Missing not at Random (MNAR) will also be explored. For example, patterns could be as simple as a group of day 59 completers vs. a

group of dropouts (i.e., not measured at the final HRQOL assessment timepoint day 59). In this case, covariates in the pattern-mixture model could include treatment groups, time, interactions of treatment group and time, dropout indicator, interactions of dropout indicator and time, interactions of dropout indicator and treatment, 3-way interactions of dropout indicator and time and treatment. The 3-way interactions indicate whether any differential change across time for VAN-TP/P (or FID-TX) relative to VAN-TX varies between dropouts and completers. The estimates from each pattern are then combined using weights based on the proportion of individuals in each pattern. Aforementioned potential baseline predictors will also be tested. Model comparison will use likelihood ratio test.

Participants in the pilot phase that were not measured by CDiff32 HRQOL questionnaire will be excluded from the analysis.

D.2.11. Analysis of Safety Data

The p-values for safety data analyses will not be adjusted for multiple comparisons. As is typical for randomized clinical trials, the study is underpowered for events that are expected to occur at low-frequency and the p-values provided will be considered by the DMC as one component of assessing safety along with examination of actual event rates that rise to a level that the DMC would consider an elevated risk.

D.2.11.1 SAE and AE Data

Calculate the proportion of participants experiencing an SAE for each treatment group. Perform a Pearson's chi-square test for the difference in proportions to compare treatment groups over all SAE and by MedDRA system group. Compare by treatment groups: the number of participants experiencing an SAE, the number if treatment-related SAE, and the number of SAE by MedDRA system group. Do the same for related AEs.

D.2.11.2 Toxicity and Kidney Function

Compare the proportion of participants who experienced an absolute increase of 0.3 or more of serum creatinine or a > 1.5 times relative increase the level recorded at baseline at any time point between treatment group using Pearson's chi-squared test of a 3 by 2 contingency table. If the *p*-value is significant at the alpha=0.05 level, perform pair-wise comparisons using Pearson's chi-squared tests (or Fisher's exact test if cell count is not sufficient), and report *p*-

values, cell frequencies, estimated difference in proportion and associated 95% confidence intervals. The p-values will not be adjusted for multiple comparisons.

D.2.11.3 Continuous Laboratory Values

Calculate the changes in laboratory values (CBC and serum chemistry panel to include Cr, AST, ALT, alkaline phosphatase, albumin, total bilirubin) from baseline at days 10, and 31. Report the descriptive statistics of the laboratory values at days 0, 10, and 31for each treatment group. Report the descriptive statistics of the change from baseline at days 10, and 31. Compare the changes from baseline at days 10, and 31 between the three treatment groups using longitudinal analysis (covariance pattern model with unstructured covariance matrix). The dependent variable is safety measure at days 0, 10 and 31. The covariates include treatment (3 level class variable: VAN-TX - reference group), 2), time (3 level class variable: day 0- reference category), and treatment by time interactions. The estimate of mean change for each group is derived from the model parameter estimates. The p-value for overall treatment comparisons in change score from day 0 to day 10 (day 31) is the simultaneous testing of treatment by day 10 (day 31) interaction from likelihood ratio test with 2 degrees of freedom; the p-value for VAN-TP/P vs. VAN-TX comparison at day 10 (day 31) is the testing of treatment (VAN-TP/P vs. VAN-TX) by day 10 (day 31) interaction. The p-value for FID-TX vs. VAN-TX comparison at day 10 (day 31) is the testing of treatment (FID-TX vs. VAN-TX) by day 10 (day 31) interaction. No multiplicity adjustment will be made.

D.2.11.4 Discontinuation of Treatment Due to Adverse Events

Compare the proportion of participants who discontinue treatment due to lack of tolerance of the drug between treatment groups using Pearson's chi-squared test of a 3 by 2 contingency table. If the *p*-value is significant at the alpha=0.05 level, perform pair-wise comparisons using Pearson's chi-squared tests (or Fisher's exact test if cell count is not sufficient), and report the *p*-values, cell frequencies, estimated difference in proportion and associated 95% confidence intervals. No multiplicity adjustment will be made.

D.2.12 Analysis of Adherence Data

Estimate proportion of adherent participants overall and in each group, defined as having taken more than 80% of study medication. Compare the treatment groups using a 3x2 Pearson's chi-

square test of equality of proportions of adherent participants in each of the three groups: VAN-TP/P, FID-TX and VAN-TX. If the *p*-value is significant at 0.05 level, make pairwise comparisons using Pearson's chi-square tests, and report all test statistics, *p*-values, cell frequencies, estimated proportion difference and 95% confidence intervals. No multiplicity adjustment will be made.

XVI. FEASIBILITY WITHIN THE VA SYSTEM

Estimates of participant Availability

The study launched a pilot phase to access the recruitment feasibility prior expanding to full scale. We observed that the original goal of recruiting 7 participants per site per year proposed for the pilot phase is overestimated. The low recruitment was due to several reasons: 1) a lower CDI recurrence rate was observed during the pilot phase than the original estimate of 30%. Thus, fewer recurrent CDI cases were available to recruit; and 2) patients with diarrhea symptoms were treated empirically by physicians not involved in the study with antibiotics for CDI without ordering a stool test.

Following the feasibility assessment, we set a more realistic recruitment goal of 6 participants (on average) per year from main hospital, and 9 participants (on average) per year if site could partner with nearby VA facilities within a station and/or across multiple stations to recruit for the full phase. The recruitment timeline was set to be 6 years, including 2 years of pilot phase with 6 sites plus transition period from pilot phase to full study, and 4 years of full study with 26 sites (24 units) to recruit 549 veteran participants as its originally planned sample size. The recruitment timeline was based on 6 sites in pilot phase and 24 units (26 sites) in the full phase. It is conservatively estimated that 20% of participants diagnosed with recurrent CDI who are screened for the study will be randomized. Therefore, an ideal site recruiting from main hospital would need at least 25 cases annually of recurrent CDI, and an ideal site recruiting from multiple facilities would need 40 recurrent CDI cases annually from which to recruit.

However, due to the suspension of recruitment by the COVID-19 pandemic in mid-March 2020 and its significant impact on the slow recruitment after the sites resumed recruitment activities, as well as the termination of 6 underperformed sites and the startup of replacement sites, the sample size of the study is reduced to 459. The recruitment timeline is also extended

with an intention of funding a full 31-month extension of study recruitment till August 31, 2024. The recruitment timeline projection for extension is based on subjects that were recruited by February 2021 plus the expected monthly recruitment rate from all sites afterwards.

Site Selection Methods

Prior launching the pilot phase, we conducted a review of VA inpatient and outpatient encounters extracted from the VHA National Patient Care Database to identify the number of unique patients who were treated in the VA system for CDI. Patients with one or more outpatient visits or one or more in-patient visits to a medical center in a calendar year with a diagnosis of CDI (selecting on ICD-9 codes: 00845 or 04184) were counted in 2011 and 2012. ICD-9 code 04184 made up 4% of the total. In 2011 and 2012 there were an estimated 9,234 and 9,471 unique patients diagnosed with CDI. An estimated 30% or 2,770 and 2,841 of those cases are recurrent CDI. We sent surveys (see appendix H) to 41 sites, of which, 36 responded expressing interest. Of those we have identified 27 potential sites which had an estimated population of 35 or more recurrent CDI patients in 2011 and 2012. All VA sites are listed in Table H.1, appendix H, sorted by expected number of cases in 2012, along with the estimated population of CDI cases and an expected number of study participants (20% of estimated recurrent CDI cases) in 2011 and 2012. Sites marked "responded" have responded to our survey indicating that they have the patient population necessary to sustain the recruitment of 21 participants over 3 years and they have identified potential site investigators and they were to recruit at the rate expected based on their reported 2011 and 2012 ICD9 codes.

Towards the end of pilot phase, a three-step process was used for determining sites to be included in the full study. *First*, the VA Informatics and Computing Infrastructure (VINCI) database was interrogated to identify the number of patients who were treated in the VA system (inpatient or outpatient) for occurrence of CDI cases (selecting on ICD-9 code "00.845" before 10/01/2015 **or** ICD10 code "A04.7" after 09/30/2015, or selecting on positive stool test for CDI from microbiology lab) from 2011 to 2016 at VA sites nationwide, categorized per facility and total by site within the Station, and sites were then ranked by numbers of CDI cases. A 39-site contact list was created for first contact by including NODES sites and the sites with the highest number of CDI cases (more than 90 CDI cases at main facility, and more than 120 cases for entire catchment area annually. This will provide at least 5 eligible recurrent

CDI cases annually, assuming 25% recurrence and 20% enrollment of recurrent CDI cases). A site investigator survey including a clinical lab survey of positive stool C. *difficile* assays was also sent to the ACOS/Research and Research Administrative Officer at each of the sites. *Second*, after a LSI candidate was identified by the site and the completed site investigator survey was returned, a follow-up survey was sent to the LSI candidate which asked questions regarding their proposed recruitment strategy, site leadership support, site investigator availability and commitment, research experience, and proposed study team members at the site. Additional questions were included to gauge the site's potential for expanding recruitment to nearby VA facilities. The follow-up survey responses were then reviewed and scored. *Third*, of the sites showed interest of participation, structured phone interviews were further conducted and scores were given based on their responses. Sites were evaluated based on CDI recurrence rate at their facility and catchment area, site interview score, and other qualitative factors. Twenty-four units (26 sites. Note that given Gainesville and Hines each recruiting from another VA location, we are referencing the dual location as one unit. e.g., Gainesville and Lake City VA locations are considered one unit) were selected for inclusion into the proposed study expansion with six to be considered as primary back-up sites.

Prior to the start of the pandemic in mid-March 2020, five sites (Tampa Bay, Palo Alto, Loma Linda, West LA, San Antonio) were dropped due to inability to enroll. One additional site (San Juan) was terminated in February 2021 due a chronic problem enrolling prior to the pandemic. To restore the complement of 24 approved enrollment units for CSP #596, five replacement sites were identified through careful interviewing and vetting of the investigators, reviewing of CDI case numbers, and establishment of administrative support. Those sites were launched between October 2020 and July 2021. One additional replacement site will be launched in September/October 2021.

Positive Laboratory Test Data

In addition to an ICD9 code search of patient availability, we sent a survey to the 36 sites that responded with interest asking them to report the number of unique patient positive tests of CDI in 2011 and 2012 as well as number of second and third positive tests sent at least 10 days after the first and second tests. As of writing, 24 sites responded with complete surveys. The survey results alongside the corresponding ICD9 counts from Table H.1, appendix H is displayed in Table H.2, appendix H.

Because the totals in the two data sources differ, we fitted a regression line through the origin to assess the relationship between the two sets of data. The regression coefficients (interpreted as number of unique patients with CDI by ICD9 count for every reported positive test result) 0.9626 and 0.9884 in years 2011 and 2012 respectively indicate close agreement between ICD9 and positive test result data. Figure 6 shows a scatter plot of the estimated total number of unique patients with positive CDI tests from both sources along with a regression line for calendar year 2012. Figure 7 shows estimated number of recurrent cases from ICD9 codes vs. reported positive test results. Some outliers lead to higher regression coefficients of 1.2795 and 1.4360 in 2011 and 2012 respectively. We intend to follow-up with these sites to confirm their reported numbers.

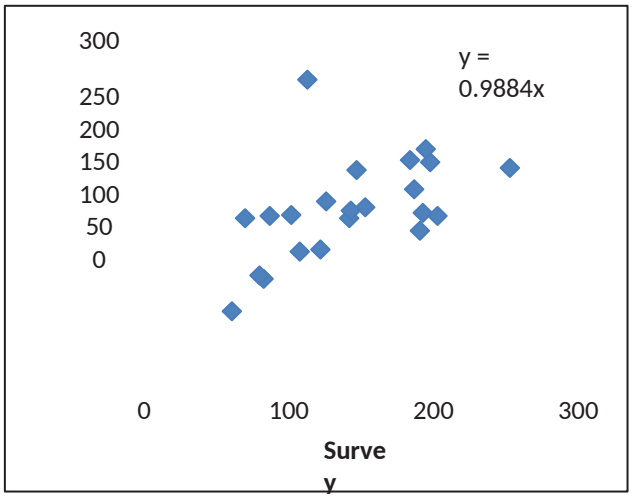


Figure 6. Total Number of Unique Patients with Primary CDI 2012

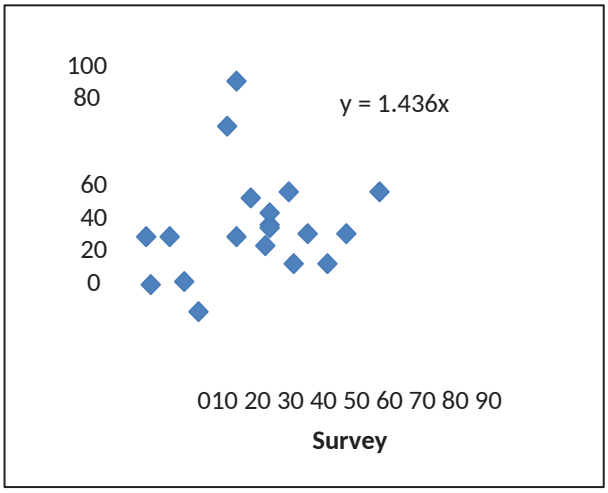


Figure 7. Total Number of Unique Patients with Recurrent CDI 2012

XVII. STUDY ORGANIZATION AND ADMINISTRATION

The organization and administrative structure of this cooperative study will be similar to others in the Cooperative Studies Program. Specifically, it will include the following components:

The Cooperative Studies Program (VA Central Office)

The Hines Cooperative Studies Program Coordinating Center (CSPCC)

The Study Chair's Office

The CSP Clinical Research Pharmacy Coordinating Center (PCC) Each participating VA Medical Center

The Cooperative Studies Scientific Evaluation Committee (CSSEC) The Study Group

The Executive Committee

The Data Monitoring Committee (DMC) Central or local IRB

The CSPCC Human Rights Committee

The CSP Site Monitoring, Auditing and Review Team (SMART)

The Cooperative Studies Program (VA Central Office) establishes overall policies and procedures that are applied to all VA cooperative studies through the Study Chairman's office and the CSPCC.

The CSPCC and the **Study Chair's office** jointly will perform the day-to-day scientific and administrative coordination of the study. These include developing the study protocol, Operations Manual, and case report forms; ensuring the appropriate support for the participating centers; scheduling meetings and conference calls; answering questions about the protocol; conducting site visits; publishing newsletters, The CSPCC will also prepare interim and final progress reports and archive study data at the end of the study. Study DMC reports will be produced at least annually and other reports will be produced at intervals determined by the study team. Participant accrual, participant safety, and data quality will be monitored closely to ensure that the study is progressing satisfactorily.

The CSP Clinical Research Pharmacy Coordinating Center (PCC) manages the pharmaceutical aspects of multicenter pharmaceutical and device clinical trials including procurement or manufacturing, packaging, labeling and distribution of clinical trial materials

and participant safety monitoring. PCC acts as a liaison for CSP (Sponsor) with the FDA, and the manufacturers of the study drug(s) or device(s) in all VA Cooperative Studies that involve drugs or devices. The PCC develops Drug or Device Treatment and Handling Procedures (DTHP), prepares a Drug or Device Information Report (DIR) for each of the study drugs or devices, and provides advice and consultation about drug or device-related matters during the study.

PCC is responsible for monitoring and reporting the safety for trial participants through the review, assessment, and communication of adverse events and serious adverse events reported by study personnel with reviewing responsibilities occurring through ongoing communication with the Study Chair(s), Executive Committee, CSPCC, and CSP Central Office. The reporting activities include filing regulatory documents involving adverse events with the FDA and manufacturers to meet federal regulations and CSP policies. The PCC provides input to the CSPCC on content and format of safety data reports for various committees including the Data Monitoring Committee (DMC), VA IRB of record, Study Executive Committee(s), and Study Group meetings.

Each **participating VA medical center** will designate a site investigator (SI) to be responsible administratively and scientifically for the conduct of the study at the center. SIs will be expected to attend all annual Study Group meetings, as well as to hire and supervise personnel. By agreeing to participate in the study, the medical center delegates responsibility for global monitoring of the ongoing study to the Data Monitoring Committee, the CSPCC Human Rights Committee, and the CSSEC. However, the Research and Development Committee (R&D) and the VA IRB of record may require the participating investigator to submit annual reports concerning the status of the study at the medical center for local monitoring purposes.

The Cooperative Studies Scientific Evaluation Committee (CSSEC) reviews the scientific merit of all new cooperative study proposals and all ongoing cooperative studies. The committee is composed of both VA and non-VA clinical research scientists, most of whom have had experience in managing their own cooperative studies.

The Study Group will be composed of the SIs from each participating center, the Study Chair(s), their staff, and CSP staff (biostatistician, project manager, clinical research pharmacist, pharmaceutical project manager and others). The Study Chair(s) will head the

group, which will meet throughout the study to discuss the progress of the study, any problems that the investigators have encountered, and any suggestions for improving the study.

Monitoring Bodies

Five bodies will oversee the ongoing scientific and ethical conduct of the study including the Executive Committee, the Data Monitoring Committee, the CSPCC Human Rights Committee (HRC), and the Study Group and SMART Monitors and CSPCC Quality Assurance Nurse Specialist. In addition, at the mid-point of the study, the Cooperative Studies Scientific Evaluation Committee (CSSEC) will review the study.

By agreeing to participate in the study, the medical center delegates responsibility for global monitoring of the ongoing study to these five bodies along with the Hines CSPCC, the PCC and the VA Central or local IRB. However, the Research and Development (R&D) Committee and the Human Studies Committee of the local medical center may require the investigators to submit additional reports concerning the status of the study.

A.1 Executive Committee

The Executive Committee will oversee study operations, the performance of participating medical centers, and the quality of data collected. This Committee will also monitor adherence to the protocol. The members of the Executive Committee will be selected by the Study Chairman from members of the Planning Committee and Participating Investigators. The Executive Committee will decide on specific intervals at which to meet. The Executive Committee formulates plans for publications and oversees the publication and presentation of all data from the study. Permission must be granted by the Executive Committee before data from the study may be used for presentation or publication.

A.2 Data Monitoring Committee

The Data Monitoring Committee (DMC) will review the progress of the study and monitor adherence to the protocol, participant intake, outcomes, complications, and other issues related to participant safety. They will also monitor the assumptions underlying sample size calculations for the study and alert the investigators if they see substantial departures as the data accumulate. The DMC will be composed of experts in infectious diseases (at least 3 members), biostatistics and clinical trials (2 members). The Study Chairman will make nominations to the Director, Cooperative Studies Program, who will make the final selection for the Board. The

DMC will make recommendations to the Director of the Cooperative Studies Program as to whether the study should continue or be terminated. The DMC can consider participant safety or other circumstances as grounds for early termination, including either compelling internal or external evidence of treatment differences or feasibility of addressing the study hypotheses (e.g. poor participant enrollment, poor adherence). Data on study progress will be provided to the DMC by the Study Biostatistician. The DMC will decide on specific intervals at which to meet. At least annually the Study Biostatistician will provide the DMC with an interim summary report on the study status and on safety data for monitoring purposes.

A.3 Human Rights Committee

The Human Rights Committee (HRC) at the Coordinating Center may review the study at the request of the Coordinating Center Director prior to submission to Central or local IRB in order to provide recommendations regarding human rights issues for Veteran participants. The HRC will also conduct a selected number of site visits to interview study staff and participants regarding the processes and personal experiences in recruitment and continued participation.

A.4 Study Group

The Study Group is chaired by the Study Chairman and includes the Study Biostatistician, Project Manager, Clinical Research Pharmacist, National Study Coordinator and all Site Investigators. This group will meet regularly to discuss the progress of the study and any problems encountered during the conduct of the trial. They will also monitor adherence to the protocol.

A.5 Clinical Monitors

The CSP Site Monitoring, Auditing and Review Team (SMART), located at the CSPCRPCC in Albuquerque, will monitor the trial for compliance with Good Clinical Practices. Site monitoring visits and activities will be conducted according to the study's monitoring plan. On-Site visits by clinical research monitors from SMART are planned for this trial. However, challenges may arise, that may impact SMART's ability to conduct planned on-site monitoring visits, for example the COVID-19 pandemic. In such circumstances, on-site monitoring visits will be converted to "Virtual" monitoring visits. Virtual monitoring visits will be kept as close to SMART's on-site processes and procedures as possible. SMART will provide an orientation to GCP at the study kick-off meeting and provide GCP tools to enhance compliance. Additionally, SMART will conduct periodic routine audits

throughout the course of the study and for-cause audits of participating sites only as requested by study leadership or CSP VACO.

The SMART Monitors will ensure that submitted data are accurate and in agreement with source documentation; verify that medications are properly stored and accounted for; verify that consent for study participation has been properly obtained and documented; confirm that research participants entered into the study meet inclusion and exclusion criteria; and ensure that all essential documentation required by good clinical practices guidelines are appropriately filed. Ready access to a participant's medical records by CSP site monitors is a requirement for sites to participate in the trial.

XVIII. STUDY PUBLICATIONS

Publication Policy

According to the policy of the Cooperative Studies Program, outcome data will not be revealed to the Study Chairs or participating site investigators until the data collection and clean-up phase of the study is completed. This policy safeguards against possible biases affecting the data collection.

All presentations and publications from this study will follow CSP policy as stated in the CSP Guidelines. The presentation or publication of any or all data collected by site investigators on participants entered into CSP #596 is under the direct control of the Executive Committee. No individual site investigator has the right to perform analyses, make interpretations, make public presentations, or seek publication of any or all of the data without the approval of the Executive Committee.

The Executive Committee has the authority to establish one or more publication committees (usually comprised of subgroups of site investigators and some members of the Executive Committee) for the purpose of producing manuscripts for presentation and publication. A presentation or publication, formulated by the Executive Committee or its authorized representatives, should be circulated to all members of the Executive Committee for review, comments, and suggestions at least four weeks prior to submission of the manuscript to the presenting or publishing body.

All publications must give proper recognition to the study's funding source, including the Department of Veterans Affairs Cooperative Studies Program, and should list or reference all principal and site investigators in the study. All VA investigators will list VA as their primary institutional affiliation. Submission of manuscripts or abstracts must acknowledge the work as "a Department of Veterans Affairs Cooperative Study." A copy of the letter to the editor and the manuscript/abstract submitted for publication or presentation should be sent through the CSPCC Director to the CSP Director and for information purposes to the members of the study's DMC. The CSP also requires that every manuscript be reviewed and approved by the Hines CSPCC Director and the CSRD Director prior to submission for publication.

Planned Publications

We anticipate a primary publication describing the results of the main, 3-arm, randomized, double blinded, placebo-controlled trial of participants with a first or second CDI recurrence. Other potential publications may include subset analyses of the primary endpoint (sustained clinical response). Although some of the subset analyses (concomitant systemic antibiotics, number of prior CDI recurrences, severity, and infection with the epidemic BI strain of *C. difficile*) may be included in the primary publication, inclusion of all of the typing results may be too detailed for the primary publication but be informative by itself. Additional publications may arise out of sub-studies of the enrolled participant population that are outside of the scope of this proposal, but which are submitted and initiated by local PIs, such as studies on changes of the intestinal microbiome following treatment with the 3 regimens.

XIX. REFERENCES

1. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene-variant strain of Clostridium difficile. N Engl J Med **2005**; 353(23): 2433-41.
2. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of Clostridium difficile-associated diarrhea with high morbidity and mortality. N Engl J Med **2005**; 353(23): 2442-9.
3. Muto CA, Pokrywka M, Shutt K, et al. A large outbreak of Clostridium difficile-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. Infect Control Hosp Epidemiol **2005**; 26(3): 273-80.
4. Lucado J, Gould C, Elixhauser A. Clostridium difficile infections (CDI) in hospital stays, 2009. In: US Department of Health and Human Services AfHRaQ. Rockville, MD, **2011**.
5. Miller BA, Chen LF, Sexton DJ, Anderson DJ. Comparison of the burdens of hospital- onset, healthcare facility-associated Clostridium difficile Infection and of healthcare- associated infection due to methicillin-resistant Staphylococcus aureus in community hospitals. Infect Control Hosp Epidemiol **2011**; 32(4): 387-90.
6. Kralovic SM. Trends of Clostridium difficile infection (CDI) in VA hospitals and proposed systems interventions. In: 10th Biennial Congress of the Anaerobe Society of the Americas. Philadelphia, PA, 2010:Abstract# SIII-2.
7. Guideline for the Prevention of Clostridium difficile Infection in VHA Inpatient Acute- Care Facilities. In: Office DoVAM-ROP, **2012**.
8. Campbell RJ, Giljahn L, Machesky K, et al. Clostridium difficile infection in Ohio hospitals and nursing homes during 2006. Infect Control Hosp Epidemiol **2009**; 30(6): 526-33.
9. Johnson S. Recurrent Clostridium difficile infection: a review of risk factors, treatments, and outcomes. J Infect **2009**; 58(6): 403-10.
10. Burke KE, Lamont JT. Fecal transplantation for recurrent Clostridium difficile infection in older adults: a review. J Am Geriatr Soc **2013**; 61(8): 1394-8.
11. Surawicz CM, Alexander J. Treatment of refractory and recurrent Clostridium difficile infection. Nat Rev Gastroenterol Hepatol **2011**; 8(6): 330-9.
12. Dubberke ER, Butler AM, Reske KA, et al. Attributable outcomes of endemic Clostridium difficile-associated disease in nonsurgical patients. Emerg Infect Dis **2008**; 14(7): 1031-8.
13. Pepin J, Routhier S, Gagnon S, Brazeau I. Management and outcomes of a first recurrence of Clostridium difficile-associated disease in Quebec, Canada. Clin Infect Dis **2006**; 42(6): 758-64.
14. Kyne L, Warny M, Qamar A, Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. N Engl J Med **2000**;

342(6): 390-7.

15. Kyne L, Warny M, Qamar A, Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea. Lancet **2001**; 357(9251): 189-93.

16. Kelly CP. Can we identify patients at high risk of recurrent Clostridium difficile infection? Clin Microbiol Infect **2012**; 18 Suppl 6: 21-7.

17. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal Microbiome in recurrent Clostridium difficile-associated diarrhea. J Infect Dis **2008**; 197(3): 435-8.

18. Louie TJ, Cannon K, Byrne B, et al. Fidaxomicin preserves the intestinal microbiome during and after treatment of Clostridium difficile infection (CDI) and reduces both toxin reexpression and recurrence of CDI. Clin Infect Dis **2012**; 55 Suppl 2: S132-42.

19. Abujamel T, Cadnum JL, Jury LA, et al. Defining the vulnerable period for re-establishment of Clostridium difficile colonization after treatment of C. difficile infection with oral vancomycin or metronidazole. PLoS One **2013**; 8(10): e76269.

20. Garey KW, Sethi S, Yadav Y, DuPont HL. Meta-analysis to assess risk factors for recurrent Clostridium difficile infection. J Hosp Infect **2008**; 70(4): 298-304.

21. Hu MY, Katchar K, Kyne L, et al. Prospective derivation and validation of a clinical prediction rule for recurrent Clostridium difficile infection. Gastroenterology **2009**; 136(4): 1206-14.

22. D'Agostino RB, Collins SH, Pencina KM, Kean Y, Gorbach S. Risk Estimation for Recurrent Clostridium Difficile Infection Based on Clinical Factors. Clin Infect Dis **2014**.

23. Shaughnessy MK, Amundson WH, Kuskowski MA, DeCarolis DD, Johnson JR, Drekonja DM. Unnecessary antimicrobial use in patients with current or recent Clostridium difficile infection. Infect Control Hosp Epidemiol **2013**; 34(2): 109-16.

24. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infect Control Hosp Epidemiol **2010**; 31(5): 431-55.

25. McFarland LV, Elmer GW, Surawicz CM. Breaking the cycle: treatment strategies for 163 cases of recurrent Clostridium difficile disease. Am J Gastroenterol **2002**; 97(7): 1769-75.

26. Lieu D, Skol L, Lieu C, Cheng S. Oral vancomycin 6-week taper regimen is superior to metronidazole and short course oral vancomycin as treatment for both initial and recurrent Clostridium difficile infection (CDI). In: The 5th Decennial International Conference on Healthcare-Associated Infections. Atlanta, GA, 2010:Abstract#132.

27. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus

vancomycin for Clostridium difficile infection. N Engl J Med **2011**; 364(5): 422-31.

28. Cornely OA, Crook DW, Esposito R, et al. Fidaxomicin versus vancomycin for infection with Clostridium difficile in Europe, Canada, and the USA: a double-blind, non- inferiority, randomised controlled trial. Lancet Infect Dis **2012**; 12(4): 281-9.

29. Cornely OA, Miller MA, Louie TJ, Crook DW, Gorbach SL. Treatment of first recurrence of Clostridium difficile infection: fidaxomicin versus vancomycin. Clin Infect Dis **2012**; 55 Suppl 2: S154-61.

30. Johnson S, Schriever C, Galang M, Kelly CP, Gerding DN. Interruption of recurrent Clostridium difficile-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. Clin Infect Dis **2007**; 44(6): 846-8.

31. Johnson S, Schriever C, Patel U, Patel T, Hecht DW, Gerding DN. Rifaximin Redux: treatment of recurrent Clostridium difficile infections with rifaximin immediately post- vancomycin treatment. Anaerobe **2009**; 15(6): 290-1.

32. Curry SR, Marsh JW, Shutt KA, et al. High frequency of rifampin resistance identified in an epidemic Clostridium difficile clone from a large teaching hospital. Clin Infect Dis **2009**; 48(4): 425-9.

33. Huang JS, Jiang ZD, Garey KW, Lasco T, Dupont HL. Use of rifamycin drugs and development of infection by rifamycin-resistant strains of Clostridium difficile. Antimicrob Agents Chemother **2013**; 57(6): 2690-3.

34. Musher DM, Logan N, Mehendiratta V, Melgarejo NA, Garud S, Hamill RJ. Clostridium difficile colitis that fails conventional metronidazole therapy: response to nitazoxanide. J Antimicrob Chemother **2007**; 59(4): 705-10.

35. Musher DM, Logan N, Hamill RJ, et al. Nitazoxanide for the treatment of Clostridium difficile colitis. Clin Infect Dis **2006**; 43(4): 421-7.

36. Musher DM, Logan N, Bressler AM, Johnson DP, Rossignol JF. Nitazoxanide versus vancomycin in Clostridium difficile infection: a randomized, double-blind study. Clin Infect Dis **2009**; 48(4): e41-6.

37. Abougergi MS, Kwon JH. Intravenous immunoglobulin for the treatment of Clostridium difficile infection: a review. Dig Dis Sci **2011**; 56(1): 19-26.

38. Johnston BC, Ma SS, Goldenberg JZ, et al. Probiotics for the prevention of Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. Ann Intern Med **2012**; 157(12): 878-88.

39. Pozzoni P, Riva A, Bellatorre AG, et al. Saccharomyces boulardii for the prevention of antibiotic-associated diarrhea in adult hospitalized patients: a single-center, randomized, double-blind, placebo-controlled trial. Am J Gastroenterol **2012**; 107(6): 922-31.

40. Surawicz CM, McFarland LV, Greenberg RN, et al. The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii. Clin Infect Dis **2000**; 31(4): 1012-7.

41. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med **2013**; 368(5): 407-15.

42. Lawley TD, Clare S, Walker AW, et al. Targeted restoration of the intestinal microbiota with a simple, defined bacteriotherapy resolves relapsing Clostridium difficile disease in mice. PLoS Pathog **2012**; 8(10): e1002995.

43. Sambol SP, Merrigan MM, Tang JK, Johnson S, Gerding DN. Colonization for the prevention of Clostridium difficile disease in hamsters. J Infect Dis **2002**; 186(12): 1781- 9.

44. Villano SA, Seiberling M, Tatarowicz W, Monnot-Chase E, Gerding DN. Evaluation of an oral suspension of VP20621, spores of nontoxigenic Clostridium difficile strain M3, in healthy subjects. Antimicrob Agents Chemother **2012**; 56(10): 5224-9.

45. Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against Clostridium difficile toxins. N Engl J Med **2010**; 362(3): 197-205.

46. Johnson S, Gerding DN, Louie TJ, Ruiz NM, Gorbach SL. Sustained clinical response as an endpoint in treatment trials of Clostridium difficile-associated diarrhea. Antimicrob Agents Chemother **2012**; 56(8): 4043-5.

47. Louie TJ, Gerson M, Grimard D, et al. Results of a phase III trial comparing tolevamer, vancomycin and metronidazole in patients with Clostridium difficile-associated diarrhea (CDAD). In: The 47th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, IL, September 17-20, 2007:Abstract# K-425a.

48. Garey KW, Ghantoji SS, Shah DN, et al. A randomized, double-blind, placebo-controlled pilot study to assess the ability of rifaximin to prevent recurrent diarrhoea in patients with Clostridium difficile infection. J Antimicrob Chemother **2011**; 66(12): 2850-5.

49. Miller M, Gravel D, Mulvey M, et al. Health care-associated Clostridium difficile infection in Canada: patient age and infecting strain type are highly predictive of severe outcome and mortality. Clin Infect Dis **2010**; 50(2): 194-201.

50. Petrella LA, Sambol SP, Cheknis A, et al. Decreased cure and increased recurrence rates for Clostridium difficile infection caused by the epidemic C. difficile BI strain. Clin Infect Dis **2012**; 55(3): 351-7.

51. Mullane KM, Miller MA, Weiss K, et al. Efficacy of fidaxomicin versus vancomycin as therapy for Clostridium difficile infection in individuals taking concomitant antibiotics for other concurrent infections. Clin Infect Dis **2011**; 53(5): 440-7.

52. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB. A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity. Clin Infect Dis **2007**; 45(3): 302-7.

53. Johnson S, Gerding DN, Broom C, Gelone SP. Efficacy and safety of oral vancomycin (V) capsules for treatment of Clostridium difficile infection (CDI): Results from two randomized clinical trials. In: 11th Biennial Congress of the Anaerobe Society of the Americas. San Francisco, CA, June 29-July 1, 2012:Abstract#

PII-16.

54. Vancocin® package insert. Exton, PA revised December 2011.

55. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. , **2012**.

56. McFarland LV, Surawicz CM, Stamm WE. Risk factors for Clostridium difficile carriage and C. difficile-associated diarrhea in a cohort of hospitalized patients. J Infect Dis **1990**; 162(3): 678-84.

57. Miller MA, Louie T, Mullane K, et al. Derivation and validation of a simple clinical bedside score (ATLAS) for Clostridium difficile infection which predicts response to therapy. BMC Infect Dis **2013**; 13: 148.

58. Crook DW, Walker AS, Kean Y, et al. Fidaxomicin versus vancomycin for Clostridium difficile infection: meta-analysis of pivotal randomized controlled trials. Clin Infect Dis **2012**; 55 Suppl 2: S93-103.

59. Westfall PH, Tobias RD, Wolfinger RD. Multiple Comparisons and Multiple Tests Using SAS®, Second Edition. Cary, N.C.: SAS Institute, INc., **2011**.

60. Proschan MA. A multiple comparison procedure for three- and four-armed controlled clinical trials. Stat Med **1999**; 18(7): 787-98.

61. Louie TJ, et al. Fidaxomicin preserves the intestinal microbiome during and after treatment of Clostridium difficile infection (CDI) and reduces both toxin expression and recurrence of CDI. Clin Infect Dis 2012;55(Suppl 2):S132-42.

62. Jump RLP, et al. Metabolomics analysis identifies intestinal microbiota-derived biomarkers of colonization resistance in clindamycin-treated mice. PLoS ONE 2014;9:e101267.

63. McDonald LC, Coignard B, Dubberke E, Song X, Horan T, Kutty PK, and the Ad Hoc Clostridium difficile Surveillance Working Group. Recommendations for surveillance of Clostridium difficile-associated disease. Infect Control Hosp Epidemiol 2007;28:140-145.

64. Kumar N, Miyajima F, He M, Roberts P, Swale A, Ellison L, Pickard D, Smith G, Molyneux R, Dougan G, Parkhill J, Wren BW, Parry CM, Pirmohamed M, Lawley TD. Genome-based infection tracking reveals dynamics of Clostridium difficile transmission and disease recurrence. Clin Infect Dis. 2016;62:746-52.

65. Soriano MM, Johnson S. Treatment of Clostridium difficile infections. Infect Dis Clin North Am 2015:29(1):93-108.

66. Design and Monitoring of Multi-Arm Multi-Stage Clinical Trials. Ghosh P, Liu L, Senchaudhuri P, Gao P and Mehta C. Biometrics, 73: 1289-1299, 2017 [66]

67. Adaptive Sequential Testing for Multiple Comparisons. Gao P, Liu L and Mehta C. Journal of Biopharmaceutical Statistics, 24: 1035–1058, 2014 [67]

68. Garey KW, Aitken SL, Gschwind L, et al. Development and Validation of a Clostridium difficile Health-related Quality-of-Life questionnaire. J Clin Gastroenterol 2016; 50(8):631-7

69. Graham, John W., Allison E. Olchowski and Tamika D. Gilreath (2007) “How many

imputations are really needed? Some practical clarifications of multiple imputation theory." Prevention Science 8: 206–213. [69]

70. Lawrence Gould A, Boye ME, Crowther MJ, et al. Joint modeling of survival and longitudinal non-survival data: current methods and issues. Report of the DIA Bayesian joint modeling working group. Stat Med. 2015; 34(14):2181-95

71. Xu Guo and Bradley P. Carlin. Separate and Joint Modeling of Longitudinal and Event Time Data Using Standard Computer Packages. American Statistician 2004; 58 (1):16-24

72. Donald Hedeker and Robert D. Gibbons (2006). Longitudinal Data Analysis (pp 302-312). New York, NY: John Wiley & Sons.

[THIS PAGE LEFT INTENTIONALLY BLANK]