

COMIRB Protocol

COLORADO MULTIPLE INSTITUTIONAL REVIEW BOARD

CAMPUS BOX F-490 TELEPHONE: 303-724-1055 Fax: 303-724-0990

Protocol #: 21-2851

DMID protocol # 21-0014: Funding provided by NIH U01 AI150589

Project Title:

Do decreased dietary fat and increased fiber reduce recurrence of *Clostridioides difficile* infection in oncology patients?

Principal Investigator:

Catherine Lozupone, PhD

University of Colorado, Anschutz medical Campus

12700 East 19th Avenue

Aurora, CO 80045

Catherine.Loizupone@cuanschutz.edu

Version Date: October 10, 2025

TABLE OF CONTENTS

TABLE OF CONTENTS.....	2
PROTOCOL SUMMARY	6
1 BACKGROUND AND SCIENTIFIC RATIONALE	10
1.1 Background.....	10
1.2 Scientific Rationale.....	11
1.2.1 Purpose of Study	11
1.2.2 Study Population.....	11
1.3 Potential Risks and Benefits	11
1.3.1 Potential Risks	11
1.3.2 Potential Benefits	12
2 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES	13
2.1 Study Design Description	13
2.2 Study Objectives	14
2.2.1 Primary	14
2.2.2 Secondary	14
2.3 Study Endpoints or Outcome Measures	14
2.3.1 Primary	14
2.3.2 Secondary	14
3 DIET INTERVENTION.....	16
3.1 Diet Intervention Overview	16
3.1.1 Dietary Targets.....	16
3.1.2 Diet design, delivery and compliance assessment.	16
4 SELECTION OF SUBJECTS AND STUDY ENROLLMENT AND WITHDRAWAL.....	17
4.1 Eligibility Criteria.....	17
4.1.1 Subject Inclusion Criteria.....	17
4.1.2 Subject Exclusion Criteria	17
4.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination	18
4.2.1 Withdrawal from the Study or Discontinuation of the Study Product.....	18
4.2.2 Subject Replacement.....	18
4.2.3 Study Termination	18

5	STUDY PROCEDURES	19
5.1	Screening	19
5.2	Enrollment	20
5.3	Planned Study Visits.....	20
5.3.1	Follow-up.....	20
5.3.2	Final Study Visit	21
5.3.3	Early Termination Visit	21
6	DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS	22
6.1	Clinical Evaluations.....	22
6.2	Laboratory Evaluations.....	22
6.2.1	Research Assays.....	22
7	ASSESSMENT OF SAFETY.....	23
7.1	Assessing and Recording Safety Parameters	23
7.1.1	Adverse Events (AEs).....	23
7.2	Specification of Safety Parameters	25
7.2.1	Solicited Events	25
7.2.2	Unsolicited Events	25
7.3	Halting Rules	26
7.3.1	Study Halting Criteria	26
7.3.2	Individual Halting Criteria.....	26
7.4	QUALITY CONTROL AND QUALITY ASSURANCE	26
7.5	Institutional Data Safety Monitoring Plan.....	28
8	HUMAN SUBJECTS PROTECTION	29
8.1	Informed Consent Process	29
8.1.1	Requirements for Permission by Parents/Guardians and Assent by Children (in case of a minor)	29
8.2	Consent for Future Use of Stored Specimens and Data	30
8.3	Exclusion of Women, Minorities, and Children (Special Populations).....	30
9	STATISTICAL CONSIDERATIONS	31
9.1	Study Hypotheses	31
9.2	Sample Size Considerations	31
9.3	Treatment Assignment Procedures	32
9.3.1	Randomization Procedures	32

	9.3.2 Masking Procedures	32
	9.3.3 Interim Safety Review	32
	9.4 Final Analysis Plan	32
10	LITERATURE REFERENCES.....	33
11	APPENDICES	34
	Appendix A. Schedule of Events.....	34

CERTIFICATE OF CONFIDENTIALITY

To protect privacy, the NIH has provided a Certificate of Confidentiality, CoC. With this CoC, the participating sites cannot be forced to release information that may identify the participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The participating sites will use the CoC to resist any demands for information that would identify the participant, except as explained below.

The CoC cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects, like this study or local laws, such as for reporting of communicable diseases.

A CoC does not prevent the participant from voluntarily releasing information about themselves or their involvement in this research. If any person or agency obtains a written consent to receive study information, then the participating sites may not use the CoC to withhold that information.

The CoC does not prevent the participating sites from reporting without the participant's consent, information that would identify the subject as a participant in the study regarding matters that must be legally reported including: child and elder abuse, sexual abuse, or wanting to harm themselves or others.

PROTOCOL SUMMARY

Title:	Does decreased dietary fat and increased fiber reduce recurrence of <i>Clostridioides difficile</i> infection in oncology patients? Randomized control trial
Design of the Study:	Oncology patients diagnosed with a malignant neoplasm and a <i>C. difficile</i> infection and who are eating a low-fiber, high-fat diet will be randomized to a diet intervention (n=30) or non- diet intervention (n=30) group. Oncology patients already consuming a higher-fiber and/or lower fat diet will be recruited as a second non-intervention group (n=15). Oncology patients diagnosed with a malignant neoplasm and a <i>C. difficile</i> infection who are receiving the majority of their nutrition from tube feeds or intravenous nutrition will be recruited to an additional non-intervention cohort (n=15). The diet intervention group will undergo a 6-week diet intervention aimed to decrease dietary fat and increase dietary fiber. All study participants will collect 1 fecal sample per week, which will be used to assess <i>C. difficile</i> carriage, toxin production and microbiome composition (secondary outcomes).
Study Phase:	1
Study Population:	The study population will include 90 individuals with a malignant neoplasm who are receiving therapy at Children's Hospital Colorado and University of Colorado Hospital. All study participants will be 9 years old or older. Study participants are expected to be split approximately evenly between male and female and to live in the areas surrounding the enrolling hospitals.
Number of Sites:	1
Description of Study Product or Intervention:	The diet intervention will target an age-matched dietary saturated fat intake at the 25 th percentile of average American intake (19 grams/day (g/d) for ages 9-18 years, for adults 18g/d for females and 24g/d for males) is in the context of a diet designed to be 30% total fat (as a percentage of total daily intake; also at the 25 th percentile). The dietary fiber intake target will be 19 g/d for ages 9-18 and females age 19+ and 23g/d for males age 19+. Enrolled patients and their parent/guardians (if applicable) will

meet with the team of Dr. Janine Higgins to assess current diet and food preferences. This information will be used to design lists of preferred foods from which the study participant will be asked to eat a certain number of items per day. For instance, the patient will receive a list of high fiber foods such as fruits and vegetables that the patient likes to choose among to meet fiber targets. The patient will also receive a list of items high in saturated fat such as meats and cheeses that they can choose a limited amount of. Based on these food preferences, we will provide groceries that support each patient's meal plan once per week via online home delivery service (e.g. Amazon Pantry). Compliance will be assessed using consumption checklists for the provided food items weekly and three 24-hour food recalls.

Study Objectives:

Primary:

- Determine whether dietary intervention to increase fiber and decrease fat reduces *C. difficile* infection recurrence in a cohort of oncology patients compared to non-intervention.

Secondary:

- Determine whether dietary intervention to increase fiber and decrease fat reduces *C. difficile* carriage/abundance and toxin production in a cohort of oncology patients.
- Determine whether dietary intervention increases fiber and decrease fat increases fecal short acid concentration (SCFA), abundance of bacterial genes for SCFA and bile acid metabolism in a cohort of oncology patients.
- Determine whether dietary intervention increases alpha diversity of the fecal microbiome in a cohort of oncology patients.
- Compare *C. difficile* recurrence, carriage/abundance and toxin production, and microbiome and metabolite (SCFA and bile acid) compositions between the intervention and non-intervention regular diet arms and individuals receiving nutrition via NG or G tube or IV

**Duration of Individual
Subject Participation:**

6 weeks

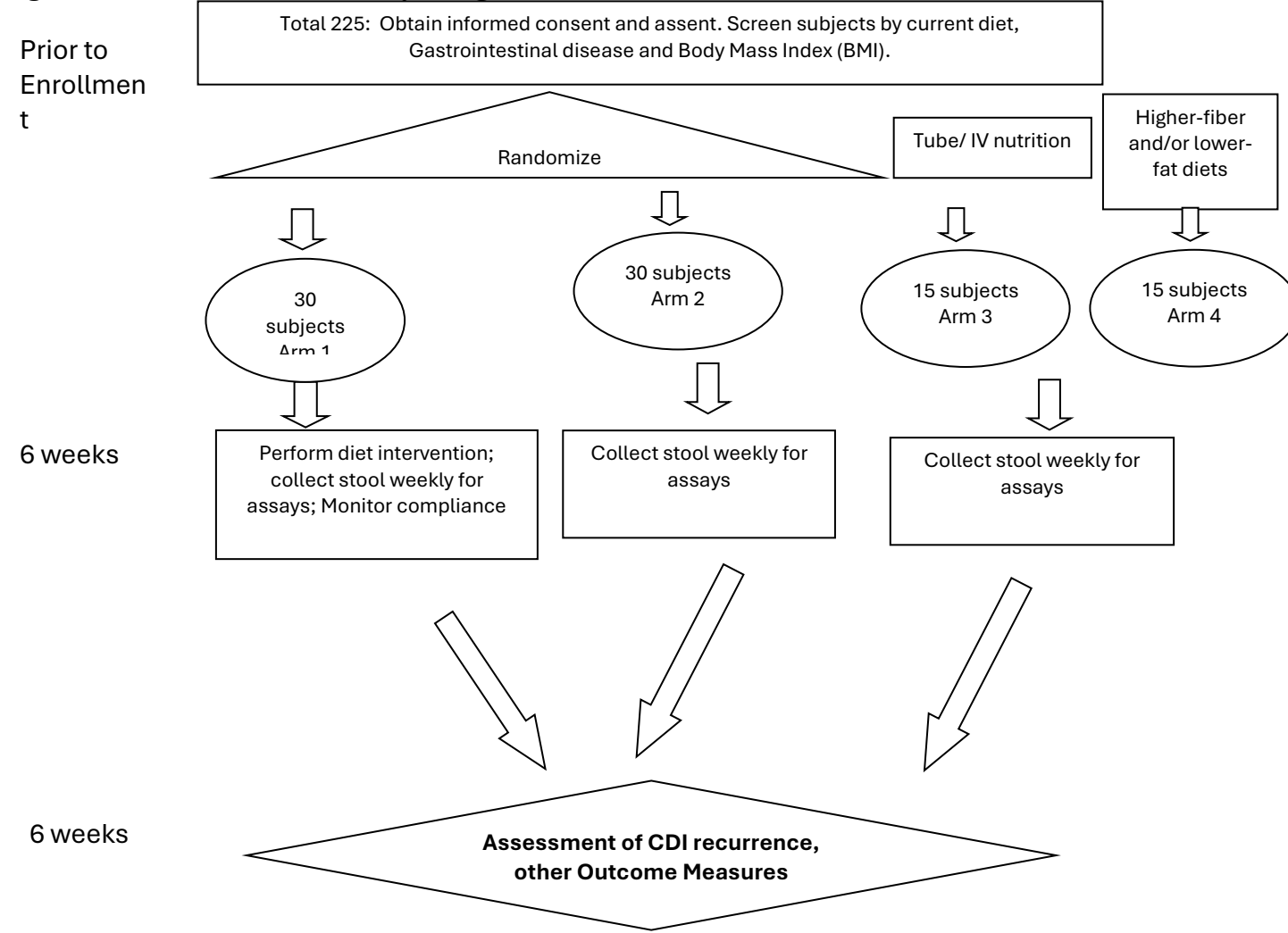
**Estimated Time to Last
Subject/Last Study Day:**

3.2 years

Table 1: Treatment Arms

Cohort A	Low-fiber/high-fat diet ARM 1	30	Diet intervention
	Low-fiber/high-fat diet ARM 2	30	No diet intervention
Cohort B	Tube/ IV Nutrition	15	No diet intervention
Cohort C	Higher-fiber and/or lower-fat diet	15	No diet intervention

Figure 1: Schematic of Study Design



1 BACKGROUND AND SCIENTIFIC RATIONALE

1.1 Background

The incidence of *C. difficile* infection (CDI) has been rising over the past decade (1) with IBD and immune suppressed patients being at increased risk (2). In pediatrics, ~5% oncology patients develop CDI at some time during treatment (3) and a recent study conducted at Children's Hospital Colorado, where we will recruit in part for this clinical trial, showed stool colonization with *C. difficile* in 29% of pediatric oncology patients without gastrointestinal symptoms and in 55% of patients with prior CDI (4). Unfortunately, continued surveillance for CDI in this population has indicated a rise in cases despite best efforts to curb infection rates (Fig. 2).

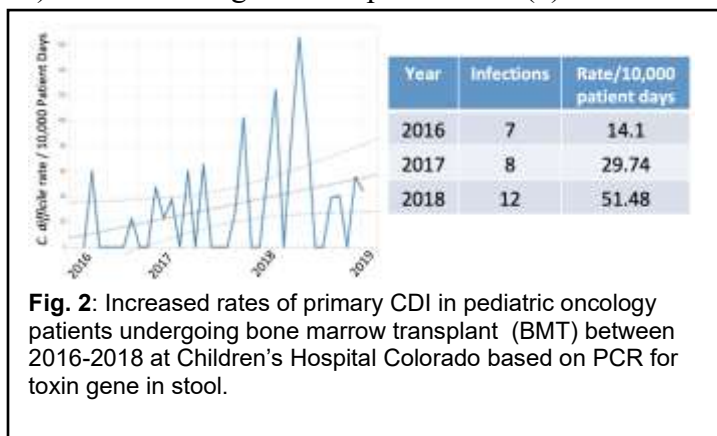


Fig. 2: Increased rates of primary CDI in pediatric oncology patients undergoing bone marrow transplant (BMT) between 2016-2018 at Children's Hospital Colorado based on PCR for toxin gene in stool.

In this study, we will test the effects of dietary modulation on colonization with *C. difficile* and microbiome composition in an oncology cohort. The diet intervention aims to increase intake of soluble and insoluble fibers and decrease fat intake. The potential for increased fiber to decrease CDI recurrence has been supported by work conducted in a mouse model. Diets deficient in fiber perpetuated CDI in a murine model of antibiotic-induced CDI (5) and introduction of the soluble fiber inulin into the diet reduced *C. difficile* toxin production. The studies shown in our preliminary data also support a detrimental effect of high dietary fat in a murine CDI model. Specifically, we have found that a high-fat/low-fiber diet coupled with antibiotic treatment is associated with increased microbiome disturbance following broad spectrum antibiotic challenge, and increased morbidity and mortality upon exposure to *C. difficile*. However, we have found that lack of dietary fiber alone does not result in increased mortality, weight loss or other signs of increased disease severity that we did observe in low fiber diets that were also high in fat. This indicates that the effects of a high-fat/low-fiber diet on CDI may be two-fold; the reduction in fiber creates an ecological space for *C. difficile* to occupy, while the increase in fat exacerbates CDI pathology through altering host bile acid excretion and impairing microbial bile acid catabolism. Dietary intervention to increase fiber and decrease fat thus seems to be rational, practical, inexpensive and likely safe for the prevention of CDI recurrence.

1.2 Scientific Rationale

1.2.1 Purpose of Study

The purpose of this study is to test the effects of dietary modulation on colonization with *C. difficile* and microbiome composition in an oncology cohort. The diet intervention aims to increase intake of soluble and insoluble fibers and decrease fat intake in individuals consuming low-fiber, high-fat diets. We hypothesize that a 6-week intervention to increase dietary fiber and decrease dietary fat will lead to decreased CDI recurrence compared to a control cohort of individuals consuming a low-fiber, high-fat diet that did not undergo diet intervention. We also would like to recruit individuals eating a range of healthier diets to the non-intervention arm, including individuals already consuming higher-fiber and/or lower-fat diets so that we can compare *C. difficile* recurrence rates across different dietary intakes. Finally, we will compare these cohorts to individuals receiving nutrition via IV or NG/G tubes. IV feeds are of interest because no food will reach the gut microbiome and the formula feeds currently used in NG/G tubes in clinical practice are highly devoid of dietary fiber. A six week intervention was selected based on the time frame during which rCDI typically occurs and on prior work from other research groups that has used a similar time frame to study the effects of use of probiotics and antibiotics on rCDI (6-8).

1.2.2 Study Population

We will target the recruitment of patients with a diagnosis of a malignant neoplasm with a first incident case of CDI or first-time recurrence of CDI. All patients who meet the defined eligibility criteria will be actively recruited into our study. The demographics of the study participants will reflect the demographics of oncology patients in Colorado. Study participants are expected to be Caucasian (~68%), Hispanic (~20%), African American (~8%) and Asian (~4%). Approximately 50% of participants will be female. The anticipated age range of study participants is 9-70 years old. Oncology patients will be recruited at Children's Hospital Colorado and University of Colorado Hospital after diagnosis of CDI by the patient's primary oncologist. The study will not include participants at international sites. Women, minorities, and/or children will not be excluded.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

The risks involved in study participation are secondary to clinical data collection, stool collection and diet intervention.

Data collection: Though data will be securely stored without identifying factors, the possibility exists for loss of confidentiality.

Stool collection: Stool is collected in a plastic container that fits in the toilet seat or swabbing used toilet paper. Stool collection should not present any risk to the study participant. Individuals may experience embarrassment about providing fecal materials to the researchers.

Diet Intervention: The intervention diet will be higher in fiber and lower in total fat than the diet typically consumed by study participants (as determined with a food frequency questionnaire). Although the dietary fiber intake target of 20g/d is lower than the daily recommended fiber intake of 31-38 g/d for males and 26 g/d for females, it will still be a substantial increase for

participants who habitually consume less than 15 g/d (as per inclusion criteria) and there is a chance that it could cause increased symptoms such as flatulence or bloating. The intervention may also be terminated if it appears to be causing intestinal distress. The diet intervention is also designed to accommodate food preferences as we appreciate that food preferences in cancer patients, particularly when on chemotherapy, can be strong. As weight stability is important in oncology cohorts, we will assess weight stability during the intervention and if changes of > 4% occur within 4 days (e.g. 6lbs in a 150 lb patient), nutritionists will meet with the participant and the intervention may be terminated.

1.3.2 Potential Benefits

Study participants on the diet intervention arm may benefit from a decreased likelihood of developing CDI recurrence and other known health benefits from diets lower in fat and higher in fiber. There is no anticipated direct benefit to individuals randomly assigned to the “no diet intervention” arm. The knowledge gained concerning the effects of diet on CDI will benefit other individuals who are at high risk of CDI.

2 STUDY DESIGN, OBJECTIVES AND ENDPOINTS OR OUTCOME MEASURES

2.1 Study Design Description

This is a randomized control single center trial, to assess the effects of increased dietary fiber and decreased fat on CDI recurrence in a population of oncology patients. Study participants will all be oncology patients with a diagnosis of a malignant neoplasm at Children's Hospital Colorado and University of Colorado Hospital. A simple randomization procedure with stratification will be used to assign study participants to the "diet intervention" (n=30) or "no diet intervention" (n=30) arm of the study. The randomization procedure will stratify by gender and age with the following 5 age blocks: 9-13, 14-18, 19-30, 31-49, and >50. This is to limit potential confounding. Patients will be approached from enrollment after first incident case of CDI or first-time recurrence. The expected duration of subject participation is 6 weeks.

For pediatric participants, assent from patients and consent from their parents/guardians will be obtained. Patients 18 years old and old will provide consent. Enrolled patients and, if applicable, their parent/guardians will meet with the team of Dr. Higgins to assess current diet and food preferences. This information will be used to design lists of preferred foods from which the child will be asked to eat a certain number of items per day. For instance, the patients will receive a list of high fiber foods such as fruits and vegetables that the patient likes to choose among to meet fiber targets. The patient will also receive a list of items high in saturated fat such as meats and cheeses that they can choose a limited amount of. Based on these food preferences, we will provide groceries that support each patient's meal plan once per week via online home delivery service (e.g. Amazon Pantry). Compliance will be assessed using consumption checklists for the provided food items weekly and three 24-hour food recalls. As weight stability is important in oncology cohorts, we will assess weight stability during the intervention and if changes of >4% occur within 4 days (e.g. 6 lbs in a 150 lb individual), nutritionists will meet with the participant and the intervention may be terminated. Fecal samples will be collected at baseline and one time per week during the 6 weeks of the study (7 samples per patient total). Stool consistency will also be monitored twice per week using the Bristol stool scale once with the provided stool samples and once during the weekly phone interview) and the intervention may also be terminated if it appears to be causing intestinal distress. 24-hour food recalls will be conducted every other week (3 times total) during the phone interview. Other factors that may affect microbiome composition, such as antibiotics and chemotherapy or other drugs, will also be closely monitored both prior to and during the diet intervention. The non-intervention study participants will also provide stool and 24-hour food recalls and be monitored for stool consistency and any antibiotics or chemotherapy drugs taken. These include non-intervention participants randomized to the "no-intervention" arm (Cohort A; Table 1) and non-intervention participants on tube/ IV nutrition or already consuming higher-fiber/lower-fat diets (Cohorts B ,C Table 1, Figure 1).

Schematic of Study Design - See
Figure 1 and Table 1

2.2 Study Objectives

2.2.1 Primary

Determine whether dietary intervention to increase fiber and decrease fat reduces CDI recurrence in a cohort of oncology patients compared to both those consuming a regular diet randomized to a non-intervention arm, and to those getting the majority of their nutrition from an IV of NG/G tube.

2.2.2 Secondary

Determine whether dietary intervention to increase fiber and decrease fat reduces *C. difficile* carriage/abundance and toxin production in a cohort of oncology patients compared to both those consuming a regular diet randomized to a non-intervention arm, and to those getting the majority of their nutrition from an IV of NG/G tube.

Determine whether dietary intervention increases fiber and decrease fat increases fecal short acid concentration (SCFA), abundance of bacterial genes for SCFA and bile acid metabolism in a cohort of oncology patients compared to both those consuming a regular diet randomized to a non-intervention arm, and to those getting the majority of their nutrition from an IV of NG/G tube.

Determine whether dietary intervention increases alpha diversity of the fecal microbiome in a cohort of oncology patients compared to both those consuming a regular diet randomized to a non-intervention arm, and to those getting the majority of their nutrition from an IV of NG/G tube.

Determine whether CDI rates are related to dietary intake among individuals consuming diets across a range of high and low fat and high and low fiber among individuals with no diet intervention.

2.3 Study Endpoints or Outcome Measures

2.3.1 Primary

- C. difficile infection recurrence: CDI recurrence (yes/no) will be the primary outcome. Study participants will be asked to report any symptoms of active CDI to the study coordinator throughout the 6-week diet intervention and also for the following 6 months. Signs of active diarrhea will also be monitored during the diet intervention based on the Bristol stool scale during the weekly collections of fecal samples and weekly phone interviews. Any symptoms will immediately be investigated as a potential recurrence of CDI using PCR.

2.3.2 Secondary

- C. difficile abundance/toxin production: Fecal aliquots will be resuspended in PBS, and 10-fold serial dilutions of fecal slurries (through 10^{-3} -fold) will be plated on selective *C. difficile*

Moxalactam Norfloxacin blood agar to determine colony forming units as described in (5, 9). Toxin will be quantified in feces using the “Separate detection of *C. difficile* toxins A and B” kit (tgc Biomics) by relating levels to a standard curve of purified TcdB. [Timeframe: baseline plus once per week during 6-week intervention – 7 samples total]

- Microbiome characterization: We will also characterize the microbiome in fecal samples as using 16S rRNA targeted and shotgun metagenomic sequencing [Timeframe: baseline plus once per week during 6-week intervention – 7 samples total].
- Metabolite characterization: We will also characterize SCFA and bile acids using targeted GC/MS and LC/MS respectively.

3 DIET INTERVENTION

3.1 Diet Intervention Overview

The patient, and if applicable the family, will receive a list of high fiber foods such as fruits and vegetables that the patient likes to choose among to meet fiber targets. The patient will also receive a list of items high in saturated fat such as meats and cheeses that they can choose a limited amount of. Based on these food preferences, we will provide groceries that support each patient's meal plan once per week.

3.1.1 Dietary Targets

The diet intervention will target a dietary saturated fat intake at the 25th percentile of average American intake (19 grams/day (g/d) for ages 9-18 years, for adults 18g/d for females and 24g/d for males) in the context of a diet designed to be 30% total fat (as a percentage of total daily intake; also at the 25th percentile). The dietary fiber intake target will be 19 g/d for ages 9-18 and females age 19+ and 23g/d for males age 19+. in the context of a diet designed to be 30% total fat (as a percentage of total daily intake; also at the 25th percentile). Although these targets are lower than the daily recommended fiber intake of 31-38 g/d for males and 26 g/d for females, they will still be a substantial increase for participants who habitually consume less than 15 g/d (as per inclusion criteria).

3.1.2 Diet design, delivery and compliance assessment.

The patient will receive a list of high fiber foods such as fruits and vegetables that the patient likes to choose among to meet fiber targets. The patient will also receive a list of items high in saturated fat such as meats and cheeses that they can choose a limited amount of. Based on these food preferences, we will provide groceries that support each patient's meal plan once per week. Groceries will be provided via an online home delivery service (e.g. Amazon Pantry). All food items supplied can be stored either at room temperature or in a standard refrigerator and safe for consumption by study participants within 1 week of receipt. Compliance will be assessed with 24-hour food recalls.

4 SELECTION OF STUDY PARTICIPANTS AND STUDY ENROLLMENT AND WITHDRAWAL

4.1 Eligibility Criteria

4.1.1 Inclusion Criteria

Study participants must meet all the inclusion criteria in order to be eligible to participate in the study.

- 1) Diagnosis of a malignant neoplasm at the at Children's Hospital Colorado or the University of Colorado Hospital
- 2) Diagnosis of first time or first recurrent CDI as defined by clinical criteria within the past 21 days
- 3) Informed consent obtained and signed. For minors, the consent will be completed by a guardian and the study participant will sign the assent form. Only the consent form is required of adults.
- 4) 9 years old or older
- 5) 40th to 100th percentiles of saturated fat intake and below the 50th percentile for fiber intake based on a food frequency questionnaire for individuals in cohort A (randomized to intervention versus non-intervention arm) or on a NG/G tube or IV regimen (Cohort C) or 1st to 39th percentiles of saturated fat intake and above the 50th percentile for fiber intake based on a food frequency questionnaire (Cohort B)
- 6) Ability to comply with study procedures for the entire length of the study.

4.1.2 Exclusion Criteria

All study participants meeting any of the exclusion criteria at baseline will be excluded from study participation.

- 1) Inflammatory bowel disease: ulcerative colitis, Crohn's disease, or microscopic colitis
- 2) Toxic megacolon or celiac sprue.
- 3) BMI Z-score greater than -1.5 (to exclude malnourished individuals)
- 4) Participants with more than 2 episodes of CDI within 6 months
- 5) Female and pregnant or lactating
- 6) Major gastrointestinal (GI) surgery (that is, significant bowel resection including total colectomy with ileostomy) within 3 months of enrollment (this does not include appendectomy or cholecystectomy)
- 7) Known current positive stool cultures for other enteropathogens, including but not limited to Salmonella, Shigella, and Campylobacter
- 8) Any condition that, in the opinion of the Investigator, might interfere with study objectives
- 9) Life expectancy is less than 6 weeks
- 10) Unable to comply with the protocol requirements

4.2 Withdrawal from the Study, Discontinuation of Study Product, or Study Termination

4.2.1 Withdrawal from the Study or Discontinuation of the Study Product

Subjects may voluntarily withdraw their consent for study participation at any time without penalty or loss of benefits to which they are otherwise entitled.

An investigator may also withdraw a subject from receiving the study product for any reason. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision must be recorded in the case report forms (CRFs).

The reasons, might include, but are not limited to the following:

- Subject no longer meets eligibility criteria
- Subject becomes noncompliant
- Medical disease or condition, or new clinical finding(s) for which continued participation, in the opinion of the investigator might compromise the safety of the subject, interfere with the subject's successful completion of this study, or interfere with the evaluation of responses
- Subject lost to follow-up
- Determined by a physician's discretion to require additional therapy not indicated in the protocol to ensure subject's health and well-being (or treatment failure, if applicable)

The investigator will inform the subject that already collected data will be retained and analyzed even if the subject withdraws from this study.

4.2.2 Subject Replacement

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the informed consent form (ICF) and administration of the study product will not be replaced.

Subjects who withdraw, or are withdrawn from this study, or are lost to follow-up after signing the ICF but before administration of the study product may be replaced.

4.2.3 Study Termination

If the study is prematurely terminated by the sponsor, any regulatory authority, or the investigator for any reason, the investigator will promptly inform the study subjects and assure appropriate therapy or follow-up for the subjects, as necessary. The investigator will provide a detailed written explanation of the termination to the IRB/IEC.

5 STUDY PROCEDURES

5.1 Screening

Potential study participants will be recruited through clinical providers within inpatient and outpatient settings, including at the UCHealth Clinical Assessment and Rapid Evaluation (CARE) clinic on the Anschutz Medical Campus. The RECRUIT tool set will also be utilized which offers a variety of recruitment tools used to assist research teams in recruitment efforts by providing a way for teams to connect with UCHealth patients using My Health Connection. We will utilize 1) Research Preference Profile: Patients use MHC to indicate their research interests by providing their preferences for the types of research studies they want to learn more about. Patients can update or change their preferences over time. 2) “I’m Interested” Button: Displays a virtual flyer to potential eligible patients in MHC and allows them to indicate interest in the study. These tools will be used by the study coordinator to identify patients in Epic that are meet our study criteria and should be contacted to gauge interest in participation in the study. We will also identify potential study participants using prescreening efforts by study coordinators. Cancer patients with new CDI diagnoses will be primarily prescreened and recruited. Additionally, to try and identify more outpatient participants, we will be reviewing the pharmaceutical records of UCHealth patients. Particularly, we will be using EPIC to look for the prescription of oral Fidaxomicin or Vancomycin, two antibiotics that are almost exclusively used in the treatment of C.diff infections. Potential study participants may sometimes be provided a CoMIRB-approved flyer giving a brief introduction to the study. Subjects and if applicable their parent/guardian will discuss the study with an investigator or study coordinator in person and if subjects are willing to participate, informed consent will be obtained from the patient or for subjects under 18 years old from the parent/guardian and assent obtained from the minor. The investigator or nurse will review the information contained in the assent and consent forms with each patient and parent/guardian. It will be explained that participation in the study will be of no benefit to them and if they decide not to participate, their medical care will not be affected. Potential study participants will be informed that they can withdraw from participation at any time. The subject will be asked to read and sign an informed consent/assent approved the institutional review board. Subjects will have the opportunity to review the informed consent/assent forms for as long as they wish prior to signing the informed consent/assent documents. Subjects must demonstrate an understanding of the reasons for the study, the risks and benefits, to the investigator before signing the informed consent/assent documents. The screening evaluation will not be performed until after the subject agrees to participate in the study and signs the informed consent/assent documents. Copies of the consent/assent documents will be given to the study participant.

Typical diet in subjects who have assigned the consent/assent documents will then be evaluated with the BLOCK (version 2014) Food Frequency Questionnaire (10). For adult participants this will be administered via an online link during the screening visit. For kids <18 years old, administration will be done by a trained member of the diet core to ensure understanding since this questionnaire was designed for adults. Individuals who meet the Inclusion/Exclusion criteria will be enrolled in the study. Screening will be carried out within 20 days prior to enrollment.

5.2 Enrollment

Signed informed consent and assent will have already been determined during screening.

Enrolled patients who are in the diet intervention arm and, if applicable, their parent/guardians will meet with the team of Dr. Higgins to assess food preferences. This information will be used to design lists of preferred foods from which the patient will be asked to eat a certain number of items per day during the 6-week diet intervention. They will also be interviewed for any new medical changes or BMI changes to confirm that a subject still meets eligibility criteria.

To account for potential confounders we will collect extensive meta-data from each patient during enrollment both from their medical records and interview. These will include antibiotic use for the prior 6 months, chemotherapy regimen, other current medications, medical and surgical history, smoking status, absolute neutrophil count, age, gender, weight, and height.

Fecal sample collection kits to use at home will be provided to individuals in both the “diet intervention” and “non diet intervention” arm during enrollment.

5.3 Planned Study Visits

No study visits are required after study enrollment. The 24-hour food recalls will be conducted over the phone every 2 weeks (3 total) by trained personnel from the nutrition core. In all cases it will be conducted with the study participant (e.g. not their parent if a child). Fecal sample collection kits and food items will be given to study participants by mail. Fecal sample collection kits may also be provided during routine outpatient visits. Fecal samples will also be returned by mail. Consumption checklists for the provided food will also be collected by mail with the fecal samples. Study participants will only come in if there is a concern about e.g. weight loss and meeting with a trained nutritionist is deemed appropriate to determine whether an individual should continue to participate in the study.

Study participants will be contacted by phone weekly during the 6-week duration of the experiment. A member of the study team will review the patient’s electronic medical record to monitor for diagnosis of CDI for 6 months after the diet intervention begins and for any changes in medications such as antibiotics. Study participants will also be monitored for potential adverse events (weight loss and GI symptoms) during these calls. Any potential adverse effects will be immediately reported to the study PI (Dr. Lozupone). Dr. Lozupone will discuss the reports with Dr. Gutman (adults) or Dr. Hartman (pediatric) to determine whether the study participant should remain in the study.

5.3.1 Follow-up

The primary outcome will be CDI recurrence. This will be determined by the patient’s primary oncology team based on the following criteria:

- 3 or more unformed stools (Bristol Stool Scale 6 or 7) in 24 hours
- Positive *C. difficile* Toxin B PCR test
- For multiplex PCR testing, no other pathogens detected

Study participants will be contacted weekly by phone by study personnel during the intervention and asked to report any CDI symptoms, changes in weight of >4% occurring within 4 days (e.g. 6 lbs in a 150 lb individual), or issues with the diet.

5.3.2 Final Study Visit

There are no scheduled visits after the study participant and family have met with our nutrition team. We will follow up by phone only. If the study participant, the study participant's family or the study team are concerned about the study participant's weight status after the study, these concerns will be communicated the study participant's oncologist and continued care will be provide through a trained nutritionist.

5.3.3 Early Termination Visit

If the study participant, the study participant's family or the study team are concerned about the study participant's weight status they will meet with a trained nutritionist that is part of the study team. During the visit the study participant will be weighed and a detailed dietary history will be obtained. The team will attempt to augment the study participants caloric intake, however if the team does not feel adjustments will have a high chance of success, the study participant will be withdrawn from the study. The study team will communicate their concerns about the study participant's weight status to the participants oncologist and nutritional support will be provided during their regular clinic visits.

6 DESCRIPTION OF CLINICAL AND LABORATORY EVALUATIONS

6.1 Clinical Evaluations

The following evaluations will be conducted at screening and enrollment:

- Participant height and weight. If there are concerns of weight loss during the intervention, height and weight will be reassessed during study visit with nutritionist.
- Allergy history – foods that the participant is allergic to or sensitive to will be excluded from the participant's meal plan
- Detailed dietary history – diet history will be used to screen patients for eligibility and, if enrolled, to tailor the meal plan for participants assigned to the intervention arm

6.2 Laboratory Evaluations

6.2.1 Research Assays

- Up to 7 bulk samples will be collected from the study participant. One sample will be collected shortly after enrollment, followed by once weekly stool collections during the six weeks of the protocol. All research assays will be conducted on the stool samples.
- *C. difficile* toxin B PCR, Lozupone laboratory, University of Colorado – 250 micrograms of stool
- 16S rRNA gene sequencing, Lozupone laboratory, University of Colorado – 250 micrograms of stool
- Bacterial Shotgun metagenome sequencing Lozupone laboratory, University of Colorado – 250 micrograms of stool

6.2.1.1 Laboratory Specimen Preparation, Handling, and Storage

Stool collection will be performed by the participant and/or their family at home. The participant will be provided with a collection kit that includes a disposable commode, collection utensils, storage tube, and disposable gloves. Participant will store the stool sample at 4°C before shipping the sample to the study team. Samples will be shipped in insulated containers with cold-packs to ensure the samples stay below 4°C during shipment. Upon arrival to the Lozupone lab the stool samples will be aliquoted by laboratory personnel into 2 g samples in a biosafety cabinet and then stored at -80°C.

Samples will be labelled with the participants anonymized study ID and the week of the study (e.g. baseline sample for participant #2 will be labelled 'CCBD02-0').

6.2.1.2 Laboratory Specimen Shipping

Up to 7 samples will be shipped during the protocol. One baseline stool sample and one stool samples each week during the 6-week protocol. Samples will be collected and packed by the study participant and their family and shipped to the Lozupone laboratory.

7 ASSESSMENT OF SAFETY

7.1 Assessing and Recording Safety Parameters

7.1.1 Adverse Events (AEs)

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. CRF Title 21 sec 312.b). An Adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgement about causality.

7.1.1.1 Adverse Events Grading

All AEs (laboratory and clinical symptoms) will be graded for severity and assessed for relationship to the diet intervention. AEs characterized as intermittent require documentation of onset and duration of each episode. The start and stop date of each reported AE will be recorded on an appropriate data collection form. Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of intensity.

Severity of Event:

The following guidelines will be used to quantify severity of AEs:

- Mild (Grade 1): Events that are usually transient and may require only minimal or no treatment or therapeutic intervention and generally do not interfere with the subject's usual activities of daily living.
- Moderate (Grade 2): Events that are usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
- Severe (Grade 3): Events interrupt usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention. Severe events are usually incapacitating.

Relationship to Study Product: The assessment of the AE's relationship to study product will be done by Dr. Gutman (adults) or Dr. Harman (pediatric) and the assessment will be part of the documentation process. Whether the AE is related or not, is not a factor in determining what is or is not reported in this trial. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

In a clinical trial, the study product must always be suspect. The relationship to study product will be assessed for AEs using the terms related or not related:

- Related – There is a reasonable possibility that the diet intervention caused the AE. Reasonable possibility means that there is evidence to suggest a causal relationship between the diet intervention and the AE.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

The study team will document AEs from Study Day 0, through the end of the 6 week protocol, Study Day 42. During weekly phone contact with the participant and their family, the study team will solicit if the participant is experiencing any GI symptoms, including but not limited to abdominal pain, bloating, gassiness, heartburn, nausea, vomiting, diarrhea, or constipation. The team will also solicit if the participant or family have concerns that the participant is losing weight.

All AEs will be captured on the appropriate data collection form. Information to be collected for AEs includes event description, date of onset, assessment of severity, relationship to study product and alternate etiology, date of resolution, seriousness and outcome. AEs occurring during the trial collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

Any medical condition that is present at the time that the subject is screened will be considered as baseline and not reported as an AE. However, if the severity of any pre-existing medical condition increases, we will record it as an AE.

7.1.2 Serious Adverse Events (SAEs)

An AE or suspected adverse reaction is considered a serious adverse event (SAE) if, in the view of either the site principal investigator or sponsor, it results in any of the following outcomes:

- Death,
- a life-threatening adverse event¹,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

¹ Life-threatening adverse event. An AE is considered “life-threatening” if, in the view of either the site principal investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

SAEs will be:

- Assessed for severity and relationship to the diet intervention and alternate etiology (if not related to the diet intervention) by Dr. Gutman (adults) or Dr. Hartman (children).

- Recorded on the appropriate SAE data collection form.
- Followed through resolution by Dr. Gutman (adult) or Dr. Hartmen (children)

7.2 Specification of Safety Parameters

7.2.1 Solicited Events

Solicited events are AEs that are common and known to occur following administration of study product.

The study team will be in contact with the study participants and their families on a weekly basis by phone. During those phone call a member of study team will inquire if the participant or family have concerns about weight loss, poor appetite or poor tolerance of the intervention diet and if participant has experienced any adverse gastrointestinal symptoms, including but not limited to:

- Abdominal pain
- Nausea
- Bloating
- Gassiness
- Vomiting
- Diarrhea
- Constipation

The study team will inquire about the onset and during of the symptoms and if applicable the location and character of pain. If reported, these symptoms will be reviewed by a licensed physician and followed up by the study team during the following week's phone call. Follow up will be continued until resolution of symptoms or the end of the intervention. The reviewing physician may refer the participant to their primary oncologist or primary care doctor for evaluation if necessary.

If there are concerns about weight loss or tolerance of the intervention diet a visit with a trained nutritionist will be scheduled to review the diet and make adjustments to increase caloric intake. The participant's height and weight will be assessed during this visit.

7.2.2 Unsolicited Events

Unsolicited events are any other AEs that occur with the diet intervention.

All study participants and their families will be provide with contact information for the study team. At enrollment and during weekly phone calls, the participant and their families will be

encouraged to contact the study team with any suspected AEs. Unsolicited AEs will be reviewed by a licensed physician and if necessary the study participant will be referred to their oncologist or primary care doctor for evaluation. The study team will follow up on the AEs in weekly phone calls until resolution or stabilization of the AE.

7.3 Halting Rules

7.3.1 Study Halting Criteria

The study will be halted if a high percent (>25%) of individuals in the Dietary Intervention Arm are experiencing significant weight loss and/or GI symptoms that are considered to negatively affect their health and cannot be ameliorated after meeting with the nutrition team when we have enrolled half the study participants.

7.3.2 Individual Halting Criteria

The study intervention will be discontinued in an individual if:

1. An individual has lost >4% of their body weight and has met with the nutritionist and appears to be linked with a difficulty in consuming enough food to maintain a healthy weight while under diet intervention.

7.4 QUALITY CONTROL AND QUALITY ASSURANCE

The proposed study is a single site study thus all quality control and assurance will be conducted at the University of Colorado Anschutz Medical Campus/Children's Hospital Colorado. The study site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The principal investigator will provide direct access to all study-related sites, source data/data collection forms, and reports for the purpose of monitoring and auditing, and inspection by local and regulatory authorities. The principal investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained.

The quality control and quality assurance plans for this protocol is as follows:

Informed Consent and Assent - all study personnel involved in obtaining consent will complete training. All paper consent/assent forms will be stored in a locked and secure location. Five charts will be selected randomly on an

annual basis to ensure consent/assent forms were completed and stored correctly.

Clinical data collection: Participant clinical data will be obtained during the enrollment visit and from the participant's electronic medical record. All patient data will be stored on a secure RedCap server hosted by the University of Colorado. The study team will audit patient entries monthly to assess for missing data. If voids are found in the data, the study team will take action to obtain the missing data if possible.

Sample collection: The study team will provide standardized education to the participants and their families regarding home stool collection and shipping. The study team will also be in contact with the participants and their families on a weekly basis and will reinforce the education given at enrollment. On receipt of the samples by the study team, all samples will be aliquoted within 6 hours of receipt and stored in -80°C freezers that are continuously monitored and on back-up generator power outlets. All samples will be processed by a single laboratory (PI Lozupone lab) with consistent internal quality controls to ensure batch to batch quality.

Study product: the study product (diet intervention/food) will be provided by an external vendor.

Staff training:

All study team members who will be in contact with the study participants will receive appropriate human subjects protection training.

All study team members that will handle study specimens will complete required hazardous materials and laboratory safety training. Additionally, all laboratory personnel processing the samples will receive thorough training on each assay. This training will be monitored semi-annually by the PI.

Adverse Event Reporting: Quarterly reviews of the reported AEs, both solicited and unsolicited, will be conducted annually. All study participants

will be given contact information for the local IRB in order to independently report AEs or other concerns.

7.5 Institutional Data Safety Monitoring Plan

The principal investigator will be responsible for the conduct of this study, overseeing participant safety, executing the data and safety monitoring (DSM) plan, and complying with all reporting requirements to local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center). The DSMC is responsible for ensuring data quality and study participant safety for all trials at the CU Cancer Center. A summary of the DSMC's activities is as follows:

- Conduct of internal audits
- Ongoing review of all serious adverse events (SAEs) and unanticipated problems (UAPs)
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

Per the CU Cancer Center Institutional DSM Plan, SAEs and UAPs are reported to the DSMC, IRB and the principal investigator per protocol. All SAEs and UAPs are to be reported to the DSMC within 7 (for fatal or life-threatening events) or 15 (non-life-threatening events) calendar days of the principal investigator receiving notification of the occurrence.

Any AE that meets a protocol-defined serious criterion will also be submitted immediately (within 24 hours of site awareness) on an SAE form to the DMID Pharmacovigilance Group, at the following address:

**DMID Pharmacovigilance Group
Clinical Research Operations and Management Support (CROMS)
6500 Rock Spring Dr. Suite 650
Bethesda, MD 20817, USA
SAE Hot Line: 1-800-537-9979 (US) or 1-301-897-1709 (outside US)
SAE FAX Number: 1-800-275-7619 (US) or 1-301-897-1710 (outside US)
SAE Email Address: PVG@dmidcroms.com**

Study audits conducted by the DSMC will consist of a review of the regulatory documents, consent forms, and source data verification. Documentation of the audit conducted by the DSMC will then need to be submitted to the IRB of record at the time of the IRB's continuing review of this trial.

8 HUMAN SUBJECTS PROTECTION

8.1 Informed Consent Process

Informed consent is a process that is initiated prior to an individual agreeing to participate in a trial and continuing throughout the individual's trial participation. Before any study procedures are performed, informed consent will be obtained and documented. Subjects will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The explanation will be organized, and presented in lay terminology and language that facilitates understanding why one might or might not want to participate.

An investigator or designee will describe the protocol to potential subjects face-to-face or on the phone. The key information about the purpose of the study, the procedures and experimental aspects of the study, risks and discomforts, any expected benefits to the subject, and alternative treatment will be presented first to the subject.

8.1.1 Requirements for Permission by Parents/Guardians and Assent by Children (in case of a minor)

Minors will be informed about the study to the extent understandable to the minor. Investigators or designee will conduct the consent process with the parent(s)/legal guardian, who will be given an IRB/IEC-approved permission form, which may be referred to as a consent form, to read, review, and sign prior to any study procedures. The parent(s)/legal guardian will be provided meaningful study information including a statement that this study involves research, the child may not benefit from the trial, and the study involves risk. The required elements will be clearly presented, including the purpose of the study, the experimental procedures, the potential risks and discomforts, known adverse effects, possible benefits of the study for the subject, alternative therapies that may be beneficial, use and disclosure of private information, and other elements that are part of obtaining proper consent. The subject's parent(s)/legal guardian will be allowed sufficient time to discuss questions with the investigator.

The investigator or designee will describe in simplified terms the details of the study intervention/product, study procedures, risks and discomforts, benefits, and other consent elements, as appropriate. A separate IRB/IEC-approved assent form will be used for the minor, who may read and sign the form, or have it read to him/her prior to participation in study procedures. Assent may be obtained verbally or waived when approved by the IRB/IEC as appropriate to age. If a child declines to participate in the trial when assent is required by the IRB/IEC, the subject will not be enrolled even though the parents have provided permission.

8.1.2 Consent/assent by phone

In some cases it may be more convenient for the study participants to perform the consent (or consent + assent for minors) over the phone. In these cases, we will obtain signed consent/assent using a phone consent process as follows: 1) We will ensure that the prospective subject has a written copy of the approved consent/assent form(s) "in-hand" at the time of the phone consent/assent process. This will be mailed or e-mailed to the participant to print at home depending on their preference. 2) An uninvolved witness will be involved in the phone consent/assent process. If the subject agrees to participate, the witness will sign the copy of the

consent/assent form(s), attesting that the subject had a copy of the written consent/assent form(s) at the time of the discussion, the study was explained consistent with the consent/assent form(s), the subject had their questions answered, and the subject explicitly agreed to participate. 3)

A copy of the signed consent/assent form(s) (and HIPAA Authorization) will be received by the study before any research procedures are conducted on the subject. This will be delivered by mail.

8.2 Consent for Future Use of Stored Specimens and Data

Residual samples/specimens are those that are left over after protocol-specified testing and this study has been completed. Subjects will be asked for permission to keep any remaining stool for possible use in future research studies, such as examining levels of particular microbially produced metabolites. These residual specimens will be stored coded indefinitely in the laboratory of Dr. Catherine Lozupone.

There are no benefits to subjects in the collection, storage and subsequent future use of their samples/specimens. Future use samples/specimens will not be sold or used directly for production of any commercial product. Each sample/specimen will be encoded (labeled) only with a barcode and a unique tracking number that connects to a code key at the study site. Restricted access to the code key is maintained by the principal investigator to protect subject confidentiality. Reports from future research studies performed using subjects' samples/specimens will NOT be kept in their health records.

8.3 Exclusion of Women, Minorities, and Children (Special Populations)

The study was limited to individuals ≥ 9 years old because completion of the FFQ and 24-hour recalls in younger children is challenged by developmental stage and because dietary recommendations are very different between ages 4 and 9, requiring more meal plans and compliance support.

9 STATISTICAL CONSIDERATIONS

9.1 Study Hypotheses

Hypothesis of Primary Objective:

- Dietary intervention to increase fiber and decrease fat will reduce *C. difficile* recurrence in a cohort of oncology patients.

Hypotheses of Secondary Objectives:

- Dietary intervention to increase fiber and decrease fat will reduce *C. difficile* carriage, abundance and toxin production in a cohort of oncology patients.
- Dietary intervention to increase fiber and decrease fat will be associated with increased diversity of the gut microbiome and increased representation of secondary bile and SCFA producers.
- Individuals receiving no nutrition to their gut (IV tubes) or low-fiber gastric/nasogastric tube feed formulas will have higher *C. difficile* recurrence, higher *C. difficile* carriage, abundance and toxin production, and a less diverse microbiome with lower secondary bile acid and SCFA production compared to individuals eating regular diets, particularly those higher in fiber and lower in fat.
- Individuals consuming higher-fiber and/or lower fat diets across non-intervention study participants will have higher *C. difficile* recurrence, higher *C. difficile* carriage, abundance and toxin production, and a less diverse microbiome with lower secondary bile acid and SCFA production compared to individuals eating regular diets, particularly those higher in fiber and lower in fat.

9.2 Sample Size Considerations

Primary Objective: We will use a chi-square test to determine whether the percent of individuals experiencing recurrent *C. difficile* is different after six weeks between the diet intervention and non-diet intervention groups (Cohort A). With a sample size of 124, 30 per arm, we will have 80% power to detect an effect size of 0.25 at $\alpha=0.05$ (Calculated with G*Power). This effect size would occur if CDI recurrence was 35% in the non-diet intervention arm, and decreased to 14% with diet intervention.

Secondary Objectives: The selection of the sample size for this trial was based solely on the power calculations performed for the Primary outcome. The microbiome differences assessed as a secondary outcome would define a mechanism for a significant result in the primary outcome and so we expect these to be significant if the primary outcome is.

9.3 Treatment Assignment Procedures

9.3.1 Randomization Procedures

The trial will be randomized. A simple randomization procedure with stratification will be used to assign study participants to the “diet intervention” (n=30) or “no diet intervention” (n=30) arm of the study.

9.3.2 Masking Procedures

The treatment arms will not be masked.

9.3.3 Interim Safety Review

The study team will document AEs from the first intervention, Study Day 0, through the end of the 6 week protocol, Study Day 42. During weekly phone contact with the participant and their family, the study team will solicit if the participant is experiencing any GI symptoms, including but not limited to abdominal pain, heartburn, nausea, vomiting, diarrhea, or constipation. The team will also solicit if the participant or family have concerns that the participant is losing weight.

The study will be halted if a high percent (>25%) of individuals in the Dietary Intervention Arm are experiencing significant weight loss and/or GI symptoms that are considered to negatively effect their health and cannot be ameliorated after meeting with the nutrition team when we have enrolled half the study participants.

The study may also be suspended (subject enrollment and/or study interventions suspended) because of safety findings, such as an SAE or an overall pattern of symptomatic, clinical, or laboratory events that the DMID or the Safety Oversight Committee consider associated with the study product. These may appear minor in terms of individual events, but might collectively represent potential concern for safety.

No statistical rules will be used to halt enrollment into all or a portion of the study.

9.4 Final Analysis Plan

We will use a chi-square test to determine whether the percent of individuals who experience a recurrence of *C. difficile* is different after six weeks between the diet intervention and non-diet intervention groups.

We will also characterize changes in the microbiome over time using standard microbiome analysis techniques. Principal coordinate analysis will be used to visualize changes in microbiome composition over time with and without diet intervention and in relation to other measured factors in these children such as any infections or antibiotics the children may be exposed to. We will also use the shotgun metagenomic data to specifically interrogate the effects of these factors on SCFA and bile acid metabolizing genes/organisms. Statistical comparisons of alpha diversity, microbial taxa and 100% ID OTUs, and genes across diet intervention arms will be conducted using ANOVA and mixed effects linear regression models when appropriate to correct for confounding variables.

10 LITERATURE REFERENCES

1. Borali E, De Giacomo C. 2016. Clostridium Difficile Infection in Children: A Review. *J Pediatr Gastroenterol Nutr* 63:e130-e140.
2. Nylund CM, Goudie A, Garza JM, Fairbrother G, Cohen MB. 2011. Clostridium difficile infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 165:451-7.
3. Tai E, Richardson LC, Townsend J, Howard E, McDonald LC. 2011. Clostridium difficile infection among children with cancer. *Pediatr Infect Dis J* 30:610-2.
4. Dominguez SR, Dolan SA, West K, Dantes RB, Epton E, Friedman D, Littlehorn CA, Arms LE, Walton K, Servetar E, Frank DN, Kotter CV, Dowell E, Gould CV, Hilden JM, Todd JK. 2014. High colonization rate and prolonged shedding of Clostridium difficile in pediatric oncology patients. *Clin Infect Dis* 59:401-3.
5. Hryckowian AJ, Van Treuren W, Smits SA, Davis NM, Gardner JO, Bouley DM, Sonnenburg JL. 2018. Microbiota-accessible carbohydrates suppress Clostridium difficile infection in a murine model. *Nat Microbiol* 3:662-669.
6. Louie TJ, Cannon K, Byrne B, Emery J, Ward L, Eyben M, Krulicki W. 2012. 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* 55 Suppl 2:S132-42.
7. Louie TJ, Miller MA, Mullane KM, Weiss K, Lentnek A, Golan Y, Gorbach S, Sears P, Shue YK, Group OPTCS. 2011. Fidaxomicin versus vancomycin for Clostridium difficile infection. *N Engl J Med* 364:422-31.
8. McFarland LV, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, Melcher SA, Bowen KE, Cox JL, Noorani Z, et al. 1994. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. *JAMA* 271:1913-8.
9. Aspinall ST, Hutchinson DN. 1992. New selective medium for isolating Clostridium difficile from faeces. *J Clin Pathol* 45:812-4.
10. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. 1986. A data-based approach to diet questionnaire design and testing. *Am J Epidemiol* 124:453-69.

11 APPENDICES

Appendix A. Schedule of Events

Evaluation	Screening/ Enrollment Visit 1 Day 1	Enrollment (conducted by phone)	Weekly phone contact Days 7-42	Weekly home events	Unscheduled/ Early Termination Visit
Signed consent form	X				
Confirmation of eligibility criteria	X				
Allergy History	X				
Randomization to study arm		X			
Creation of meal plan		X			
Height and Weight assessment	X				X
Detailed dietary history	X				X
weekly home stool collection				X	
Biweekly 24-hour dietary recall				X	
Completion of food consumption checklist				X	
Delivery of groceries				X	
Solicitation of rCDI symptoms, and potential AEs including weight loss.			X		

No clinical testing will be done during the protocol

Study Intervention – The diet intervention will target a dietary saturated fat intake at the 25th percentile of average American intake for children 9-18 years which is 20 grams/day (g/d) for females and 22 g/d for males in the context of a diet designed to be 30% total fat (as a percentage of total daily intake; also at the 25th percentile). The dietary fiber intake target will be 20 g/d. Enrolled patients and, if applicable, their parent/guardians will meet with the team of Dr. Higgins to assess current diet and food preferences. This information will be used to design lists of preferred foods from which the patient will be asked to eat a certain number of items per day. For instance, the family will receive a list of high fiber foods such as fruits and vegetables that the patient likes to choose among to meet fiber targets. The patient will also receive a list of items high in saturated fat such as meats and cheeses that they can choose a limited amount of. Based on these food preferences, we will provide groceries that support each patient's meal plan once per week via online home delivery service (e.g. Amazon Pantry). Compliance will be assessed using consumption checklists for the provided food items weekly and three 24-hour

food recalls. Twice weekly stool samples will be collected by the participants and their family using a study-provided collection kit.