CLINICAL STUDY PROTOCOL

Protocol Title

Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population

Clinical Trials.gov Number

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Investigational Agent: Vancomycin

Funding Sponsor:
MCW Cancer Center

REVISION HISTORY

Revision history is presented in chronological order so that the information pertaining to the most current version of the protocol is presented last in this section.

Original protocol: Version 1.1, Version Date 07/28/17

Original protocol: Version 1.2, Version date 09/11/17

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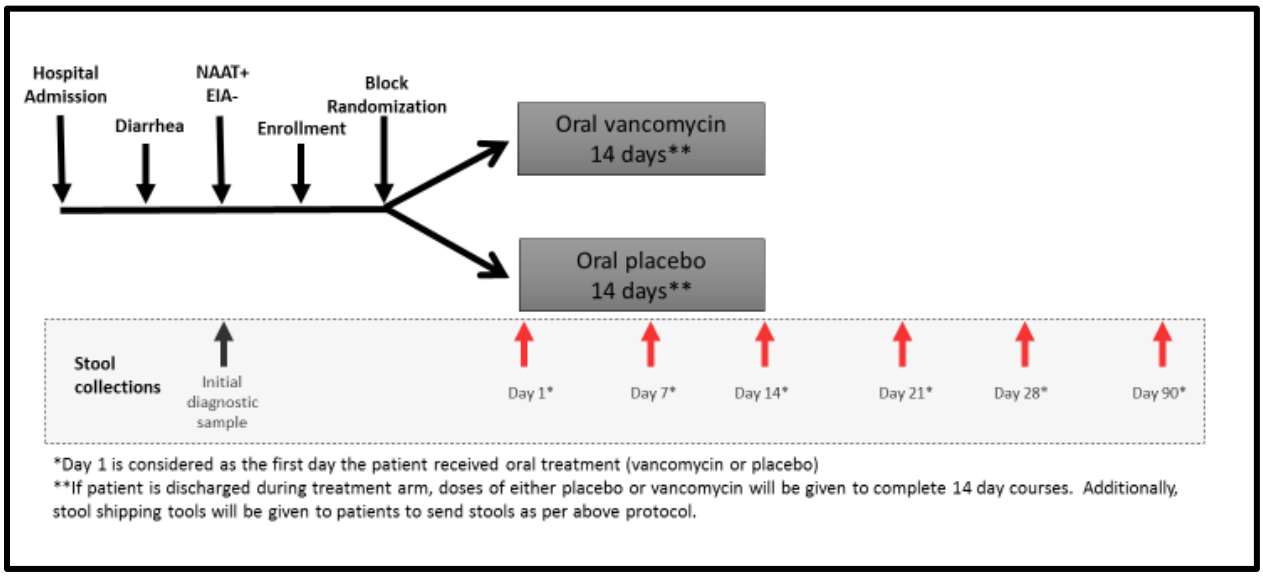
PROTOCOL SUMMARY

Title	Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population
IND Sponsor	Not applicable
Principal Investigator	Luisa Silvia Munoz-Price, MD, PhD
Study Sites	Froedtert Hospital Cancer Center (CC) & Center For Advanced Care (CFAC)
Clinical Trial Phase	Pilot
Study Disease	*Clostridium difficile infection (CDI)*
Inclusion Criteria	Patients must meet all inclusion criteria to be eligible to participate in the study. • Patients must be at least 18 years of age at time of consent. • Presence of loose stools triggered clinical *C. difficile* NAAT/toxin EIA testing. • Having both *C. difficile* NAAT (+) and *C. difficile* toxin EIA (-). • Patient admitted in a hematology-oncology unit which for the purposes of this study will be defined as 7-CFAC, 8-CFAC, and 9-CFAC (when applicable). • Patients must be willing to keep a study supplied drug diary.
Exclusion Criteria	Patients must NOT meet any exclusion criteria to be eligible to participate in the study. • Presence of sepsis. Sepsis will be defined as a Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more as per 2016 definitions.[1] • Patient is only boarding in hematology-oncology units and would have not otherwise been admitted to these units. • Inability to take oral medications. • Unwillingness or inability to provide written informed consent. • Patient has a documented allergy to vancomycin. • Patient has a documented life expectancy shorter than treatment course (14 days). • Patient is unwilling or unable to collect stool samples in the outpatient setting after discharge. • Diagnosis of *C. difficile* colitis [NAAT(+) and toxin EIA(+)] in the preceding 3 months from enrollment.

	• Received oral vancomycin during their current hospitalization, excluding empiric treatment given while pending *C. difficile* NAAT/toxin EIA results. Intravenous vancomycin is not an exclusion criterion. • Women known to be pregnant or lactating during the study.
Study Rationale	*Clostridium difficile* infection (CDI) is considered the most frequent healthcare associated infection in the US, causing almost half a million cases per year with an estimated annual cost of 4.8 billion dollars. Despite the existence of a few treatment options against CDI, yearly attributable deaths are estimated at 29,300 in the US. From April 2014 to April 2016, Froedtert Health reported 899 CDIs. In our institution, we perform 300 hematopoietic stem cell transplants (HSCT) a year and our hematology oncology inpatient units have our highest CDI rates.
Primary Objectives	To characterize the impact of oral vancomycin on *C. difficile* and VRE loads after end of treatment compared to a placebo group.
Secondary Objectives	To determine the effect of oral vancomycin on structural and functional microbiome changes after end of treatment compared to a placebo group. To characterize the impact of oral vancomycin against a placebo group on the daily frequency of loose stools by the end of treatment.
Endpoints	C. difficile loads, VRE loads, microbiome structure and function, number of stools per day.
Study Design	This is a randomized double-blind controlled trial of vancomycin against placebo for the treatment of NAAT(+)/EIA(-) in hematology oncology patients.
Study Agent	Vancomycin capsules against placebo capsules
Number of Subjects	30 (15 per arm)
Subject Participation Duration	Patients may continue study treatment for approximately 14-15 days from the time of study entry.
Duration of Follow up	90 days from the time of study entry
Estimated Time to Complete Enrollment:	Approximately 36 months
Statistical Methodology:	The primary outcomes of this randomized double-blind controlled intervention trial will be change in *C. difficile* between day 1 and day 14 and change in *C. difficile* between day 14 and day 28. Thirty patients will be randomized to either 14 days of vancomycin or placebo capsules. Subjects enrolled will be consecutive hematology

	oncology inpatients with a *C. difficile* NAAT(+) and a toxin EIA(-). Block randomization will be used to assign patients to the treatment or placebo arms. Randomized assignments will be placed in sealed envelopes which will only be handled by the research pharmacist. Clinical providers, research team, and patients will remain blinded to allocation. Study related stool collections will be obtained on days 1, 7, 14, 21, and 28 (+/- 2 days) [Day 1=first day study drug was administered]. Patients will be followed for 90 days starting on day 1. Patients unable to take at least one dose of study treatment will be removed from analysis and replaced. All statistical analyses will be performed by Dr. Sergey Tarima.
Safety Assessments	Oral vancomycin has very limited systemic absorption. The most common side effects associated with this oral drug include nausea, abdominal pain, vomiting, hypokalemia, diarrhea, and hypokalemia. Patients will be followed for a total of 90 days after the first day of study drug administration or until death, whichever occurs first. Patients removed from the study treatment for unacceptable SAEs will be followed until resolution or stabilization of the adverse event. SAEs will be followed until completion. If a patient is unable to take oral medications, then vancomycin may be held for up to 7 days until patient is able to tolerate oral medicines. If vancomycin is held for more than 7 days, the patient will be removed from treatment but will continue to provide stool samples and complete study questionnaires and diaries as required per protocol to receive the gift cards. Given that patients included in this study will all have diarrhea, then diarrhea will not be considered an Adverse Event. Special consideration for early study withdrawal due to worsening of diarrhea will be followed. We will perform dose modifications or dosing delays for nausea, vomiting, abdominal pain, renal failure, or hypokalemia.

SCHEMA



*Day 1 is considered as the first day the patient received oral treatment (vancomycin or placebo)
**If patient is discharged during treatment arm, doses of either placebo or vancomycin will be given to complete 14 day courses. Additionally, stool shipping tools will be given to patients to send stools as per above protocol.

STUDY CALENDAR

STUDY CALENDAR							
		Treatment (vancomycin vs. placebo[12]			Post-antibiotic treatment		
Parameter	**Pre-study Baseline[4]**	**Day 1[1,4] Pre-dose**	**Day 7 +/- 2 days**	**Day 14 +/- 2 days**	**Day 21 +/- 2 days**	**Day 28 +/- 2 days**	**Day 90 +/- 5 days**
Informed consent	X						
Medication History	X						
Baseline Questionnaire	X						
WBC, BMP and albumin from index admission	X	X	X[3]	X[3]			
Urine pregnancy test[7]	X						
Concomitant medications[10]	X	X	X	X	X		
Stool samples[2]	X	X[8]	X	X	X	X	X
On-study Questionnaire[6]		X[5]	X	X	X	X	X
AE/SAE assessment[11]		X	X	X	X		
Gift Card Dispensation[9]				X		X	X
Drug Diary 1 Return				X			

[1] First day of study drug administration (vancomycin or placebo).

[2] All stool samples should be placed in a refrigerator or on ice and given to CTO study staff who will submit them to Dr. Munoz-Price's lab staff (TRBC).

[3] If hospitalized.

[4] Pre-study baseline and Day 1 can be done on the same day. No need to repeat Day 1 assessments (labs, concomitant medications) if done within one day of the first dose of study drug.

[5] Complete only if >one day since the Baseline Questionnaire was completed.

[6] May be conducted in person or via telephone after the patient is discharged.

[7] Only for women of child-bearing potential who have not had a pregnancy test within two weeks prior to enrollment. Prior pregnancy test may be done on serum or urine.

[8] Day 1 samples can be obtained within 48 hrs prior to the first administration of study drug.

[9] A $100 gift card will be dispensed when the day 14 sample is received by the study staff and again when the day 28 sample is received by study staff. A $100 gift card will be dispensed

when the day 90 sample is received by study staff. Patients must be compliant with all sample collections to be eligible to receive the gift cards.

[10] All concomitant medications will be collected from time of consent until Day 21. The only drugs that will be followed until Day 90 are any antibiotics.

[11] AE/SAEs will be followed from consent until Day 21. The only AE that will be followed until Day 90 will be a diagnosis of Clostridium difficile which is defined as NAAT + and EIA +.

[12] Patients who stop study drug early will be asked to continue to bring stool samples and complete questionnaires and diaries through Day 90. If patients are compliant with requirements, they will still be eligible to receive gift cards for their stool sample collections.

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LIST OF ABBREVIATIONS

16S rRNA	Modality of gene sequencing
BUN	blood urea nitrogen
CCCTO	Cancer Center Clinical Trials Office
CDI	Clostridium difficile infection
BMP	Basic metabolic panel
CRC	Clinical Research Coordinator
EIA	Enzyme immunoassay
NAAT	Nucleic acid amplification test
PCR	Polymerase chain reaction
qPCR	Quantitative Polymerase chain reaction
VRE	vancomycin resistant enterococcus
WBC	white blood cell (count)

1 BACKGROUND

1.1 Study Disease

Clostridium difficile infection (CDI) is considered the most frequent healthcare associated infection in the US, causing almost half a million cases per year with an estimated annual cost of 4.8 billion dollars.[2, 3] Despite the existence of a few treatment options against CDI, yearly attributable deaths are estimated at 29,300 in the US.[2] From April 2014 to April 2016, Froedtert Health reported 899 CDIs. In our institution, we perform 300 hematopoietic stem cell transplants (HSCT) a year and our hematology oncology inpatient units have our highest CDI rates. Since 2015 we have cultured the stool of over 1000 hematology oncology patients finding a colonization rate of 15%, with two thirds of these patients testing positive for *C. difficile* upon admission. Using this cohort, we determined that the relative risk of developing CDI was 4.9 times higher if *C. difficile* colonized ($p<0.0001$). However, are these episodes of diarrhea a manifestation of CDI or are we detecting *C. difficile* colonization among patients with concurrent diarrhea caused by a different etiology?

Clinical diagnosis of CDI is ascertained using a highly sensitive test --nucleic acid amplification test (NAAT)--, which is unable to differentiate *C. difficile* colonization from CDI.[4] Patients with hematologic malignancies commonly experience diarrhea triggering a *C. difficile* NAAT test. Diarrhea in this population can be caused by many etiologies (e.g. chemotherapy, graft versus host disease (GVHD), and antibiotics). Thus, it is difficult to establish CDI in hematology oncology patients even if they test *C. difficile* NAAT(+).[5] Additionally, the presentation of CDI in this population tends to be mild, reinforcing the suspicion that these cases might constitute colonized patients with concomitant diarrhea due to other etiologies.[5] More importantly, unnecessary antibiotic treatment can be detrimental for hematologic patients by further disrupting the structure and function of their already perturbed gut microbiome, and subsequently increasing their risk for colonization by other pathogens such as vancomycin resistant enterococcus (VRE), GVHD, or bacteremia.[6] Moreover, the degree of microbiome disruption during HSCT engraftment was recently associated with higher mortality.[7] Early 2017 Froedtert replaced *C. difficile* NAAT with a two-step test, in which a positive NAAT will reflex to a *C. difficile* toxin enzyme immunoassay (EIA), to determine if clinically significant amounts of toxin are being produced. However, NAAT(+), toxin(-) results will create a treatment dilemma, especially in immunocompromised populations. This pilot project aims to determine both the microbiome and the clinical implications of treating *C. difficile* toxin(-) patients. We hypothesize that antibiotic treatment of *C. difficile* NAAT(+), toxin(-) patients will increase *C. difficile* and VRE loads after the antibiotic course, will prolong *C. difficile* carriage status, will worsen the gut microbiota structure and function, without a clear clinical improvement in the hematology-oncology inpatient population.

1.2 Investigational Agent(s)

<u>Oral vancomycin versus placebo</u>
This study aims to compare the standard antibiotic treatment (oral vancomycin) versus placebo (no antibiotics). Oral vancomycin is currently FDA approved for the treatment of NAAT (+) *C. difficile* colitis, regardless of toxin production. However, recent European literature shows that treatment of NAAT(+) EIA toxin (-) *C. difficile* patients might not be necessary.[8-10] Furthermore, we hypothesize that vancomycin treatment of NAAT(+) EIA toxin(-) might be deleterious for the gut microbiome.

Detection of free *C. difficile* toxin in the stool was the standard of care for the diagnosis of CDI from 1983 through 2010. Since then, NAAT became the standard diagnostic test for over 60% of US hospitals (National Healthcare Safety Network, unpublished data). However, there is growing evidence that symptomatic patients who are NAAT(+), toxin(-) have outcomes similar to patients who are NAAT(-), toxin(-); some of these outcomes include 30-day mortality, ICU admission, and hospital readmission triggered by *C. difficile*.[8, 11-13] Furthermore, the United Kingdom has successfully used a similar two step diagnostic algorithm with a confirmatory toxin EIA since 2012.[14]

The adverse health consequences resulting from antibiotic overtreatment of NAAT(+), toxin(-) patients may be particularly important in transplant recipients.[15] The usual treatment prescribed for CDI at the Froedtert Memorial Lutheran Hospital is oral vancomycin. While this drug has excellent activity against *C. difficile* and commonly suppresses its growth to non-detection, it does not eradicate carriage and its use results in marked and prolonged disruption of the lower intestinal microbiota.[15, 16] Meanwhile, the degree of lower intestinal microbiota disruption at the time of HSCT engraftment has been demonstrated to be an independent predictor (controlling for other markers of underlying disease) of overall and transplant-related 3-year mortality.[17] In addition, recent findings suggest that bone marrow suppressive effects of antibiotics, in this case potentially unnecessary oral vancomycin (which is not appreciably absorbed), may be solely mediated via microbiota disruption.[18] All these data supports the notion that antibiotic treatment of NAAT(+), toxin(-) *C. difficile* patients might have significant negative repercussions without a clear clinical benefit.

1.3 Similar ongoing randomized clinical trial

A similar randomized clinical trial comparing the effectiveness of vancomycin versus placebo in a NAAT+/EIA- is underway at Washington University in St. Louis. This study is funded and designed in collaboration with the Centers for Disease Control and Prevention (CDC). Even though this CDC funded study is done in the general inpatient population, its protocol was recently modified to include hematology oncology patients. The new amendments in our current version of the protocol are aimed to mirror the less restrictive exclusion criteria used by Washington University and CDC. These include the use of gift cards to increase the compliance with stool collections and courier services or pre-paid shipping labels to send stool samples.

2 HYPOTHESIS AND OBJECTIVES

2.1 Primary Hypothesis

Treatment of NAAT(+)/EIA(-) patients with oral vancomycin will prolong and increase the C. difficile and VRE fecal loads when compared to a placebo group.

Primary Objectives

To characterize the impact of oral vancomycin on *C. difficile* and VRE loads after end of treatment compared to a placebo group.

2.1.1 Compare the impact of vancomycin against placebo on the *C. difficile* load fluctuations from day 1 to end-of-treatment (day 14-15) and to day 28. This will be ascertained using quantitative PCR.

2.1.2 Characterize the differential VRE load from day 1 to day 28. This will be ascertained using selective solid media.

2.1.3 Establish the long-term persistence of *C. difficile* by quantitative PCR at day 90.

2.2 Secondary Hypothesis

Oral vancomycin administered to NAAT(+)/EIA(-) patients will cause a greater disruption of the structure and function of the gut microbiome without improving the persistence of diarrhea when compared to a placebo group.

Secondary Objectives

Determine the effect of oral vancomycin on structural and functional microbiome changes after end of treatment compared to a placebo group.

2.2.1 Characterize the impact of oral vancomycin against placebo on structural alterations of the microbiome after end of treatment using 16S rRNA sequencing. Structural microbiota changes will be defined as 1) Alpha diversity index (Shannon index), 2) Abundance of specific phyla and taxa.

2.2.2 Characterize the impact of oral vancomycin against placebo on functional microbiome changes after end of treatment by measuring: 1) Primary and secondary bile acids, 2) Amino acids, 3) Sugars, and 4) Lipids.

2.2.3 Characterize the impact of oral vancomycin against a placebo group on the frequency of loose stools by the end of treatment.

2.3 Rationale for the Outcome Measures Selection

The gut microbiome has been shown to play an important role in the protection of individuals from the development of *C. difficile* infection. In previous studies, it has been shown that patients experiencing active *C. difficile* infection show marked shifts in their microbiome: reduced overall microbial diversity, reduced abundance of numerous taxa, including the Bacteroidetes, Lachnospiraceae, and Ruminococcacae, an increased abundance of members of Clostridia, and concomitant shifts in the metabolome of the microbiota.

***C. difficile* loads:** Antibiotics cause perturbations of the gut microbiota that allow *C. difficile* spore germination and vegetative bacterial growth in the colon with subsequent toxin production. [19, 20] Therefore, a necessary step for the biological progression of *C. difficile* is an increase of *C. difficile* fecal loads. Oral vancomycin for CDI treatment has been associated with an initial decrease of *C. difficile* loads followed by a rebound of *C. difficile* loads 1-2 weeks after completion of therapy.[21] Our study will expand on this finding by evaluating the *C. difficile* loads by qPCR (not culture) only among NAAT(+) toxin(-) patients.

VRE loads: Oral vancomycin in mice markedly increases their susceptibility to intestinal colonization by VRE upon challenge. [22, 23] This effect is believed to occur through microbiota perturbation;[20]however, the longitudinal association between oral vancomycin and higher VRE fecal loads is still unknown in humans.[22]

Microbiome structure: Vancomycin use in mice has been shown to produce marked structural microbiota perturbations.[22] [23]However, its effect in humans is still not well characterized.[22] We hypothesize that oral vancomycin treatment will have a profound disruption on Shannon index and specific taxa.

Microbiome function: Structural microbiota changes impact the metabolic environment of the gut.[24] Primary and secondary biliary acids, amino acids, lipids and sugars play key roles promoting and inhibiting germination, and are associated with outgrowth of vegetative forms and development of toxin.[24-26] Derangements of these metabolites have major impact on the susceptibility of the host to pathogens. [25, 26]

Frequency of stools: A recent observational study also showed that NAAT(+)/ toxin(-) and NAAT(-) groups had similar duration of diarrhea.[8] Our study will explore this observation with a controlled design.

3. STUDY DESIGN

3.1 General Description

This pilot study will be a randomized double blind intervention trial for consecutive hematology oncology inpatients with a *C. difficile* NAAT(+) and a toxin EIA(-). After enrollment, patients will be randomized to treatment with vancomycin 125 mg capsules by mouth approximately every 4-6 hours for 14-15 days or to placebo with identical looking capsules for the same length of treatment. Block randomization will be used to assign patients to the treatment or placebo arms. Randomized assignments will be placed in sealed envelopes which will only be handled by the research pharmacist. Clinical providers, research team, and patients will remain blinded to allocation.

3.1.1 Number of Subjects
We plan to randomize 30 patients (15 to each arm).

3.2 Primary Endpoint(s)

3.2.1 Endpoint 1: C. difficile fecal loads: Stool samples collected for diagnosis and days 1, 7, 14, 21, 28, and 90 will be thawed in batches and one cc aliquot will be extracted refreezing the remaining sample at -80 degrees F. One cc aliquot will then be divided into 4 sub-samples of equal volume and with the same unique identifier. This identifier will be able to chronologically cluster samples by each unique patient. One sample will be used for C. difficile qPCR determination and VRE cultures using methods previously described in the literature.[5, 23]

3.3 Secondary Endpoint(s)

3.3.1 *Endpoint 2a: VRE fecal loads:* As per 3.2.1.

3.3.2 *Endpoint 2b: Alpha diversity index (Shannon index)* Sub-sample of stool from diagnosis, days 1, 7, 14, 21, 28 & 90 will be used for 16S rRNA gene sequencing to determine the microbiome community structure.

3.3.3 *Endpoint 2c: Abundance of specific phyla and taxa:* As per 3.3.2.

3.3.4 *Endpoint 2d: Metabolites:* These will include 1) Primary and secondary bile acids, 2) Amino acids, 3) Sugars, and 4) Lipids. They will be measured using mass spectrometry using stool from diagnosis, days 1, 7, 14, 21, 28 & 90.

3.3.5 *Endpoint 2e: Frequency of loose stools: this will be obtained using the baseline CRF and on study questionnaires on* days 1, 7, 14, 21, 28 & 90.

3.4 Randomization

Block randomization (15 patients per block) will be used to assign patients to the treatment or placebo arms. Prior to the study onset, 30 randomized assignments will be placed in individually sealed envelopes which will only be handled by the research pharmacist. Clinical providers, research team, and patients will remain blinded to allocation. If patient substitution is deemed to be required, then an additional block randomization and placement of randomized assignments in sealed envelopes will be performed.

3.5 Study Timeline

The study will take approximately 4 years to complete from protocol submission to manuscript preparation. The majority of the time will be spent enrolling and randomizing patients. Upon completion of follow up of all 30 included patients, samples will be sent to our collaborators for metabolomics and sequencing.

3.6 Primary Completion

The study is expected to reach primary completion approximately 36 months from the time the study opens to accrual.

3.7 Study Completion

The study is expected to reach study completion approximately 42 months from the time the study opens to accrual.

4 PATIENT SELECTION AND IDENTIFICATION

4.1 Inclusion Criteria

Patients must meet all inclusion criteria to be eligible to participate in the study.

4.1.1 Patients must be at least 18 years of age at time of consent.

4.1.2 Presence of loose stools triggered clinical *C. difficile* NAAT/toxin EIA testing.

4.1.3 Having both *C. difficile* NAAT (+) and *C. difficile* toxin EIA (-).

4.1.4 Patient admitted in a hematology-oncology unit which for the purposes of this study will be defined as 7-CFAC,8-CFAC, and 9-CFAC (when applicable).

4.1.5 Patients must be willing to keep a study supplied drug diary.

4.2 Exclusion Criteria

Patients must NOT meet any exclusion criteria to be eligible to participate in the study.

4.2.1 Presence of sepsis. Sepsis will be defined as a Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more as per 2016 definitions.[1]

4.2.2 Patient is only boarding in hematology-oncology units and would have not otherwise been admitted to these units.

4.2.3 Inability to take oral medications.

4.2.4 Unwillingness or inability to provide written informed consent.

4.2.5 Patient has a documented allergy to vancomycin.

4.2.6 Patient has a documented life expectancy shorter than treatment course (14 days).

4.2.7 Patient is unwilling or unable to collect stool samples in the outpatient setting after discharge.

4.2.8 Diagnosis of *C. difficile* colitis [NAAT(+) and toxin EIA(+)] in the preceding 3 months from enrollment.

4.2.9 Received oral vancomycin during their current hospitalization, excluding empiric treatment given while pending *C. difficile* NAAT/toxin EIA results. Intravenous vancomycin is not an exclusion criterion.

4.2.10 Women known to be pregnant or lactating during the study.

4.3 Patient Identification

It is preferred that all samples be collected Monday-Friday from 7 am to 5 pm only, with few exceptions. All samples, including those collected outside of 7 am to 5 pm, should be kept in the study refrigerators located on designated CFAC floors until the CCCTO study staff collects them. Please see Appendix 1 for outpatient collections using courier/shipping services. The Clinical Research Coordinator (CRC) will de-identify the samples and assign them a unique patient number (UPN). These UPNs will be assigned to patients sequentially (CD001, CD002, CD003. etc.) as they sign consent. A sub-identifier will be used to identify the time point of the sample, see Table 1 below. This will result in every sample having a 5-digit identifier (i.e. CD001-03 is the first patient's Day 7 sample, CD004-01 is the fourth patient's baseline sample).

Sub-identifier	Time point
-01	Baseline
-02[a]	Day 1[a]
-03	Day 7 (+/- 2 days)
-04	Day 14 (+/- 2 days)
-05	Day 21 (+/- 2 days)
-06	Day 28 (+/- 2 days)
-07	Day 90 (+/- 5 days)

[a] Day 1 sample may be skipped if < 48 hours has passed since the Baseline sample was collected.

5 STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

5.1 Study Entry Procedures

5.1.1 Consent and Authorization

The study-specific assessments are detailed in this section and outlined in the Study Calendar. A written, signed informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A member of the clinical research staff or designee will present the potential subject with the consent and allow the potential subject adequate time to read and review the informed consent form. The research staff member will then discuss the study and answer questions about time obligations and study requirements in detail to the potential subject. Subjects must be able to understand the document and provide appropriate informed consent. If the potential subject signs the consent, then all appropriate signatures are obtained, and a copy of the signed consent is given to the subject. A signed ICF copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the MCW Cancer Center Clinical Trial Management System. The system is password protected and meets HIPAA requirements.

5.1.2 Pretreatment Period

Screening Assessments
Screening will be done based on microbiology reports with NAAT(+)/EIA(-) results and physical location is 7-CFAC,8-CFAC, or 9-CFAC (when applicable).

If there are any eligibility questions, please contact the Principal Investigator Dr. Silvia Munoz-Price, MD, PhD; cell: (305)733-5113 and/or primary coordinator.

The screening procedures and assessments must be completed prior to, or on the same day as, the first administration of study drug. This will include:

- Medical History review
- Baseline Questionnaire
- WBC, BMP, and albumin labs
- Urine pregnancy test for women of child-bearing potential (unless a serum or urine pregnancy test was done within two weeks of enrollment)
- Concomitant medications
- Collection of stool sample. If a patient is unable to provide a stool sample before to treatment onset, then the PI or her physician designee could obtain rectal swabs in lieu of a stool sample. The patient and the attending physician would need to verbally consent prior to the procurement of the rectal swabs.

5.2 Study Procedures during Treatment

Patients must meet eligibility criteria on Day 1 to be treated.

5.2.1 Study Procedures, Day 1, 7, 14 (if hospitalized, otherwise follow 5.2.2.):

- WBC, BMP, and albumin labs
- Concomitant medications
- Collection of stool sample or rectal swabs if inpatient
- On-Study Questionnaire
- AE/SAE assessment

5.2.2 Study Procedures, Days 21, 28, 90

- Concomitant medications (only collected through Day 21)
- Collection of stool sample
- On-Study Questionnaire
- AE/SAE assessment (only collected through Day 21)

5.2.3 Study Diaries

- While inpatient, the nursing staff will log the date, number and type of bowel movements per day on the study drug diary supplied by the study staff. All medications will be tracked on the patient's electronic Medication Administration Record (MAR).

- While outpatient, the patient will complete two study diaries supplied by study staff prior to discharge.
    - When applicable, the patient will log the date, time study drug was taken, and number and type of bowel movements per day on the Study Drug Diary 1. Patient should return the Study Drug Diary 1 with their day 14 sample collection.
    - The patient will log all other antibiotics taken from the date of discharge until the day 90 sample collection on the Other Antibiotic Diary. Patient should return the Other Antibiotic Diary with their day 90 sample collection.

5.2.4 Gift Card Remuneration

- Remuneration is as follows (total=$300):
    - $100 at the day 14 sample collection
    - $100 at the day 28 sample collection
    - $100 at the day 90 sample collection
- If a patient withdraws early, remuneration will only be for the portions of the study already completed by the patient.

5.2.5 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for 14-15 days or until:

- *C. difficile* disease progression defined as development of sepsis (as per clinical team's or investigator's judgment), or the occurrence of any of the following conditions after 48-72 hours of first intake of study drug: a). worsening abdominal pain or b). worsening of diarrhea or c). new onset of ileus. The presence of any of these situations will grant unblinding of the study drug and the subject will be offered vancomycin if on placebo.
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the investigator's or clinical team's judgment.
- Inter-current illness that prevents further treatment administration.
- Patient decides to withdraw from the study.
- Significant patient noncompliance with protocol.
- Unacceptable adverse event(s) not covered by the first item (*C. difficile* progression).

For any patient randomized AND given at least one dose of the study drug AND who must stop study treatment due to adverse outcomes: patients will be followed with Study Drug Diary 1, stool collections, and will remain eligible for gift cards for stool samples.

For any patient randomized AND given at least one dose of the study drug AND who must stop study treatment NOT due to adverse outcomes (i.e. patient decides he/she doesn't want to be part of the study): patient is off the study. No further diaries, stool collections, or gift cards.

For any patient randomized AND not given at least one dose of the study drug: patient is off the study. No further diaries, stool collections, or gift cards are needed.

5.2.6 Patient-Initiated Withdrawal: A patient may decide to withdraw from the study at any time.

5.2.7 Investigator-Initiated Withdrawal: The Investigator will withdraw a patient whenever continued participation is no longer in the patient's best interests. Reasons for withdrawing a patient include, but are not limited to, disease progression (as described above in section 5.2.5), the occurrence of an adverse event or a concurrent illness, a patient's noncompliance or simply significant uncertainty on the part of the Investigator that continued participation is prudent.

5.2.8 Withdrawal Documentation Procedure: The reason for study withdrawal and the date the patient was removed from the study must be documented in the medical record.

6 TREATMENT PLAN

6.1 Study drug and Placebo

Treatment will be administered on an inpatient basis. Patients being discharged home prior to the completion of the 14-15 day regimen should be sent with enough medication to last through the completion of the regimen.

Regimen Description					
Study Drug	**Premedication; precautions**	**Dose**	**Route**	**Schedule**	**Length**
Vancomycin	None	One capsule (125 mg) q4-6hr	Oral	Days 1-14	14-15* days
Placebo	None	One capsule q4-6hr	Oral	Days 1-14	

*Study drug may be given on day 15 if all 4 doses were not given on day 1.

Probiotics can be given to patients if needed.

6.1.1 Dietary Restrictions: None

6.1.2 Prohibited Medications: None

6.2 Monitoring Subject Compliance

Study drug will be administered or dispensed only to eligible patients under the supervision of the investigator or identified sub-investigator(s). The appropriate study personnel will maintain records of study drug receipt and dispensing. Comprehensive instructions will be provided to the patient in order to ensure compliance with dosing procedures.

Outpatients still receiving the study drug will be requested to maintain a Study Drug diary to record each dose of medication with the time of self-administration. The Study Drug diary will be returned to clinic staff at the completion of study drug (approximately day 14-15).

6.3 Follow-Up Period

Patients will be followed for a total of 90 days after the first day of study drug administration or until death, whichever occurs first. After day 90, patients will not be followed, even for survival, and will have completed study protocol and procedures.

Patients removed from the study treatment for unacceptable SAEs will be followed until resolution or stabilization of the adverse event. SAEs will be followed until completion.

7 DOSING DELAYS/DOSE MODIFICATIONS

If a patient is unable to take oral medications, then vancomycin may be held for up to 7 days until patient is able to tolerate oral medicines. If vancomycin is held for more than 7 days, the patient will be removed from treatment but will continue to provide stool samples and complete study questionnaires and diaries as required per protocol to receive the gift cards.

Given that patients included in this study will all have diarrhea, then diarrhea will not be considered an Adverse Event. Consideration for early study withdrawal due to worsening of diarrhea would need to follow section 5.2.3.

Tables for Dosing Delays for Specific Adverse Events

Adverse Event: Nausea/Vomiting	
Grade of Event	**Management/Next Dose for Vancomycin/placebo**
≤ Grade 1	No change in dose
Grade 2	No change in dose
Grade 3	Hold* until ≤ Grade 2
Grade 4	Hold* until ≤ Grade 2
Recommended management: antiemetics.	
*Patients requiring a delay of 7 days should go off protocol therapy	

Adverse Event: Renal failure[1]	
Grade of Event	**Management/Next Dose for Vancomycin/placebo**
≤ Grade 1	No change in dose
Grade 2	No change in dose
Grade 3	Hold* until ≤ Grade 2
Grade 4	Hold* until ≤ Grade 2
[1] Only if renal failure is related to study drug. Determination will be done by Sara Revolinski, PharmD (in a blinded fashion) *Patients requiring a delay of 7 days should go off protocol therapy	

Adverse Event: Abdominal Pain	
Grade of Event	**Management/Next Dose for Vancomycin/placebo**
≤ Grade 1	No change in dose
Grade 2	No change in dose
Grade 3	Hold* until ≤ Grade 2
Grade 4	Hold* until ≤ Grade 2
*Patients requiring a delay of 7 days should go off protocol therapy	

Adverse Event: Hypokalemia[1]	
Grade of Event	**Management/Next Dose for Vancomycin/placebo**
≤ Grade 1	No change in dose
Grade 2	No change in dose
Grade 3	Hold* until ≤ Grade 2
Grade 4	Hold* until ≤ Grade 2
[1] Only if hypokalemia is related to study drug. Determination will be done by Sara Revolinski, PharmD (in a blinded fashion) *Patients requiring a delay of 7 days should go off protocol therapy	

7.1 Monitoring and Toxicity Management

Each patient receiving Vancomycin/placebo will be evaluable for safety. Given to the lack of absorption of the drug, the safety parameters will be limited to renal failure or hypokalemia coinciding with the study drug, nausea, and vomiting, and spontaneous reports of adverse events reported to the investigator by patients.

Both renal failure and hypokalemia are commonly encountered among hospitalized hematology oncology patients. Dr. Sara Revolinski (Blinded Pharmacist) will be in charge of making the determination of relatedness to the study drug based on temporal association and the lack of other nephrotoxic drugs. Dr. Revolinski can be contacted by phone 414-955-2873 or cell 262-327-5504.

Each patient will be assessed periodically for any toxicity development. Toxicity will be assessed according to the NCI CTCAE v4.0. Dose adjustments will be made according to the system showing the greatest degree of toxicity.

8 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

8.1 Defining and reporting an Adverse Event

Definition. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE.

Reporting source. AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

Prior to the trial. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned).

Treatment events. For all AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Follow-up of Adverse Events. All adverse events will be collected and followed with appropriate medical management until the day 21 visit. Patients will be followed until day 90 for a diagnosis of *C. difficile* colitis [NAAT (+) and toxin EIA (+)].

Signs or symptoms reported as adverse events will be graded and recorded by the investigator, according to the CTCAE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®.

8.2 Defining and reporting a Serious Adverse Event (SAE)

8.2.1 Definition

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization (see clarification in the paragraph below on planned hospitalizations).
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent one of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations

8.2.2 Reporting SAEs

Serious AEs must be reported from the date the participant signs Informed consent until the day 21 visit or until they are resolved unless they are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Since this is an investigator-initiated study, the principal investigator, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor-investigator's IRB when required.

8.3 Reporting AEs/SAEs to the Data and Safety Monitoring Committee

Review all unexpected grade 3, and all grade 4 and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. Non-hematological grade 4 and all grade 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge. Hematological grade 4 events can be routine reported. Given that patients included in this study will all have diarrhea, then diarrhea will not be considered an adverse event unless it worsens (increase in grade).

Report Method: The study staff will use email to report SAEs to the DSMC. The SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE as a guideline whenever possible.

The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

8.4 Reporting AEs/SAEs to MCW Committee Institutional Review Board

The study staff must report events to the MCW IRB within five business days of his/her awareness of the event if it meets expedited reporting criteria.

8.5 Unanticipated Problem Involving Risk to Subject or Other (UPIRSO)

The investigator and his or her team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

8.6 AE Grading and Attribution

8.6.1 Adverse Event Grading

Grade	Description
0	No AE (or within normal limits).
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE

8.2.2 Adverse Event Attribution

Attribution is an assessment of the relationship between the AE and the medical intervention.

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

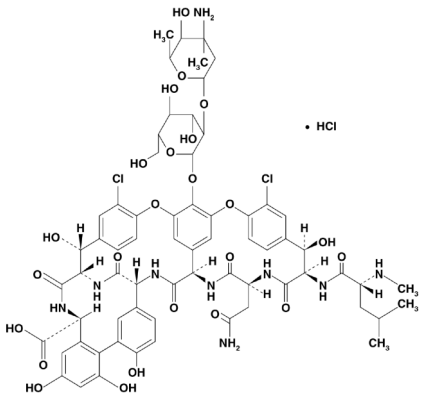
9 PHARMACEUTICAL INFORMATION

9.1 Vancomycin [VANCOCIN® (vancomycin hydrochloride, USP) CAPSULES]

VANCOCIN CAPSULES are indicated for the treatment of C. difficile-associated diarrhea. VANCOCIN CAPSULES are also used for the treatment of enterocolitis caused by Staphylococcus aureus (including methicillin-resistant strains). Parenteral administration of vancomycin is not effective for the above infections; therefore, VANCOCIN CAPSULES must be given orally for these infections.

Orally administered VANCOCIN is not effective for other types of infections. To reduce the development of drug-resistant bacteria and maintain the effectiveness of VANCOCIN CAPSULES and other antibacterial drugs, VANCOCIN CAPSULES should be used only to treat infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.

Product Description: VANCOCIN CAPSULES for oral administration contain chromatographically purified vancomycin hydrochloride, a tricyclic glycopeptide antibiotic derived from Amycolatopsis orientalis (formerly Nocardia orientalis), which has the chemical formula C66H75Cl2N9O24•HCl. The molecular weight of vancomycin hydrochloride is 1485.73; 500 mg of the base is equivalent to 0.34 mmol.



Vancomycin hydrochloride has the structural formula:

The capsules contain vancomycin hydrochloride equivalent to 125 mg (0.08 mmol) or 250 mg (0.17 mmol) vancomycin. The capsules also contain F D & C Blue No. 2, gelatin, iron oxide, polyethylene glycol, titanium dioxide, and other inactive ingredients.

Oral vancomycin (vancocin) is available in 125 mg and 250 mg capsules for oral administration. VANCOCIN 125 mg CAPSULES have an opaque blue cap and opaque brown body imprinted with “3125” on the cap and “VANCOCIN HCL 125 MG” on the body in white ink.

Classification: Vancomycin is an antibacterial drug

Mechanism of Action: The bactericidal action of vancomycin against Staphylococcus aureus and the vegetative cells of Clostridium difficile results primarily from inhibition of cell-wall biosynthesis. In addition, vancomycin alters bacterial-cell-membrane permeability and RNA synthesis.

Metabolism: Vancomycin is poorly absorbed after oral administration. During multiple dosing of 250 mg every 8 hours for 7 doses, fecal concentrations of vancomycin in volunteers exceeded 100 mg/kg in the majority of samples. No blood concentrations were detected and urinary recovery did not exceed 0.76%. In anephric subjects with no inflammatory bowel disease who received vancomycin oral solution 2 g for 16 days, blood concentrations of vancomycin were less than or equal to 0.66 µg/mL in 2 of 5 subjects. No measurable blood concentrations were attained in the other 3 subjects. Following doses of 2 g daily, concentrations of drug were >3100 mg/kg in the feces and

Contraindications: Vancomycin capsules are contraindicated in patients with known hypersensitivity to vancomycin.

Side Effects: Oral vancomycin is not absorbed and overall well tolerated. The main adverse reactions associated with this drug were nausea, abdominal pain and hypokalemia.

System/Organ Class	**Adverse Reaction**	**Vancocin % (N=260)[a]**
Gastrointestinal	Nausea	17
	Abdominal pain	15
	Vomiting	9
	Diarrhea	9
	Flatulence	8
General disorders and administration site conditions	Pyrexia	9
	Edema, peripheral	6
	Fatigue	5
Infections and infestations	Urinary tract infection	8
Metabolism and nutritional disorders	Hypokalemia	13
Musculoskeletal and connective tissue disorders	Back pain	6
Nervous system disorders	Headache	7
[a] Adverse reaction rates were derived from the incidence of treatment-emergent adverse events.		

9.1.1 Investigational Agent Administration

The study drug will be handled by the Investigational Drug Services staff under the direction of the Investigational Pharmacist, Kristen Busse, PharmD. (email: IDS.pharmacy@froedtert.com).

9.1.2 Storage Requirements

Capsules need to be stored at controlled room temperature, 59° to 86°F (15° to 30°C).

9.1.3 Stability

Capsules are stable for 6 months from the date of compounding.

9.1.4 Route of Administration

Capsules will be taken by mouth.

9.1.5 Nursing Implications

Vancomycin is excreted in human milk based on information obtained with the intravenous administration of vancomycin. However, systemic absorption of vancomycin is very low following oral administration of VANCOCIN. It is not known whether vancomycin is excreted in human milk, as no studies of vancomycin concentration in human milk after oral administration have been done. Caution should be exercised when VANCOCIN is administered to a nursing woman. Because of the potential for adverse events, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

9.1.6 Agent Supply

The investigational product will be supplied unblinded to the study site's pharmacy in bulk containers from Skywalk Pharmacy, a compounding pharmacy. Vancomycin and placebo will be manufactured by Skywalk Pharmacy to be identical in size, shape, color, appearance and taste. It will be the responsibility of the study site's pharmacy to maintain the blind once a subject has been randomized.

9.1.7 Agent Accountability

The Investigational Drug Services Pharmacy staff will manage drug accountability records.

9.1.8 Agent Destruction and Return

Any drug dispensed to the patient but not taken by the patient will be returned to the Investigational Drug Services Pharmacy at the end of the treatment period.

At the conclusion of the study, any returned or unused agent will be destroyed according to institutional policies. The destruction will be recorded on the Drug Accountability Record Form.

9.2 Placebo Capsules

Product Description: The placebo capsules will be filled with Microcrystalline Cellulose, an inactive ingredient that is allergen free.

9.2.1 Investigational Agent Administration

The study drug will be handled by the Investigational Drug Services staff under the direction of the Investigational Pharmacist, Kristen Busse, PharmD. (email: IDS.pharmacy@froedtert.com).

9.2.2 Storage Requirements

Capsules need to be stored at controlled room temperature, 59° to 86°F (15° to 30°C).

9.2.3 Stability

Capsules are stable for 6 months from the date of compounding.

9.2.4 Route of Administration

Capsules will be taken by mouth.

9.2.5 Agent Supply

The investigational product will be supplied unblinded to the study site's pharmacy in bulk containers from Skywalk Pharmacy, a compounding pharmacy. Vancomycin and placebo will be manufactured by Skywalk Pharmacy to be identical in size, shape, color, appearance and taste. It will be the responsibility of the study site's pharmacy to maintain the blind once a subject has been randomized.

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Any drug dispensed to the patient but not taken by the patient will be returned to the Investigational Drug Services Pharmacy at the end of the treatment period.

At the conclusion of the study, any returned or unused agent will be destroyed according to institutional policies. The destruction will be recorded on the Drug Accountability Record Form.

10 STATISTICAL CONSIDERATIONS

10.1 Study Endpoints

Primary outcomes: (1) Change in C. difficile between day 1 and day 14, (2) change in C. difficile between day 14 and day 28.

Endpoint 1. *C. difficile* fecal loads.

Endpoint 2a. VRE fecal loads.
Endpoints 2b and 2c. Alpha diversity index and abundance of phyla/taxa.
Endpoint 2d. Fecal levels of primary and secondary bile acids, amino acids, sugars and lipids.
Endpoint 2e. Frequency of loose stools

Endpoints 1-2a will be measured on stool samples using both culture methods and qPCR.
Endpoints 2b-2d will be measured from stool samples using 16S rRNA sequencing at the MCW's MCW Genomic Sciences & Precision Medicine Center and mass spectrometry at MCW's Core Lab.
Endpoint 2e will be measured by both patient questionnaires and diaries.

Stool samples will be thawed in batches and one cc aliquot will be extracted refreezing the remaining sample at -80. One cc aliquot will then be divided into 4 sub-samples of equal volume and with the same unique identifier. One sample will be used for *C. difficile* qPCR determination and VRE quantification. A sample will undergo DNA extraction, which will be sequenced and analyzed using bioinformatics at the MCW Genomic Sciences & Precision Medicine Center.

For metabolomics, 100 mg of thawed stool sample will be mixed with 100 µL of water, 20 µL of internal standards, and 800 µL of methanol. Samples will be vortexed for 2 min and then cooled in a refrigerator for 30 min. Samples will be spun at 20,000 rcf for 10 min at 4°C. The supernatant will be transferred to clean vials and stored at -80°C. The functional microbiome characterization will be done using mass spectrometry.

10.2 Study Design

This pilot study will be a randomized double blind intervention trial for consecutive hematology oncology inpatients with a *C. difficile* NAAT(+) and a toxin EIA(-). After enrollment, patients will be randomized to treatment with vancomycin 125 mg capsules by mouth approximately every 4-6 hours for 14-15 days or to placebo with identical looking capsules for the same dosing and length of treatment. Block randomization will be used to assign patients to the treatment or placebo arms. Randomized assignments will be placed in sealed envelopes which will only be handled by the research pharmacist. Clinical providers, research team, and patients will remain blinded to allocation.

Study related stool collections will be obtained on days 1, 7, 14, 21, and 28 (+/- 2 days) and on day 90 (+/- 5 days) [Day 1=first day study drug was administered] and sent to Dr. Munoz-Price's research laboratory located at the Center for Infectious Diseases Research located in TBRC C3693. The transport of each sample to the lab will be coordinated by the CRC and lab staff. After testing as stated in this protocol, the remaining stool may be frozen and stored at -80 degrees F in Dr. Munoz-Price's research laboratory. Any leftover stool samples may be stored for further testing as stated in this protocol. The CRC will collect baseline demographics, all medications from enrollment to day 21. If the patient is discharged prior to the completion of treatment, the CRC will follow the patient by phone with stool samples dropped off by the patient or sent to the site using the designated courier or shipper.

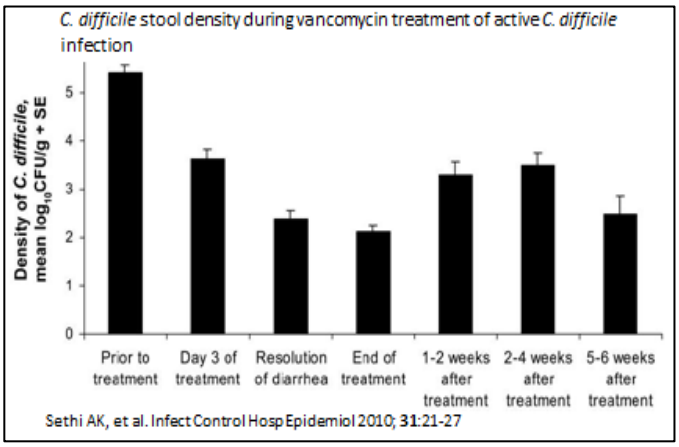
10.3 Stratification Factors

None.

10.4 Sample Size and Power Estimate

We anticipate *C. difficile* fecal loads in the vancomycin group to decrease from day 1 to 14 with a subsequent rebound by day 28. Contrary to this, we expect to see stable loads in the placebo arm.

As shown on Figure, the standard errors of log of *C. difficile* load [log(*C. difficile*)] at different time points based on 52 patients were not higher than 0.2. This means that the upper bound for standard deviation of log(*C. difficile*) is approximately 1.44, and the standard deviation of change in log(*C. difficile*) is approximately bounded at 2 under the assumption of positive correlation between *C. difficile* assessments. With 15 subjects per groups we will be able to detect a difference between the groups of 5.9 units on log(*C. difficile*) scale (POWER = 0.8, ALPHA = 0.025).



10.5 Replacement Policy

Patients that are unable to take at least one dose of study drug due to adverse events or progression of *C. difficile* disease or whom withdraw from treatment will be removed from primary analysis and replaced. Patients who do take at least one dose of study drug and stop study treatment will continue to collect stool samples and study diaries as per protocol unless the patient decides to withdraw from the study. Enrollment will continue until there are 15 patients per group who are included in the primary analysis.

Patients removed from the study treatment for unacceptable AEs will be followed until resolution or stabilization of the adverse event. SAEs will be followed until completion. Stool collections, Diaries, and gift cards will continue as per protocol..

10.6 Accrual Estimates

On March 1, 2017, Froedtert Health started performing *C. difficile* testing using NAAT, and if positive reflexing to an EIA. On April 2017, 8CFAC and 7CFAC combined had 8 NAAT(+)/EIA(-) cases, and 6 and 3 cases in May and June, respectively (≈5 per month). This would mean that in order to screen 60 patients (30 included and 30 excluded) we would need 36 months. Please note that there is an expected seasonal decrease of *C. difficile* infections during the summer.

FMLH C. difficile utilization and results - by unit

	APRIL			MAY			JUNE		
Units with NAAT (+)	Reflex C. diff tests done	NAAT (+)	EIA (+)	Reflex C. diff tests done	NAAT (+)	EIA (+)	Reflex C. diff tests done	NAAT (+)	EIA (+)
3NW	8	2	0	0			4	2	0
3SW	14	3	2	17	3	3	10	1	0
4NE	17	4	2	11	1	1	16	4	1
4NW	4	1	1	4	2	0	8	2	0
4PV	7	1	1	13	5	2	0	0	0
4SE	6	1	1	9	2	2	9	2	0
4SW	11	1	1	8	2	1	0	0	0
5NW	0	0	0	3	1	1	0	0	0
5SW	0	0	0	5	1	0	0	0	0
5SE	8	3	0	0	0	0	13	3	0
7CFAC	23	2	1	23	4	2	19	3	1
7NT	4	1	0	6	2	1	4	1	0
8CFAC	35	8	1	36	6	2	22	3	2
8NT	10	2	0	14	1	0	13	1	1
9NT	13	2	0	17	5	2	20	5	0
ER	36	11	4	30	6	4	19	5	3
MICU	26	2	0	15	2	0	10	2	0
NICU	2	1	0	0	0	0	0	0	0
SICU	0	0	0	7	1	0	1	1	0

This table only includes the primary units that have NAAT(+) samples at FMLH. Please note that the sum of B, or E, or H will not coincide with the numbers shown in Hospital worksheet.

10.7 Interim Analyses and Stopping Rules

None

10.8 Analyses Plans

Dr. Sergey Tarima will be in charge of the statistical analyses.

After the preplanned tests for the primary outcomes the secondary outcomes will be investigated on exploratory basis.

We plan to enroll 15 patients per group with 1, 14, & 28 day load assessments so that missing data will not affect the power properties. Other measurements or at other time points some data may be missing but under the assumption of missing at random (MAR) and a correctly chosen statistical model, all relevant information is retained for statistical inference. Departures from MAR will be investigated with a sensitivity analysis (e.g. tipping-point analysis).

10.8.1 Primary Analysis (or Analysis of Primary Endpoints)

Endpoint 1: Linear model analysis will be used to compare vancomycin and placebo groups. We will test if the pre-to-post treatment changes (changes between the 1st

and 14th day samples) differ between the groups controlling for the baseline (at day 1) value of *C. difficile* load. The likelihood ratio test will be used and statistical significance will be declared if p-value <2.5%. The post treatment changes (changes in *C. difficile* load between days 14, 21 & 28) will be compared between the groups via linear regression model as well, controlling for the baseline and post treatment values of *C. difficile* load. Significance will be declared if p-value < 2.5%. Thus, by the use of Bonferroni adjustment, Type I error for the primary analyses will be controlled at 5%. Given the behavior of *C. difficile* loads previously reported by Sethi et al.[21] this variable will also be analyzed on logarithmic scale.

10.8.2 Secondary Analysis (or Analysis of Secondary Endpoints)

Secondary analysis will be performed with and without cross-over subjects. If a subject changes his/her treatment (cross-over), analysis will be performed on a time-dependent status of exposure to vancomycin.

For Endpoint 2a, we will use statistical modelling to model overtime dynamics of log(*VRE*) and compare the modelled profiles between the groups.

For Endpoint 2b, 2c, 2d, temporal trends will be investigated and compared between the groups using linear mixed models. Both structural and functional microbiome changes will be compared using generalized estimating equations with a proper link function for different study outcomes. Leave one out cross validation will be used to estimate robust standard errors.

For Endpoint 2e, linear trends of stool rates over time will be compared between the groups using Poisson regression model with repeated measures. Leave one out cross validation will be used to estimate robust standard errors.

10.9 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the NCI CTCAE v4.0.

11 DATA AND SAFETY MONITORING PLAN (DSMP)

11.1 Data and Safety Management Overview

The Medical College of Wisconsin (MCW) Data Safety Monitoring Committee (DSMC) and the MCW Institutional Review Board (IRB) will approve protocol-specific DSM plans. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with regular safety and progress reports submitted to the DSMC.

The DSMP for this study will involve the following entities:

11.2 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, regulatory specialist and the study biostatistician. While subjects are on treatment, the principal investigator will meet regularly with the research coordinator and the study biostatistician to

review study status. This review will include but not be limited to reportable SAEs and UPIRSOs and an update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed.

11.3 Quality Assurance

This protocol was classified as intermediate risk and will be reviewed internally by the MCW Cancer Center Clinical Trials Office Quality Assurance Staff according to the MCWCC Data and Safety Monitoring Plan and current version SOP, 6.5.2 Internal Quality Assurance Reviews.

11.4 Clinical Trials Office

The MCWCC Clinical Trials Office [CTO] provides administrative assistance and support to the DSMC.

11.5 DSMC

The Medical College of Wisconsin Cancer Center places the highest priority on ensuring the safety of patients participating in clinical trials. Every cancer interventional trial conducted at MCW includes a plan for safety and data monitoring.

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data and Safety Monitoring Committee (MCWCC DSMC). A summary of the MCWCC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4 and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. Non-hematological grade 4 and all grade 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge. Hematological grade 4 events can be routine reported. Given that patients included in this study will all have diarrhea, then diarrhea will not be considered an adverse event unless it worsens (increases in grade).
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCWCC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

Any available DSMC letters will be submitted to the IRB of record as required.

12 REGULATORY COMPLIANCE, ETHICS AND STUDY MANAGEMENT

12.1 Ethical Standard

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

12.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol.
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and §312 (Investigational New Drug Application; and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

12.3 Institutional Review Board

The protocol, the proposed informed consent form and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the MCW Institutional Review Board. Prior to obtaining MCW approval, the protocol must be approved by the Medical College of Wisconsin Cancer Center Scientific Review Committee. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

12.4 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product.

Consent forms will be IRB-approved and the subject (and Legally Authorized Representative, if necessary) will be asked to read and review the document. Upon reviewing the document, the investigator or designee will explain the research study to the subject and answer any questions that may arise. In accordance with 46 CR 46.111, the subject will sign and date the informed consent document prior to any procedures being done specifically for the study.

A copy of the informed consent document will be given to the subjects for their records. After the subject's visit in which the consent is signed, it is documented in the clinic chart that the consent has been signed and that all questions have been answered to the subject's satisfaction after adequate time for review of the consent. It is also documented that a copy of the consent is given to the subject. The original consent is kept with the subject's study file, and a copy of the

consent is sent to the OCRICC office, which will then submit to HIM a copy of the signed consent to be scanned into EPIC, the legal medical record.

12.5 Subject Confidentiality and Access to Source Documents/Data

Subject confidentiality is strictly held in trust by the sponsor-investigator, participating investigators, and any staff. This confidentiality includes the clinical information relating to participating subjects, as well as any genetic or biological testing.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor-investigator.

The conditions for maintaining confidentiality of the subjects' records are required for the life of the data. These rules apply equally to any and all MCWCC projects.

12.6 Protection of Human Subjects

12.6.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the informed consent process. The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

12.6.2 Protection of Privacy

As noted, patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document.

12.7 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies).

12.8 Onsite Audits

Auditing is essential to ensure that research conducted at the Medical College of Wisconsin (MCW) Cancer Center is of the highest quality and meets MCW and regulatory agency standards.

Regulatory authorities, the IRB and/or sponsor may request access to all source documents, data capture records and other study documentation for on-site audit or inspection. Direct

access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

13 DATA HANDLING AND RECORD KEEPING

13.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution, and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss or theft. This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

13.2 Case Report Forms

The principal investigator and/or his/her designee will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study-specific Case Report Forms (CRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized CRFs, in accordance with the study calendar, using single data entry with a secure access account.

All source documentation and data will be available for review/monitoring by the MCWCC DSMC and regulatory agencies.

13.3 Study Record Retention

The principal investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity and use by subjects, as well as written records of the disposition of the drug when the study ends.

In accordance with FDA regulations, the investigator shall retain records for a period of two years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and FDA is notified.

13.4 Publishing Data

At least one manuscript will be developed from the experience gathered in this study. Data owners will be the PI and co-investigator. Authorship for future publications will include Drs. Revolinski, Aldrete, Charlson, in addition to the PI and Co-PI.

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APPENDIX 1. SPECIMEN COLLECTION

Instructions for Stool Specimen Collection and Handeling

Stool collection:

- **If Stool Sample is Collected while Patient is Inpatient:**

  The nursing staff will utilize the standard inpatient stool collection kit. CRC will notify the nursing staff every day a specimen collection is required. After the specimen is collected, the nurse will place the specimen in the study-supplied refrigerator and call/page the CRC who will pick up the sample.

  - **Rectal Swab Collection**
    1. Rectal swabs may be collected from hospitalized patients if they are unable to provide a bowel movement within 24 hours of the designated collection time point. (Only stool specimens (not rectal swabs) will be collected from patients who have been discharged.)
    2. Instructions for collecting rectal swabs:
       a. Overall goal: obtain 4 swabs in a manner the patient can tolerate.
       b. Can obtain two swabs together/two insertions, or to do each one individually/4 insertions.
       c. Insert the swab(s) a few centimeters into the patient's rectum and turn gently. Withdraw the swab carefully.
       d. If the swab does not have stool on it, reinsert the swab slightly further into the patient's rectum and try again.
       e. Rectal swabs will be double bagged and placed in the study-supplied refrigerator. Nurse will call/page the CRC who will pick up the sample. However, if the PI is the staff member collecting the rectal swab, the PI will take the sample directly to the lab.

  ***Prior to patient leaving the hospital, CRC will give the patient Stool Sample Collection Kit(s), Styrofoam container(s), Shipping supplies, Drug Diaries and Instructions (as applicable).**

- **If Stool Sample is Collected while Patient is Outpatient:**
  Patients will collect stool sample(s) at home at appropriate time points according to the instructions provided. Samples may be returned to the clinic by either of the following:
  - Patients will call CRC at number provided on instructions to arrange a time to drop off samples. Appropriate locations for patients to drop off appropriately packed stool samples include (but are not limited to) the Cancer Center lab (2nd floor) or the Grace clinic (Cancer Center 4th floor).

  OR
  - If patients do not live nearby and will not be returning to the clinic at a specified collection timepoint, a courier or shipping company pre-paid box may be used (i.e. USPS, FedEx, etc.). The CRC will determine which method is appropriate for each patient.
    1. For the courier, patients must live within a 1-hour drive from the site. Patients will call CCCTO study staff to arrange a time for the courier service (CS Logistics) to pick up the sample(s). The courier service (CS Logistics) will

contact the CCCTO study staff to arrange delivery of the sample(s).

2. For the shipping company pre-paid box, patients will be provided with the proper shipping materials and shipping label.

Specimen Labeling:

All specimen containers must be labeled with the study name ("C. diff study"), patient study ID#, time point (Baseline, D1, D7, etc.), and date/time of specimen collection.

Transporting and Storage Requirements:

Study staff will pick up stool sample from CFAC unit and store in Cancer Center Clinical Trials Office (CCCTO) lab until pick up. Study staff will call and/or page research lab staff as soon as sample is received. Research lab staff will pick up samples in the CCCTO lab. No processing will be done in the CCCTO lab.

Study staff will complete a requisition for the samples (see form on next page); A copy of the requisition will be retained by the study staff in the research binder.

For inpatient samples, it is preferred that they are collected Monday thru Friday, 7 AM to 5 PM. Regardless of collection time, nurses should place the stool samples inside the CFAC study refrigerators located in the dirty supply rooms and notify the CCCTO staff.

All samples should be transported to the research lab as soon as possible after collection. Samples should be kept in a refrigerator while waiting for transportation to the research lab. Samples should be packed and transported according to IATA regulations. Personnel from Dr. Silvia Munoz-Price's lab will pick up the study samples from the CCCTO lab. CRC will coordinate sample pick up with the research lab.

Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population

"C. diff study" Specimen Requisition Form

Patient Information:

Patient Initials: _____

Patient Study ID #: _____

Research Sample Information:

Date of Sample Collection: _____ Time of Sample Collection: _____

Study time point (circle one): Pre-study Baseline Day 1 Day 7

Day 14 Day 21 Day 28 Day 90

Date and Time Lab was Notified of Sample Availability: _____

Name of Lab Staff Receiving Notification: _____

Person Completing Requisition: _____

Phone Number of Person Completing Requisition: _____

Lab Staff Picking Up Research Sample: _____

Date and Time of Pick-Up: _____

APPENDIX 2. QUESTIONNAIRES

Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population

Patient study ID # _____ Date Questionnaires completed: _____
Time Questionnaire completed: _____

Baseline Questionnaires

To be asked to patients; responses should be <u>verbal</u> and recorded by study staff.
Do NOT hand these questionnaires to the patient.

History of C. difficile infection (CDI)

NOTE: If the medical record clearly documents the presence of CDI in contradiction of the patient's response, then the study case report forms should be completed using the contents of the medical record.

1. Have you had a diagnosis of C. difficile infection prior to this admission? If yes, go to question 2. If no, skip to Medical History Questionnaire on next page.	YES	NO	
2. Have you had at least two episodes of C. difficile infection within the past 2 years?	YES	NO	
3. Have you had at least one episode within the past 6 months? If yes, go to question 4. If no, skip to Medical History Questionnaire on next page.	YES	NO	
4. During any of these previous episodes:			
a. Were you hospitalized?	YES	NO	Don't know
b. Were you in the intensive care unit?	YES	NO	Don't know
c. Were you diagnosed by colonoscopy?	YES	NO	Don't know

Questionnaire version: 06/20/17

Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population

Patient study ID # _____ Date Questionnaires completed: _____
Time Questionnaire completed: _____

Selected Medical History

1. Have you had any surgeries in the past 90 days? If yes, go to question 2. If no, skip to question 3.	YES NO
2. Have any of those been open abdominal surgeries?	YES NO
3. How many times are you currently having bowel movements **within a 24-hour period**? (circle one)	None 1 2 3 4 5 6 7 8 9 10 11 more than 11
4. Regarding your current stool pattern, when did the frequency of bowel movements increase or change?	
5. What is the consistency of the majority of your bowel movements within the past 5 days? (Use Bristol chart)	Type 1 Type 2 Type 3 Type 4 Type 5 Type 6 Type 7
6. Are you currently having abdominal pain? If yes, go to question 2. If no, skip to question 3.	YES NO
7. When did the abdominal pain start?	Date: _____
8. Currently having nausea?	YES NO
9. Currently vomiting?	YES NO
NUTRITIONAL INTAKE:	
10. Type of caloric intake (circle one):	Normal/solids Soft Liquid Parenteral/enteral
11. How much of your meals are you eating?	<50% >50% Can't tell

Questionnaire version: 06/20/17

Signature of staff member completing questionnaires: _____

Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population

Patient study ID # _____ Date Questionnaires completed: _____

Expected Time point (D7, 14, 21, 28, or 90): _____ Actual Time point: _____

On-Study Questionnaire

To be asked to patients; responses should be verbal and recorded by study staff.
Do NOT hand these questionnaires to the patient.
Questionnaires can be done in person or over the phone.

NOTE: If the medical record clearly contradicts the patient's response, then the study case report forms should be completed using the contents of the medical record.

1. Since the last questionnaire, the frequency of bowel movements has:	Increased Decreased Remains unchanged
2. How many times are you currently having bowel movements **within a 24-hour period**? (circle one)	None 1 2 3 4 5 6 7 8 9 10 11 more than 11
3. What is the consistency of the majority of your bowel movements within the past 5 days? (Use Bristol chart)	Type 1 Type 2 Type 3 Type 4 Type 5 Type 6 Type 7
4. Since the last questionnaire, have you experienced fever (temp >100.3)?	YES NO Not sure
5. Since the last questionnaire, have you had new onset of abdominal pain?	YES NO Not sure
6. Since the last questionnaire, have you been diagnosed with pseudomembranous colitis?	YES NO Not sure
7. Since the last questionnaire, have you been admitted to the hospital? If yes, go to question 8. If no, skip to question 10.	YES NO
8. If yes, which hospital?	
9. If yes, which dates were you admitted?	

Randomized double blind placebo controlled trial for the treatment of NAAT(+)/toxin EIA(-) *Clostridium difficile* in the hematology oncology population

Patient study ID # _____ Date Questionnaires completed: _____

Expected Time point (D7, 14, 21, 28, or 90): _____ Actual Time point: _____

10. Since the last questionnaire, have you been started on any new antibiotics? If yes, go to question 11. If no, skip to question 12.	YES NO Not sure
11. If yes, which antibiotic were you started on and when?	
12. Since the last questionnaire, have you been started on a new chemotherapy agent? If yes, go to question 13. If no, skip to question 14.	YES NO Not sure
13. If yes, which chemotherapy were you started on and when?	
14. Since the last questionnaire, has there been any changes in your diet?	Normal/solids Soft Liquid
15. Since the last questionnaire, have you had any new or worsened medical problems?	

Signature of staff member completing questionnaires: _____

Questionnaire version: 11/20/18