A PHASE II RANDOMIZED TRIAL TO EVALUATE THE OPTIMAL DOSING OF FECAL MICROBIOTA TRANSPLANTATION USING THE PENN MICROBIOME THERAPY PRODUCTS FOR RECURRENT CLOSTRIDIUM DIFFICILE INFECTION

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Investigational Products	Penn Microbiome Therapy (PMT) -001, 002, 003
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List of Abbreviations

AE: Adverse event
AESI: Adverse event of special interest
AFT: accelerated failure time
ANC: Absolute neutrophil count
BMI: body mass index
CDI: *Clostridium difficile* infection
CCH: Chester County Hospital
CFR: Code of federal regulations
CFU: colony-forming units
CRC: clinical research coordinator
DSMB: Data safety monitoring board
EIA: enzyme immunoassay
FMT: Fecal Microbiota Transplantation
g: grams
HIPAA: Health Insurance Portability and Accountability Act of 1996
HUP: Hospital of the University of Pennsylvania
ICH: International Conference on Harmonisation
IDSA: Infectious Diseases Society of America
ITT: intention to treat
LGH: Lancaster General Hospital
m-ITT: modified intention to treat
mL: milliliters
NAAT: nucleic acid amplification testing
PAH: Pennsylvania Hospital
PHI: protected health information
PMPMC: PennMedicine Princeton Medical Center
PMT: Penn Microbiome Therapy
PPI: proton pump inhibitor
PPMC: Penn-Presbyterian Medical Center
R-CDI: Recurrent *Clostridium difficile* infection
SAE: Serious Adverse Event
SIRS: systemic inflammatory response syndrome
S/SC/F-CDI: severe or severe-complicated/fulminant *Clostridium difficile* infection
SUSAR: Suspected unexpected serious adverse reaction
WBC: white blood cells

Study Summary

Title	A Phase II, Randomized Trial to Evaluate the Optimal Dosing of Fecal Microbiota Transplantation using the Penn Microbiome Therapy Products for Recurrent *Clostridium difficile* Infection
Short Title	PMT for Recurrent-CDI
IRB Number	832963
Phase	Phase II
Methodology	Randomized, open label, comparative
Study Duration	Two years
Study Center	PennMedicine -- University of Pennsylvania Health System
Objectives	• **Primary**: To evaluate the efficacy of fecal microbiota transplantation (FMT) performed using the Penn Microbiome Therapy (PMT) suite of products to treat subjects with recurrent *Clostridium difficile* infection (R-CDI) by comparing the clinical outcomes of two treatment strategies: (1) single-dose PMT; (2) two doses of PMT administered within 24 hours. • **Secondary**: To determine whether mass of fecal material or quantitative culture better predicts efficacy of PMT products used to treat subjects with R-CDI. • **Exploratory**: To characterize differences in the efficacy of fecal microbiota transplantation for R-CDI based on route of administration, unique bacterial community features of the administered PMT dose, and host immune response to infection.
Number of Subjects	Target enrollment: 300 subjects.

Main Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Second or greater episode of CDI (first or greater recurrence) within 12 months, with symptoms including bowel movements altered in frequency or consistency from baseline.
2. Stool positive for *C. difficile* toxin by EIA or toxin gene by NAAT within 60 days of enrollment.
3. At least one additional prior positive stool test for *C. difficile* within the prior 12 months (EIA or NAAT as above).
4. Age ≥ 18 years.
5. Minimum of 72 hours of receipt of standard-of-care (vancomycin or fidaxomicin) antibiotic treatment for R-CDI prior to intervention.

Exclusion Criteria:

1. Evidence of colon/small bowel perforation at the time of study screening
2. Goals of care are directed to comfort rather than curative measures.
3. Moderate (ANC < 1000 cells/uL) or severe (ANC < 500 cells/uL) neutropenia.
4. Known food allergy that could lead to anaphylaxis.
5. Pregnancy
6. Meeting criteria for S/SC/F-CDI within 24 hours of planned trial enrollment, which we define as any one of the following: (1) leukocytosis with peripheral WBC ≥ 15,000 cells/mL; (2) hypotension with systolic blood pressure sustained < 90mmHg for three or more hours or requiring pressors; (3) provider documentation of ileus or radiologic evidence of bowel dilation or megacolon; (4) acute kidney injury with increase in baseline serum creatinine level by ≥50% or new dialysis initiation; (5) serum lactate > 2.2 mmol/L; or (6) ≥ 3 systemic inflammatory response syndrome (SIRS) criteria (which include heart rate > 90 beats per minute, respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg, temperature >38ºC or <36ºC, WBC > 12,000 cells/uL, <4,000 cells/uL, or >10% immature (band) forms).
7. Receipt of FMT or enrollment in a clinical trial for FMT within the last 3 months.

Investigational Product	1. Penn Microbiome Therapy – 001 (PMT-001): no more than 152 mL suspension for rectal enema from 40g human stool, comprising a total of $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs/dose. 2. Penn Microbiome Therapy – 002 (PMT-002): no more than 152 mL suspension for intragastric, intraduodenal, or intrajejunal administration from 40g human stool, comprising $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs/dose. 3. Penn Microbiome Therapy – 003 (PMT-003): no more than 32 capsules for oral administration from 40g donated stool, comprising $1x10^8$– $1x10^{12}$ anaerobic bacterial CFUs/dose. PMT-001, PMT-002, PMT-003, are packaged for single-dose administration.
Duration of administration	10 – 90 minutes (target 30 minutes)
Reference therapy	Single dose of Penn Microbiome Therapy product.
Statistical Methodology	Primary outcome will be resolution of diarrhea without recurrence assessed at 8 weeks (56 days) after intervention. Logistic regression analysis will be used to assess if repeat dosing is superior to single dose.
Safety Evaluations	We will monitor safety of administration as well as safety of the investigational products, in terms of adverse events as listed in Section 9.
Data and Safety Monitoring Plan	See Section 10.

Background and Study Rationale

This study will be conducted in full accordance all applicable University of Pennsylvania Research Policies and Procedures and all applicable Federal and state laws and regulations including 45 CFR 46, 21 CFR Parts 50, 54, 56, 312 and Good Clinical Practice: Consolidated Guidelines approved by the International Conference on Harmonisation (ICH). All episodes of noncompliance will be documented.

1. Introduction

Fecal microbiota transplantation (FMT) is recommended treatment for recurrent *Clostridium difficile* infection (R-CDI) based on its success in multiple trials targeting subjects with mild or moderate CDI symptoms (McDonald 2018; Kelly BJ 2018). FMT product dosing has typically been defined by the mass of human stool used to generate the dose. However, the FMT products used to treat R-CDI are not standardized, and trials to date have demonstrated a large range of efficacy (Cammarota 2014; van Nood 2013; Youngster 2016; Kelly CR 2016). A recent trial utilizing a greater mass of starting stool material demonstrated greater efficacy than prior trials (Kao 2017), raising the question of how the mass of stool material used to generate FMT product contributes to the efficacy of the product. Another possible explanation for the varying efficacy of FMT products is the fact that they originate from different stool donors, each with a unique gut microbiome and concentration of microbes (Seekatz 2014). We will perform a randomized controlled trial to evaluate the safety and efficacy of Penn Microbiome Therapy (PMT) products for FMT in subjects with R-CDI. The PMT products comprise three different formulations of FMT for oral, enteric (via tube), or rectal delivery. The trial will compare the efficacy of standard-dose FMT (a single dose derived from 40g starting stool mass administered once) versus double-dose FMT (i.e., two single doses, or the equivalent of 80g starting stool mass, administered within 24 hours) in the treatment of R-CDI. We will further evaluate the concentration of anaerobic bacteria, which include the taxa most frequently identified as protective against CDI (Lee YJ 2017), in FMT products to determine whether administered stool mass or bacterial concentration better predict FMT efficacy. We will also explore microbiome differences between FMT donors and differences in host immune response to CDI predictors of FMT efficacy. There is no placebo arm in the trial because FMT is already established as recommended therapy for R-CDI.

1.1. Background and Relevant Literature

As recently as 2015, the Centers for Disease Control and Prevention (CDC) identified *Clostridium difficile* infection (CDI) as an "urgent" threat associated with upwards of 500,000 infections in the United States each year and resulting in over 15,000 deaths. CDI is characterized by a clinical syndrome that typically includes loose, frequent bowel movements and abdominal pain, which occur as a result of toxin production by colonic *C. difficile*. CDI occurs in patients colonized with toxigenic *C. difficile* and in patients who have newly acquired *C. difficile*. Estimates of the relative contributions of persistent colonization and new acquisition to incident CDI vary. In both cases, the pathogenesis of CDI involves depletion of non-*C. difficile* colonic microbiota, altered bile acid metabolism, germination of (resident or recently ingested) *C. difficile* spores, expansion of a population of vegetative *C. difficile*, toxin production, and colonic inflammation. Key challenges

associated with CDI include its recurrence after appropriate antibiotic treatment (R-CDI), and its progression to a more severe disease state with systemic signs of inflammation or even sepsis. The latter, termed severe or severe-complicated/fulminant CDI (S/SC/F-CDI), often requires extended hospitalizations, may require colectomy, and is associated with high rates of mortality (Kelly BJ 2018). At present, antibiotic treatment is the recommended therapy for early R-CDI, and antibiotic treatment followed by FMT is the recommended therapy for later R-CDI. Antibiotics are the recommended therapy for S/SC/F-CDI (McDonald 2018).

1.2. Name and Description of the Investigational Product

Penn Microbiome Therapy suite of investigational products

- Penn Microbiome Therapy – 001, hereafter referred to as PMT- 001
  - Dose: $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs derived from 40g of human stool diluted with 10% glycerol into no more than 152 mL suspension of investigational product.
  - Enema administered rectally

- Penn Microbiome Therapy – 002, hereafter referred to as PMT - 002,
  - Dose: $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs derived from 40g of human stool diluted with 10% glycerol into no more than 152 mL suspension of investigational product.
  - Suspension administered intragastric, intraduodenal, or intrajejunal

- Penn Microbiome Therapy – 003, hereafter referred to as PMT - 003,
  - Dose: $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs derived from 40g of human stool diluted with 10% glycerol and concentrated to fill no more than 32 capsules
  - All 32 capsules to be administered orally

Chemical Name and Structure:

PMT-001: Fecal Microbiota for Transplant, enema product
PMT-002: Fecal Microbiota for Transplant, suspension product
PMT-003: Fecal Microbiota for Transplant, capsule product

The three investigational products, PMT-001,002, 003, are composed of donated stool from healthy donors that meet the donation criteria. The stool sample is composed of approximately 75% water and 25% organic and inorganic solids. Typically, 1g of stool contains approximately 10^9 bacterial cells. The stool sample is subsequently processed with phosphate-buffered saline (PBS) containing 10% glycerol and formulated as a suspension for PMT-001 and PMT-002 or concentrated and encapsulated for PMT-003.

1.2.1. Non-Clinical Data

FMT as a treatment modality for CDI did not take the conventional path of drug development. Fecal transplants seem to date as far back as 4th century China, and have recently become recommended therapy for R-CDI on the basis of several randomized trials (McDonald 2018; van Nood 2013; Youngster 2016; Kelly CR 2016; Kao 2017), even as there remains significant heterogeneity among FMT products. There is no established standard for non-clinical testing of FMT products given uncertainty as to what microbial features determine potency (Bojanova 2016). The treatment protocol outlined here is based on the results published from prior studies that have driven the standards adopted in our R-CDI and S/SC/F-CDI protocols (Hamilton 2012; Kelly BJ 2018; Cammarota 2017; Krajicek 2018).

1.2.2. Clinical Data to Date

There is no available clinical research data to date on the investigational products PMT-001, 002 or 003. FMT has been established as effective and is recommended for treatment of multiply recurrent CDI on the basis of several randomized controlled trials (McDonald 2018; van Nood 2013; Youngster JAMA 2014; Kelly CR 2016; Kao 2017). See Table 1 for a summary of the risks described in the literature.

Table 1: FMT dosing and associated risks.

Authors	Publication Year	Population	Study Design	FMT Dose (g stool)	FMT Formulation	FMT Administration	Risks
Hamilton et al	2012	R-CDI	single-group trial	50g stool	250mL suspension	colonoscopy	diarrhea, flatulence
van Nood et al	2013	R-CDI	randomized trial	(not reported)	500mL suspension	duodenal tube	diarrhea, cramping, belching
Youngster et al JAMA	2014	R-CDI	single-group trial	48g stool	30 capsules	oral	cramping, bloating
Kelly et al	2016	R-CDI	randomized trial	64g stool	500mL suspension	colonoscopy	chills, abdominal pain, bloating, nausea, flatulence
Kao et al	2017	R-CDI	randomized trial	80-100g stool	40 capsules or 180mL suspension	oral or colonoscopy	nausea, vomiting, fever, abdominal discomfort
Weingarden et al	2013	R-CDI	case series	50g stool	250mL suspension	colonoscopy	(not reported)
Agrawal et al	2015	R-CDI	case series	~30-60g stool	150-500mL suspension	upper endoscopy, lower endoscopy, enema	diarrhea, constipation, abdominal pain, ileus
Aroniadis et al	2015	R-CDI	case series	(not reported)	suspension	upper endoscopy, lower endoscopy, enema, colonoscopy	diarrhea, abdominal pain
Fischer et al	2015	R-CDI	case series	50-100g stool	300mL suspension	sigmoidoscopy or colonoscopy	treatment failure and death
Fischer et al	2017	R-CDI	case series	50-100g stool	300mL suspension	sigmoidoscopy or colonoscopy	treatment failure and death

1.3. Dose Rationale

There are no pre-clinical or clinical data for the PMT suite of FMT products to inform optimal dose. However, there are extensive observational studies and several randomized trials that suggest an FMT dose derived from 30-100g of human stool is effective to treat R-CDI (Cammarota 2014; van Nood 2013; Youngster JAMA 2014; Kelly CR 2016). A recent trial utilizing a greater mass (80-100g) of starting stool material demonstrated greater efficacy than prior trials (Kao 2017), raising the question of how the mass of stool material used to generate FMT product contributes to the efficacy of the product. We will evaluate the safety and efficacy of PMT products for FMT to treat R-CDI, comparing single-dose (40g starting stool mass) versus double-dose (i.e., two PMT doses administered within 24 hours). The PMT products produced from these 40g stool mass contain a range of 1e8 – 1e12 anaerobic bacterial CFUs, so the double-dose intervention will contain twice that. Though the difference in anaerobic CFUs is marginal between single and double-dose interventions, other bacterial taxa may scale more directly with stool mass, and recent trial data (Kao 2017) suggest that such a difference might be clinically meaningful.

2. Study Objectives

2.1. Primary Objective

To evaluate the efficacy of fecal microbiota transplantation (FMT) performed using the Penn Microbiome Therapy (PMT) suite of products to treat subjects with recurrent *Clostridium difficile* infection (R-CDI) by comparing the clinical outcomes of two treatment strategies: (1) single-dose PMT; (2) two doses of PMT administered within 24 hours.

2.2. Secondary Objective

To determine whether mass of fecal material or quantitative culture better predicts efficacy of PMT products used to treat subjects with R-CDI.

2.3. Exploratory Objective

To characterize differences in efficacy of fecal microbiota transplantation for R-CDI based on (1) route of administration, (2) unique bacterial community features of the administered PMT dose, and (3) features of host immune response to CDI.

3. Investigational Plan

3.1. General Design

We will perform a randomized, controlled, open label trial. Subjects will be screened to evaluate if they meet inclusion criteria for recurrent CDI. If eligible, subjects will participate in the informed consent process. Following informed consent subject preference and guidance of the referring/primary care team will be used to identify route of administration. Stratified randomization will then be used by route of administration to randomize subjects to one of two

arms: (1) single dose of PMT, or (2) two doses of PMT administered within 24 hours. We will compare the two treatment strategies' success in achieving clinical resolution of diarrhea in subjects with R-CDI. Subjects randomized to receive two doses of FMT will receive two doses from the same donor (but not necessarily from the same manufacturing batch). The route of administration may be different for the second dose per subject's treating physician and subject preference. Subjects will be followed until 180 days after their last PMT dose.

3.1.1. Screening Phase

Because FMT is recommended therapy for R-CDI, potential subjects will be identified through active screening and by referral from a care provider within the University of Pennsylvania Health System (PennMedicine). Potential subjects' health records will be initially screened by a study team member to evaluate if the inclusion/exclusion criteria may be met. If a potential subject may meet inclusion/exclusion criteria, the potential subject (or their designated medical decision- maker) will be contacted in person or by telephone to discuss participation. If a potential subject is currently admitted to the hospital and if the primary team is not already aware of potential eligibility, the study team will contact the subject's inpatient care team to ascertain whether the care team has any objection to the study team enrolling the potential subject. The potential subject (or their designated medical decision-maker) will be contacted to discuss participation, final screening for inclusion/exclusion criteria will be performed, and informed consent will be obtained.

3.1.2. Study Intervention Phase

R-CDI trial interventions may be performed inpatient or outpatient at the listed study sites in section 4.5. Once subjects are enrolled and informed consent is obtained, subjects will be assessed for their ability to tolerate oral capsules, gastric/duodenal/jejunal infusion, and enema (per active prescribed medication list and/or opinion of referring care provider); the subject's preference and the guidance of the referring care provider will determine if PMT-001, PMT-002, or PMT-003 will be used. Following this, stratified randomization to single- or double-dose strategy will be performed within route of delivery groups (i.e., within PMT-001, PMT-002, or PMT-003 groups). Administration of a single dose of the product will take place over 10 to 90 minutes (target 30 minutes). A second dose will be given between 4 and 24 hours after the first dose. The route of administration may be different for the second dose per subject's treating physician and subject preference. If a subject is unable to take at least 50% of a dose, they will be permitted to opt to receive a different route of administration for one dose within 24 hours. Any antibiotics previously prescribed for the treatment of CDI will be stopped 12-48 hours (target 24 hours) prior to the intervention.

3.1.3. Post-PMT Administration monitoring: Bedside for 60 Minutes

Subjects will be monitored at 30 and 60 minutes following completion of PMT administration. Subjects will be visually observed for signs of aspiration or other respiratory distress (PMT-002 and PMT-003), abdominal pain, vomiting, other signs of allergic reaction, and changes in vital signs (PMT-001, PMT-002, and PMT-003). Vital signs (temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation) will be checked. The following vital sign changes will be recorded if observed, and will prompt notification of the primary clinical care team: (1) heart rate

increase or decrease by greater than or equal to 30 beats per minute; (2) respiratory rate increase or decrease by greater than or equal to 10 breaths per minute or if respiratory rate drops below 10 or increases above 25 (if respiratory rate not already outside these ranges); (3) temperature: increase or decrease by greater than or equal to 2 degrees Fahrenheit, or if temperature drops below 96°F or increases above 100.4°F (if temperature not already outside these ranges); (4) oxygen saturation decrease by greater than or equal to 5% (sustained for more than 20 seconds) or if oxygen saturation drops below 90% (on current amount of oxygen delivered, sustained for more than 20 seconds); (5) blood pressure decrease by more than 30 mmHg systolic or 15 mmHg diastolic, or if blood pressure drops below 90/60 (if blood pressure not already outside these ranges).

3.1.4. Post-PMT Administration Follow-Up

Following a subject's final PMT administration, subjects enter the follow up phase. During this phase, the following will occur: (1) if subjects are inpatient, daily maximum temperature, number of bowel movements, and any episodes of emesis will be recorded (based on electronic medical record) for 7 calendar days post-FMT; (2) in preparation for discharge from the hospital, subjects will record daily temperature, number of bowel movements, and any episodes of emesis for 7 days post last FMT (all subjects will be given a symptom diary card; a digital thermometer will also be provided); (3) subjects will be contacted by telephone or in- person for an interview focused on potential adverse events at 7 days (or next working day, not to exceed 10 days) after final FMT; (4) subjects will be contacted by telephone or in-person at 30 days (+/- 3 days) after final FMT for an interview focused on potential adverse events; (5) subjects will be contacted by telephone or in-person at 56 days (+/- 7 days) after final FMT for an interview focused on the primary outcome and adverse events; (6) subjects will be contacted by telephone or in-person at 90 days (+/- 7 days) after final FMT for an interview focused on potential adverse events; (7) subjects will be contacted by telephone or in-person at 180 days (+/- 7 days) after final FMT for an interview focused on potential adverse events.

Telephone versus in-person follow-up will be determined based on subject preference. (If subjects prefer to be seen in person, but have been discharged from the hospital, a clinic visit with a study team member will be arranged). Subjects enrolled as outpatients will have at least one in-person follow-up visit scheduled between 1- and 4-weeks post-FMT. All FMT recipients (or their surrogates) will be given contact information for the study coordinator. As detailed above, subjects will be followed for 180 days following last investigational product administration. All subjects will be also provided a study contact telephone number on a copy of the informed consent form, in case concerns for adverse event or other questions arise. Subjects will also be permitted to initiate electronic communication with the study team, although planned follow up visits will be conducted by telephone or in person. The study team may reach out to subjects electronically if difficulties arise in reaching the subject by telephone in order to schedule visits, but study-related questions will not be asked electronically. Subjects will be classified as lost to follow up if at least three attempts are made at contact without success; however, medical record review will still be used to obtain follow up information on these subjects. If a subject is eligible and enrolled a second (or greater) time, then their subsequent

follow up will be conducted according to the schedule set by their most recent enrollment, and any remaining follow ups from earlier enrollment(s) will not be performed.

3.1.5. Allocation to Interventional Group

Stratified randomization will be performed in a 1:1 fashion (one to single-dose and one to double-dose). The stratification will be by route of delivery, which will be decided on following enrollment but prior to randomization. The route of administration (i.e., PMT-001 versus PMT-002 versus PMT-003) will be determined by counsel from the subject's referring health care provider and the subject's own preference. The randomization strata will include: upper delivery (PMT-002 or PMT-003) or lower delivery (PMT-001). Stratified randomization by route will be performed in order to ensure balance in route of delivery between the two groups. The randomization schedule will be prepared using a random-size block strategy to ensure balance throughout the trial. Prior to enrollment of the first subject, a randomization table will be produced for each category using Stata statistical software and the "Ralloc" package. There is no randomization to a no-FMT control because FMT is already guideline-recommended for the treatment of R-CDI (McDonald 2018).

3.2. Study Endpoints

3.2.1. Primary Study Endpoints

The primary endpoint will be proportion of subjects with clinical resolution of diarrhea without recurrence in subjects with R-CDI at 8 weeks (56 days) following FMT. Clinical resolution will be defined as follows:

- ≤ 4 stools per calendar day for the prior two days with no stool of Bristol stool scale type 7
- No additional stool tests with a positive EIA for *C. difficile* toxin since study enrollment
- No additional prescription or use of anti-CDI antibiotics (unless given for prophylaxis) since study enrollment
- No need for an additional FMT administration since study enrollment
- Subject is alive

3.2.2. Secondary Study Endpoints

Secondary endpoints include:

- All-cause mortality at 30- and 60-days following last FMT
- Colectomy or diverting ileostomy within 30 days after last FMT
- Cumulative days of hospitalization from enrollment until 30 days after FMT
- Cumulative days in intensive care unit from enrollment until 30 days after last FMT
- Bacteremia from enrollment until 30 days after last FMT
- Hospital admission within 60 days of discharge from index hospitalization

3.2.3. Primary Safety Endpoints

The primary safety endpoints will include:

- Frequency of solicited adverse events (AEs)

- Frequency of serious adverse events (SAEs)
- Frequency of AEs of special interest (AESIs)

See section 9 for additional details on adverse event definitions.

3.2.3.1. Post-PMT Administration monitoring: Bedside for 60 Minutes

Safety endpoints for all routes of administration will include worsened abdominal pain, fever, tachycardia, and hypotension (Section 3.2.1). Safety endpoints for upper enteral delivery (i.e., PMT-002 or PMT-003) will include evidence of aspiration (including reduced oxygen saturation, tachypnea, or respiratory distress, as defined above).

3.2.3.2. Post-PMT Administration Follow-Up

Safety endpoints assessed at the 7-day follow-up and all subsequent follow-ups will include fever, diarrhea, nausea, and vomiting. Safety endpoints assessed at the 30-day, 56-day, 90-day, and 180-day follow-ups will include evidence of new metabolic disease, including hyperglycemia, thyroid disease, weight gain or loss.

4. Study Population and Duration of Participation

4.1. Inclusion Criteria

1. Second or greater episode of CDI (first or greater recurrence) within 12 months, with symptoms including bowel movement altered in frequency or consistency from baseline.
2. Stool positive for *C. difficile* toxin by EIA or toxin gene by NAAT within 60 days of enrollment.
3. At least one additional prior positive stool test for *C. difficile* within the prior 12 months (EIA or NAAT as above).
4. Age ≥ 18 years.
5. Minimum of 72 hours of receipt of standard-of-care (vancomycin or fidaxomicin) antibiotic treatment for R-CDI prior to intervention.

4.2. Exclusion Criteria

1. Evidence of colon/small bowel perforation at the time of study screening
2. Goals of care are directed to comfort rather than curative measures.
3. Moderate (ANC < 1000 cells/uL) or severe (ANC < 500 cells/uL) neutropenia.
4. Known food allergy that could lead to anaphylaxis.
5. Pregnancy
    a. For subjects of childbearing potential (ages 18 to 55), the subject must have a negative urine pregnancy test within 48 hours of consent and no more than 48 hours prior to first product administration
6. Meeting criteria for severe, severe-complicated/fulminant CDI within 24 hours of planned trial enrollment. We define severe or severe-complicated/fulminant CDI as any one of the following: (1) leukocytosis with peripheral WBC ≥ 15,000 cells/mL; (2) hypotension with systolic blood pressure sustained < 90mmHg for three or more hours or requiring pressors; (3) provider documentation of ileus or radiologic evidence of bowel dilation or megacolon;

(4) acute kidney injury with increase in baseline serum creatinine level by ≥50% or new dialysis initiation; (5) serum lactate > 2.2 mmol/L; or (6) ≥ 3 systemic inflammatory response syndrome (SIRS) criteria (which include heart rate > 90 beats per minute, respiratory rate > 20 breaths per minute or PaCO2 < 32 mmHg, temperature >38ºC or <36ºC, WBC > 12,000 cells/uL, <4,000 cells/uL, or >10% immature (band) forms).

7. Receipt of FMT or enrollment in a clinical trial for FMT within the last 3 months.

4.3. Subject Recruitment

Because FMT is recommended therapy for R-CDI, potential subjects will be identified by active screening or referral from care providers within the University of Pennsylvania Health System (PennMedicine). Potential subjects' health records will be initially screened by a study team member to evaluate if the inclusion/exclusion criteria may be met. If a potential subject may meet inclusion/exclusion criteria, the potential subject (or their designated medical decision-maker) will be contacted in person or by telephone to discuss participation. If a potential is currently admitted to the hospital and the primary team was not the referring team, the study team will contact the subject's inpatient care team to ascertain whether the care team has any objection to the study team enrolling the potential subject. The potential subject (or their designated medical decision-maker) will be contacted to discuss participation. If the potential subject is eligible and wants to participate, informed consent will be obtained. At this time, subjects of childbearing potential (ages 18 to 55) will have a urine pregnancy test performed. If a urine pregnancy test is not able to be performed or if it is positive, the potential subjects will be excluded.

4.4. Duration of Study Participation

The subjects will participate in the study for 180 days (+/- 7 days) after the last administration of the PMT product. Study participation will conclude with the 180-day follow-up visit (in-person or by telephone).

4.5. Total Number of Subjects and Sites

Recruitment will be performed at one site with six participating PennMedicine University of Pennsylvania Health System locations: (1) the Hospital of the University of Pennsylvania (HUP), (2) Penn-Presbyterian Medical Center (PPMC), (3) Pennsylvania Hospital (PAH), (4) Lancaster General Hospital (LGH), (5) Chester County Hospital (CCH), and (6) PennMedicine Princeton Medical Center (PMPMC). Enrollment targets 300 subjects.

4.6. Vulnerable Populations

Children, fetuses, or neonates are not included in this research study because the gastrointestinal microbiome is significantly different between children and adults. Pregnant women will not be eligible to participate in this study due to unknown risks of FMT in pregnancy. Prisoners will be eligible to participate in this study. Disabled, economically- or educationally-disadvantaged persons will be eligible for enrollment. Vulnerable populations will be protected via the informed consent process, which ensures that enrolled subjects or subject-surrogates understand study risks and benefits. Vulnerable populations will be protected from coercion because there is no financial benefit to participation in the study.

5. Study Intervention

5.1. *Description*

Penn Microbiome Therapy suite of investigational products have been described previously in Section 1.2. The product label will include (1) a unique dose identifier (and barcode), (2) the PMT formulation (PMT-001, PMT-002, or PMT-003), (3) a donor identifier, (4) the date of donation and dose processing, and (5) the expiration date.

5.2. Intervention Regimen

This study is a randomized, controlled, open label trial. Subjects will be screened to evaluate if they meet inclusion criteria for recurrent CDI. If eligible, subjects will participate in the informed consent process. Following informed consent, the route of administration will be determined by the ability of the subject to safely receive oral capsules, intragastric/duodenal/jejunal infusion, or enema, as determined by the subject's treating physician, and by subject preference. Subjects will then be randomized to one of two arms using stratified randomization by route of delivery: (1) a single administration of FMT developed from 40g of human stool, which contains $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs (single dose), versus (2) two administrations of the same FMT formulation administered within 24 hours of each other (double dose). Subjects randomized to receive double-dose FMT will receive two doses from the same donor (but not necessarily from the same manufacturing batch). If a subject is unable to take at least 50% of a dose, they will be permitted to opt to receive a different route of administration for one dose within 24 hours. Subjects randomized to the arm with a double dose will receive the second dose as long as they do not have bowel surgery in the interim. Subjects will be followed until 180 days after their last PMT dose. Any antibiotics prescribed as part of routine clinical care for CDI will be stopped 12-48 hours (target 24 hours) prior to the intervention.

5.3. Receipt

The prescribed formulation of the investigational product [PMT-001 (Enema), PMT-002 (Suspension) or PMT-003 (Capsule)] will be packaged on dry ice from the PMT manufacturing facility at the University of Pennsylvania. The time of release from frozen storage will be documented and included in PMT product packaging. PMT products will be delivered by study staff or courier in single doses. Upon receipt of the product by the Investigator, packaging (bags for PMT-001 and PMT-002; capsule containers for PMT-003) will be inspected for integrity, and product will be checked to ensure that it is still frozen and logged in the investigational product accountability log. The investigational product label will also be checked to ensure that the proper product is received, and to ensure that the product has not expired.

5.4. Storage

Investigational products are transported frozen on dry ice by courier or study staff to the clinical location. Investigational products are transported in containers labelled and maintained at a temperature in accordance with federal and local regulations for the transport of biological products.

At the clinical location, administration of PMT-001 or PMT-002 will be initiated within 180 total minutes of product release from frozen storage. At the clinical site, administration of PMT-003 will be initiated within 120 minutes of product release from frozen storage. The time of release from frozen storage will be included in PMT product packaging (Section 5.3).

PMT-001 and PMT-002 doses will be thawed at room temperature for 30-60 minutes prior to administration. The bag may be massaged during thawing. PMT-003 will remain on dry ice until the time of administration.

Any doses released from frozen storage but not administered within the allotted time (180 minutes for PMT-001 and PMT-002, 120 minutes for PMT-003) after release (e.g., due to delays in transport) will be destroyed. In such cases, eligible subjects will be offered another dose, and the release process will be re-initiated.

5.5. Preparation and Packaging

Preparation of the three investigational products [PMT-001 (Enema), PMT-002 (Suspension) and PMT-003 (Capsule)] will be performed at the PMT manufacturing facility at the University of Pennsylvania. PMT-001 and PMT-002 are packaged in EVA bags suitable for storage at < -70°C (target -80°C). PMT-003 is encapsulated within three nested gelatin capsules (sizes 00, 0, and 1), then packaged in an HDPE capsule container suitable for storage at < -70°C (target -80°C). The three investigational products will be packaged in single doses.

5.6. Blinding

Neither subjects nor investigators will be blinded.

5.7. Administration and Accountability

The investigational products will be checked on receipt as above (Section 5.3). Prior to administration, PMT-001 and PMT-002 will be allowed to thaw at room temperature (50°F - 75°F) for 30-60 minutes. Administration of PMT-001 and PMT-002 will begin within 60 minutes of thawing. Prior to administration, PMT-003 will be kept on dry ice; PMT-003 will not be thawed before administration.

As stated in Section 5.4, the total time from release from frozen storage at the manufacturing facility to initiation of administration will not exceed 180 minutes for PMT-001 and PMT-002; it will not exceed 120 minutes for PMT-003.

The duration of administration will take 10 - 90 (target 30) minutes for all products. Administration of all three PMT products will be performed by a licensed physician, advanced medical practitioner, or nurse.

PMT-001 will be administered by enema using an approved enema administration kit. PMT-002 will be administered via intragastric, intraduodenal, or intrajejunal route, contingent on the presence of a pre-existing approved intragastric, intraduodenal, or intrajejunal tube. PMT-003 will be administered orally. Post-procedure monitoring will be performed as described

elsewhere. For each investigational product dose, the product identity, dose targeted for administration, the date and time administered, route of administration, subject identity, quantity of product actually administered, and quantity of product remaining will be recorded via a standard form.

5.8. Subject Compliance Monitoring

Post-procedure monitoring will be performed as described elsewhere (Sections 3.1.3 to Section 3.1.4). For each investigational product dose, the product identity, dose targeted for administration, the date and time administered, route of administration, subject identity, quantity of product actually administered, and quantity of product remaining will be recorded via a standard form. The study team will record if the subject was not able to tolerate or receive the full dose due to adverse events or because the allowed duration of administration was exceeded.

5.9. Return or Destruction of Investigational Product

For PMT-001 or PMT-002, the volume of investigational product will be recorded at the time of drug product manufacturing, and the volume of residual investigational product (if any) will be recorded by the licensed medical professional performing administration after administration is completed (or stopped). For PMT-003, the number of capsules in the dose will be recorded at the time of drug product manufacturing, and the number of remaining capsules (if any) will be recorded by the licensed medical professional performing administration after administration is completed (or stopped). Any remaining investigational product will be disposed of at the clinical site.

6. Study Procedures

The study procedures are also outlined in detail below. The schedule of study procedures is presented in Table 2.

6.1. Screening and Enrollment

6.1.1. Surveillance Test Review

Potential subjects will be identified by active screening or referral for FMT treatment at participating study sites.

6.1.2. Medical Record Review for Inclusion/Exclusion Criteria

Referral for FMT treatment will prompt review of the electronic health record to determine whether a potential subject may meet inclusion/exclusion criteria (Sections 4.1 and 4.2).

6.1.3. Informed Consent

If a potential subject may meet inclusion/exclusion criteria, the study team will contact the potential subject (or their designated medical decision-maker) to discuss participation. If the potential subject is eligible and wants to participate, informed consent will be obtained. Subjects of childbearing potential will be tested using a urine pregnancy test if possible; if urine pregnancy test positive or not possible, potential subjects will be excluded. A record of all screened subjects will be maintained to evaluate the frequency of identified exclusions or care-team objections.

6.1.4. Decision on Mode of Delivery

The enrolled subject will be offered PMT-001, PMT-002, or PMT-003, so long as the enrolled subject and their referring care provider deem the modes of delivery safe. The decision on mode of delivery will be determined by subject preference.

6.1.5. Randomization

Following enrollment and the subject's decision on mode of delivery, stratified randomization will be performed as per Section 3.1.5.

6.2. Study Intervention Phase

6.2.1. PMT Product Receipt

After the treatment plan is determined by randomization, the PMT product to which the subjects is assigned will be prepared and received as described above (Sections 5.2 - 5.7).

6.2.2. Administration

During the intervention period, following informed consent and randomization, subjects will receive either (1) single-dose PMT administration alone, or (2) one-dose PMT administration followed by a second dose within 24 hours (with at least 4 hours separating doses). Each administration of the product (PMT-001, PMT-002, or PMT-003) will take place over 10 to 90 minutes (target 30 minutes). Antibiotics prescribed as part of routine clinical care for CDI will be stopped 12-48 hours (target 24 hours) prior to the intervention.

6.2.3. Post-Intervention 60 minutes

Subjects will be monitored for a period of 60 minutes following completion of each PMT administration. Subjects will be visually observed for signs of aspiration or other respiratory distress, abdominal pain, vomiting, other signs of allergic reaction, and changes in vital signs. Vital signs (temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation) will be checked immediately post-administration and at 30 and 60 minutes following completion of PMT administration. The following vital sign changes will be recorded if observed, and will prompt notification of the primary clinical care team: (1) increase or decrease by greater than or equal to 30 beats per minute; (2) respiratory rate increase or decrease by greater than or equal to 10 breaths per minute or if respiratory rate drops below 10 or increases above 25 (if respiratory rate not already outside these ranges); (3) temperature: increase or decrease by greater than or equal to 2 degrees Fahrenheit, or if temperature drops below 96°F or increases above 100.4°F (if temperature not already outside these ranges); (4) oxygen saturation decrease by greater than or equal to 5% (sustained for more than 20 seconds) or if oxygen saturation drops below 90% (on current amount of oxygen delivered, sustained for more than 20 seconds); (5) blood pressure decrease by more than 30 mmHg systolic or 15 mmHg diastolic, or if blood pressure drops below 90/60 (if blood pressure not already outside these ranges).

6.3. Study Follow-up Phase

6.3.1. Follow-up 7 days

Following a subject's final PMT administration, subjects enter the follow up phase. During this phase, vital signs, symptoms, and laboratory values suggestive of adverse events will be tracked and recorded. Specifically, while remaining inpatient, daily maximum temperature, number of bowel movements, and any episodes of emesis will be recorded (based on electronic medical record) for 7 calendar days post-FMT. In preparation for discharge, subjects will be given a diary for tracking daily temperature, number ofbowel movements, and any episodes of emesis for 7 days post last FMT (all subjects will be given a symptom diary card; a digital thermometer will also be provided). Subjects will be contacted by telephone or in-person for an interview focused on potential adverse events at 7 days after final FMT; the first attempt at contact will be made by the first working day at 7 days or later (not to exceed 10 days) following the final FMT. (In case of telephone follow-up, the 7-day symptom diary card will be transcribed by study staff over the phone/and or collected during the in-person follow-up visit. See Section 6.3.2). Telephone versus in-person follow-up will be determined based on subject preference. If subjects prefer to be seen in person, but have been discharged from the hospital, a clinic visit with a study team member will be arranged.

To address the exploratory aims, if blood that was collected for clinical purposes would otherwise be discarded, it will be captured for immune response phenotyping. Blood will be collected from residual material available in EDTA ("purple top") vacutainer tubes already collected in the clinical laboratory. If no residual blood is available, or if subjects are outpatients or have been discharged from the hospital, no blood will be collected. Blood specimen analysis will be directed by the principal investigator and study team.

6.3.2. Follow-up 30 days

Subjects will be contacted again by telephone or in-person at 30 days (+/- 3 days) after final FMT for an interview focused on potential adverse events. Telephone versus in-person follow-up will be determined based on subject preference. Subjects enrolled as outpatients will have at least one in-person follow-up visit scheduled between 1- and 4weeks post-FMT. (If subjects prefer to be seen in person, but have been discharged from the hospital, a clinic visit with a study team member will be arranged).

6.3.3. Follow-up 56 days

Subjects will be contacted by telephone or in-person at 56 days (+/- 7 days) after final FMT for an interview focused on the primary outcome and potential adverse events. Telephone versus in-person follow-up will be determined based on subject preference. (If subjects prefer to be seen in person, but have been discharged from the hospital, a clinic visit with a study team member will be arranged).

6.3.4. Follow-up 90 days

Subjects will be contacted by telephone or in-person at 90 days (+/- 7 days) after final FMT for an interview focused on potential adverse events. Telephone versus in-person follow-up will be

determined based on subject preference. (If subjects prefer to be seen in person, but have been discharged from the hospital, a clinic visit with a study team member will be arranged).

6.3.5. End-of-study visit at 180 days

The study will conclude after subjects are contacted by telephone or in-person at 180 days (+/- 10 days) after final FMT for a final interview focused on potential adverse events. Telephone versus in-person follow-up will be determined based on subject preference. (If subjects prefer to be seen in person, but have been discharged from the hospital, a clinic visit with a study team member will be arranged).

Subjects will be permitted to initiate electronic communication with the study team, although planned follow up visits will be conducted by telephone or in person. The study team may reach out to subjects electronically if difficulties arise in reaching the subject by telephone in order to schedule visits, but study-related questions will not be asked electronically. Subjects will be classified as lost to follow up if at least three attempts are made at contact without success; however, medical record review will still be used to obtain follow up information on these subjects. If a subject is eligible and enrolled a second (or greater) time, then their subsequent follow up will be conducted according to the schedule set by their most recent enrollment, and any remaining follow ups from earlier enrollment(s) will not be performed.

6.4. Rescue Therapy

If a subject is thought to have a severe adverse event it will be recorded by the study team and the subject will be followed until resolution and referred to subject's primary clinical care team. In the case of an inadequate clinical response, the subject's primary clinical care team will be contacted, so that the subject can pursue treatment at their discretion. Regardless of rescue therapy used, subjects will continue to be followed until the end of the study.

6.5. Unscheduled Visits

Data collected from subjects who contact the study team (by telephone, electronically, or in-person) outside of the scheduled visits (as above) will be associated with the next scheduled study visit, as each study visit is intended to retrospectively cover the time period since the last study visit.

6.6. Subject Withdrawal

Subjects may withdraw from the study at any time without impact to their regular medical care, but they will receive no further treatment with investigational product. They may also be discontinued from the study at the discretion of the Investigator for lack of adherence to intervention or study procedures or visit schedules, adverse events, or due to other concerns. The Investigator or the Sponsor may withdraw subjects who violate the study plan, or to protect the subject for reasons of safety or for administrative reasons. It will be documented whether or not each subject completes the clinical study. The targeted study enrollment is 300 subjects randomized; subjects who withdrawal after randomization will not be replaced.

6.6.1. Data Collection

Data will be obtained from direct subject observation, review of the subject's electronic medical record, scheduled and unscheduled follow-up visits (which may be in-person or by telephone), as detailed above. For subjects who withdraw consent to participate in the study, data collected up until the time of withdrawal will be kept, but no further data will be collected.

6.6.2. Early Termination Visits

If the subject decides to withdraw from the study after randomization, the subject will be asked to complete all follow-up activities that would have been performed at the next scheduled follow-up visit.

Table 2: Schedule of study procedures

Study Phase	Screening & Enrollment	Intervention	Follow-Up: 30 & 60 minutes	Follow-Up: 7 days	Follow-Up: 30 days	Follow-Up: 56 days	Follow-Up: 90 days	Follow-Up: 180 days
Study Days	**1**	**1 (Retreatment Day 2)**	**1 (Retreatment Day 2)**	**Final Intervention + 7**	**Final Intervention + 30**	**Final Intervention + 56**	**Final Intervention + 90**	**Final Intervention + 180**
Surveillance Test Review	X							
Medical Record Review	X			X	X	X	X	X
Review Inclusion/Exclusion	X							
Informed Consent	X							
Enteral Access Check	X							
Randomization	X							
Dispense PMT to Clinical Site		X						
PMT Receipt Check		X						
PMT Administration		X						
Compliance Check		X						
Vital Signs Review			X	X				
Symptom Review			X	X	X	X	X	X
Laboratory Review				X	X	X	X	X
CRF3 / Outcomes Check				X	X	X	X	X
Adverse Event Check		X	X	X	X	X	X	X
Immune Phenotypic Analysis on Otherwise Discarded Whole Blood				X				

7. Study Evaluations and Measurements

7.1. Medical Record Review

The medical record will be reviewed prior to enrollment, in order to assess eligibility, as outlined above. The medical record will be reviewed at 7-day, 30-day, 56-day, 90-day, and 180-day follow-ups to help ascertain primary and secondary outcomes, as well as adverse events (Sections 3.2 and 9.1.3). HIPAA authorization will be obtained with informed consent.

7.2. Physical Examination

Physical examination will include only of vital sign measurements, as below.

7.3. Vital Signs

Vital signs will be checked by study team personnel during the 60 minute observation period after product administration. Parameters for notifying the clinical care team of abnormalities are outlined in the study intervention phase section above. Vital signs will be checked while subjects are either sitting or lying down, as per their preference.

7.4. Laboratory Evaluations

No laboratory evaluations will be performed as a part of this study. Laboratory values will be collected from review of the subject's electronic medical record, in order to ascertain outcome data and adverse events, as described above.

7.5. Pregnancy Testing

Urine pregnancy testing using an FDA-cleared test will be performed as a part of this study.

7.6. Other Evaluations, Measures

Subjects who are discharged from the hospital prior to 7 days following their last FMT will be provided with a diary and thermometer to keep track of their temperature (once a day), stool frequency, and any emesis. This diary will be reviewed with subjects at their 7-day follow-up visit.

For the exploratory aim, immune phenotypic analysis using flow cytometry may be performed on a subset of subjects using whole blood collected for clinical purposes, which would otherwise be discarded. Blood will be collected from residual material available in EDTA ("purple top") vacutainer tubes already collected in the clinical laboratory.

7.7. Efficacy Evaluations

The primary outcome (time to resolution of R-CDI symptoms) will be assessed at 8 weeks (56 days) following the intervention. The criteria will be assessed via patient interview and electronic medical record review as per Section 3.2.1.

7.8. Genetic Testing (only if applicable)

No human genetic testing will be performed. For the exploratory aim, stool microbiome analysis will be performed on stool samples obtained as described in Section 7.6. These stool samples would otherwise be discarded. This will involve sequencing of microbial nucleic acids. This will not involve human DNA sequencing and thus does not comprise genetic testing. Likewise, phenotyping of immune response via flow cytometry may be performed on a subset of subjects, using whole blood collected for clinical purposes that would otherwise be discarded. This will not involve human DNA sequencing and or genetic testing.

7.9.Safety Evaluations

7.9.1. Deviations and Exceptions

Exception (Prospective action):
An ***exception*** is defined as a one-time, intentional action or process that departs from the approved study protocol, intended for one occurrence. If the action disrupts the study progress, such that the study design or outcomes may be compromised, or the action compromises the safety and/or welfare of study subjects, advance documented approval from the Regulatory Sponsor and local regulatory review committees, per institutional guidelines, is required. Approval from the Regulatory Sponsor must be received prior to submission to the local regulatory review committees.

Deviation (Retrospective action):
A ***deviation*** is defined as a one-time, unintentional action or process that departs from the approved study protocol, involving one incident and identified retrospectively. If the deviation disrupts study progress, such that the study design or outcomes may be compromised, or the deviation compromises the safety and/or welfare of study subjects, the deviation must be reported to the Regulatory Sponsor within 2 days of PI knowledge and to local regulatory review committees per institutional guidelines.

Report the following information on the Sponsor's exception/deviation form:

- Protocol number
- Subject number
- Description of the exception or deviation
- Impact on subject safety
- Impact on data integrity

Deviations that are assessed by the PI to not disrupt the study progress, such as not affecting the study design or outcome, or compromising the safety and/or welfare of study subjects, should be documented in site records and contain documentation of the PI's assessment.

7.9.2. Safety Parameters

Safety will be monitored in several ways:

- 30- and 60-minute observations with vital sign checks after investigational product administration.

- Follow-up at 7 days, 30 days, 90 days, and 180 days as outlined in the schedule of study procedures (Table 1).
- Examination of safety-related secondary outcomes, including: bacteremia, mortality, hospital admission, colectomy or diverting ileostomy.

8. Statistical Plan

8.1. Primary Endpoint

The primary endpoint will be proportion of subjects with clinical resolution of diarrhea without recurrence in subjects with R-CDI at 8 weeks (56 days) following FMT. To address the potential for misclassification of subjects who are only NAAT-positive for *C. difficile* (McDonald 2018), the primary analysis will be stratified based on the most recent positive CDI test (NAAT versus EIA) prior to enrollment.

8.2. Secondary Endpoints

Secondary endpoints are detailed in Section 3.2.2. Secondary endpoints will include all-cause mortality (30- and 60-days following last FMT), colectomy or diverting ileostomy within 30 days after last FMT, cumulative days of hospitalization from enrollment until 30 days after FMT, cumulative days in intensive care unit from enrollment until 30 days after last FMT, bacteremia from enrollment until 30 days after last FMT, hospital admission within 60 days of intervention.

8.3. Sample Size and Power Determination

The primary analysis will be stratified based on the most recent positive CDI test (NAAT versus EIA) prior to enrollment. Given the anticipated enrollment of 300 subjects (150 per intervention group), a conservative estimate that 10% of subjects will be lost to follow-up (i.e., minimum subject number 90% of total), and the expectation that the ratio of EIA-positive/NAAT-positive subjects will be 1:2, the expected sample size will be 90 for EIA and 180 for NAAT (150*0.9=135 per arm, with a 45:90 ratio for EIA/NAAT). The low-dose cure rate is anticipated to be about 50% in the NAAT group and 70% in the EIA group. Based on these assumptions, the detectable difference between the cure rates for the low and high dose groups for 80% power and varying type I error rates, based on a Chi-square test (two-sided), are provided below.

Stratified Population	Number of subjects (subjects per arm)	Type 1 Error	Power	Detectable difference in proportions compared to 50%
EIA	90 (45)	0.05	0.8	0.7725
EIA	90 (45)	0.1	0.8	0.7458
EIA	90 (45)	0.2	0.8	0.7133
NAAT	180 (90)	0.05	0.8	0.7003
NAAT	180 (90)	0.1	0.8	0.6793
NAAT	180 (90)	0.2	0.8	0.6543

For phase II studies it is typical to accept a type 1 error of greater than 0.05 (Schoenfeld 1980; Khan 2012). For this phase II study, we will accept a difference between intervention groups that meets a type I error threshold of 0.2. Therefore, we will have 80% power to detect a difference of 0.2133 (50% to 71.33%) in the EIA group, and a difference of 15.43% (50% to 65.43%) in the NAAT group. The power calculations were performed using PASS 16 (Power Analysis and Sample Size Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.).

8.4. Statistical Methods

8.4.1. Baseline Data

Baseline and demographic characteristics, as well as vital signs, laboratory values, and radiologic study results, will be summarized by standard descriptive statistics (including mean and standard deviation for continuous variables such as age and standard percentages for categorical variables such as gender). Collected variables will include but not be limited to: body mass index (BMI), comorbid medical conditions, use of immunosuppressive medications, use of proton pump inhibitor (PPI) or H2 antagonist medications, use of concurrent non-CDI directed antibiotics, hospital length of stay and level of care. Time between CDI diagnosis and intervention will also be assessed.

8.4.2. Efficacy Analysis

An intention to treat analysis will be performed for the primary outcome of clinical resolution of diarrhea without recurrence in subjects with R-CDI at 8 weeks following FMT. A logistic regression model will be used to compare the two study groups (single-dose versus double-dose) to detect superiority of a dose. A p-value (one-sided) of <0.2 will be considered significant. As above, the primary analysis will be stratified based on the most recent positive CDI test (NAAT versus EIA) prior to enrollment. Per protocol analysis will also be performed, based on received (rather than randomized) treatment.

8.4.3. Interim Analysis

No interim analysis is planned.

8.4.4. Safety Analysis

All subjects entered into the study and randomized will have detailed information collected on adverse events starting at enrollment and for the duration of follow-up, as described elsewhere. The primary safety endpoints of frequency of AEs, SAEs, and AESIs will be described.

8.4.5. Exploratory Analyses

To characterize (1) the bacterial community composition of the administered PMT product, we will apply 16S ribosomal RNA (rRNA) gene sequencing to the drug substance. To characterize (2) the host immune phenotype associated with R-CDI, we will perform Luminex bead assay (cytokine/chemokine levels) and flow cytometric analysis (granulocyte/mononuclear cell counts) to serum and blood specimens collected as part of routine clinical care prior to FMT.

8.5. Subject Population(s) for Analysis

For the primary analysis, an intention-to-treat (ITT) population Secondary per-protocol analyses will also be performed. All randomized subjects will be included in the latter analyses.

9. Safety and Adverse Events

9.1. Definitions

9.1.1. Adverse Event

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Intercurrent illnesses or injuries should be regarded as adverse events.

A pre-existing condition should be recorded as an adverse event if the frequency, intensity or the character of the condition changes.

9.1.2. Serious Adverse Event

Adverse events are classified as serious or non-serious. A serious adverse event (SAE) is any AE that, in the view of either the investigator or the sponsor, is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

9.1.3. Adverse Events of Special Interest (AESI)

Based on prior published experience with FMT-associated risks (Table 1), we have defined several adverse events of special interest.

In the initial, 60-minute bedside observation period following administration of investigational product, subjects will be monitored with close attention to the following:

- allergic reaction (temperature, skin rash, heart rate, or blood pressure change as already described) -- PMT-001, 002, and 003
- sepsis or shock (temperature, heart rate, or blood pressure change as already described) – PMT-001, 002, and 003
- bowel injury (abdominal pain change as already described) – PMT-001, 002, and 003

- aspiration (respiratory rate or oxygen saturation change as already described) – PMT-002 and 003 only

In the subsequent, 7-day monitoring period following administration of investigational product, subjects will be monitored with close attention to the following:

- donor-derived infection (temperature or abdominal pain change as already described) – PMT-001, 002, and 003
- worsening CDI (temperature, change in stool frequency/quantity or abdominal pain, or emesis as already described) – PMT-001, 002, and 003

In the subsequent, 30-, 56-, 90-, and 180-day monitoring periods, subjects will be monitored with close attention to the following:

- transmitted infection (temperature or abdominal pain change as already described) – PMT-001, 002, and 003
- recurrent CDI (temperature, change in stool frequency/quantity or abdominal pain, or emesis as already described) – PMT-001, 002, and 003
- metabolic changes (polydipsia, polyuria, weight gain or weight loss) – PMT-001, 002, and 003

9.1.4. Expected Adverse Events

Based on our own experience and the published literature (Table 1), we expect that gastrointestinal symptoms (including fever, aspiration, belching, bloating, nausea or vomiting, gastroesophageal reflux, abdominal cramps, abdominal pain, diarrhea, constipation) may be common post administration of all three investigational products, both as a consequence of the underlying disease being treated, and potentially as an adverse effect of the administered product.

To distinguish a causal relationship between these AEs and the investigational product, we will set a threshold of >= 50% increase in gastrointestinal symptom severity (e.g., number of stools or volume of diarrhea; number of vomiting episodes or hours/day of nausea) as compared to symptom severity in the 48-hour period surrounding the intervention.

9.2. Recording of Adverse Events

Safety will be assessed by monitoring and recording potential adverse effects using the Common Toxicity Criteria version 5.0 (CTCAE V5.0) at each study visit. Participants will be monitored by medical histories, physical examinations, and other studies. If CTCAE V5.0 grading does not exist for an adverse event, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, will be used whenever possible.

At each contact with the subject, the investigator will seek information on adverse events by non-directive questioning and, as appropriate, by examination. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all adverse events will be recorded in the source documentation. To the extent possible, adverse events will

be recorded as a diagnosis and symptoms used to make the diagnosis recorded within the diagnosis event.

As much as possible, each adverse event or follow-up information will be evaluated to determine:

1. Severity grade (CTCAE V5.0 Grade 1-5)
2. Duration (start and end dates)
3. Relationship to the study treatment or process – [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected) - is the event possibly, probably or definitely related to the investigational treatment?
4. Action taken with respect to study or investigational treatment or process (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
5. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
6. Whether the event is serious

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

9.3. Reporting of Adverse Events and Unanticipated Problems

Reporting period
Adverse events will be reported from the time of informed consent until study completion.

Investigator reporting: notifying the study sponsor
The sponsor must be notified within 24 hours of learning of an SAE occurrence, regardless of suspected causality (e.g., relationship to study drug(s) or study procedure or disease progression).

At the time of the initial notification, the following information should be provided:

- Study identifier
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Follow-up information on SAEs should be reported when updates are available, as a follow-up to the initial SAE report, and should include both the follow-up number and report date. New information on ongoing serious adverse events should be provided promptly to the sponsor. The

follow-up information should describe whether the event has resolved or continues, if there are any changes in assessment, if and how it was treated, and whether the patient continued or withdrew from study participation. The investigator must follow the event to resolution or until the event is deemed and documented irreversible, whichever is longer.

Recurrent episodes, complications, or progression of the initial SAE must be reported as the follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE considered completely unrelated to a previously reported one should be reported separately as a new event.

In addition, all unexpected fatal or life-threatening suspected adverse reaction will be reported to the FDA as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Other SAEs that are both, unexpected and related to the study drug (SUSAR) will be reported to the FDA as soon as possible but no later than 15 calendar days after the sponsor knowledge of the event.

Investigator Reporting: Local Reporting Requirements
The investigator will report AEs and SAEs to his/her IRB/EC of record and other local regulatory groups per the local requirements.

9.3.1. Pregnancy

Pregnancy, in and of itself, is not regarded as an AE unless there is suspicion that study drug or process may have interfered with the effectiveness of a contraceptive medication or method. When a pregnancy has been confirmed in a subject during maternal or paternal exposure to study drug and/or process, the following procedures should be followed to ensure subject safety:

Data on fetal outcome are collected for regulatory reporting and drug safety evaluation. Follow-up should be conducted for each pregnancy to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. A case report form will capture these outcomes for pregnant subjects, and the sponsor will be notified within 24 hours of recorded an adverse event related to pregnancy.

9.4. Toxicity Management, Stopping Rules and Study Termination

Enrollment and administration of the study product will be halted pending review by the study staff and Data Safety Monitoring Board (DSMB) if any subject experiences an AE that is assessed as (1) serious (i.e., that results in significant symptoms preventing normal daily activities or requiring hospitalization), (2) unexpected treatment emergent, and (3) related to administration of the study product. Enrollment and administration of the study product will be halted pending review by the study staff and Data Safety Monitoring Board (DSMB) if any subject experiences a suspected or proven infection assessed as related to the study product. The review of these adverse events, and any decision to prematurely stop subject enrollment, will be determined by the Medical Director and DSMB.

9.5. Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above.

9.5.1. Data and Safety Monitoring Plan

A Drug Safety Monitoring Plan has been developed based on risk and is described in a separate document. The monitoring plan will include the following components:

- Principal Investigator monitoring
- The Sponsor Medical Director
- Data Safety and Monitoring Board (DSMB)

9.5.2. Data Safety Monitoring Board

An independent DSMB will perform evaluations of safety data at specified intervals throughout the study and make recommendations to the Sponsor regarding further conduct of the study. The DSMB will evaluate subject safety as specified in the DSMB Charter.

10. Study Administration, Data Handling and Record Keeping

10.1. Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

10.2. Future Use of Stored Specimens and Data

Stool and blood specimens collected as above (Section 7.6) may be preserved in frozen storage for future analyses. The data generated from those specimens will only be used for research purposes. It will not be shared with subjects or be used to direct treatment.

10.3. Data Collection and Management

Case Report Forms (CRFs) will be completed, with copies either printed and stored in binders in a secure location or securely stored in an approved electronic system to which only study

personnel will have access. Subject diaries will also be collected and stored in a secure location. Subjects will be assigned a unique study identification number, and PHI will be kept separately with a master file stored on a secure, password-protected computer. Linkage to PHI will be stored until study analysis and publication completion, and then will be destroyed.

Exploratory microbiome analysis will be performed on stool specimens and exploratory immune response phenotyping using flow cytometry will be performed using a unique study identification number, with no associated PHI. The exploratory analysis of subject microbiome composition (will or will not) be shared with the subjects treating physician.

Stool and blood specimens will be coded to maintain patient confidentiality and will be stored according to regulations. The subject specific stool samples be tracked with a master file linking PHI to study ID numbers, which will be kept on a secure, password-protected computer.

10.4. Records Retention

Data (electronic and paper) will be stored in a secure location until study analysis and publication completion, after which any linkage to PHI will be destroyed. Anonymized microbial nucleic acid sequence data and associated metadata will be uploaded on public databases (i.e., the National Center for Biotechnology Information's Sequence Read Archive) as required by funding agencies.

11. Study Monitoring, Auditing, and Inspecting

11.1. Study Monitoring Plan

This study will be monitored according to the data safety monitoring plan. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

11.2. Data Quality Assurance

During monitoring visits, the monitor will review CRFs to ensure data accuracy, completeness, and clarity, including laboratory reports and other subject records with the stipulation that subject confidentiality will be strictly maintained in accordance with local and federal regulations, including HIPAA requirements. Instances of missing or uninterruptible data will be resolved in coordination with the Investigator.

11.3. Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator shall notify the Sponsor of all audits and inspections by regulatory bodies. The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.). Participation as an

investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

12. Ethical Considerations

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study.

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by the University of Pennsylvania and the Sponsor. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, provided the IRB/IEC is notified within 5 days.
Any permanent change to the protocol must be handled as a protocol amendment. The written amendment must be submitted to the IRB/IEC and the investigator must await approval before implementing the changes. The Sponsor will submit protocol amendments to the appropriate regulatory authorities for approval.

12.1. Risks

The main risks of this study are related to administration of the investigational product. The most severe possible risks include: allergic reaction, donor-derived infection, aspiration (for upper gastrointestinal administration), bowel perforation (for lower gastrointestinal administration), and death. Other risks include: nausea, vomiting, abdominal pain, diarrhea, flatulence, fever. Product specific risks are described in Section 9.1.4.

Theoretical long-term risks may include: changes in metabolism, weight changes, development of autoimmune conditions.

Other risks include loss of privacy or confidentiality, as a result of study staff interacting with subjects.

These risks will be discussed with subjects during the informed consent process. The eligibility criteria will help mitigate these risks (e.g., excluding subjects with neutropenia or bowel perforation, and not permitting subjects at risk for aspiration to receive investigational product via upper gastrointestinal delivery). To minimize the risk of privacy and confidentiality loss, the minimum number of required study staff will interact with subjects, and all collected data will be protected as above.

12.2. Benefits

Potential direct benefits include: cure or improvement of CDI symptoms more quickly than with standard of care, reduction in risk of complications of CDI (death, need for surgery), reduction in risk of recurrence of CDI, which have been suggested by observational studies to date (Table 1). Indirect benefits include contributing to medical knowledge about the utility of FMT in this study population and therefore helping patients who have this disease in the future.

12.3. Risk Benefit Assessment

Based on the risks and benefits outline above, the benefits of this study outweigh the potential risks. This is a patient population with significant illness at high risk for complications, and the investigational products are likely to reduce this risk. While there are potential adverse outcomes associated with this product, prior studies have demonstrated that the risk of these occurring is relatively low.

12.4. Informed Consent Process / HIPAA Authorization

Informed consent must be obtained before any of the baseline procedures are performed. The consent process will occur in the setting of the potential subject's regularly scheduled clinical care or by telephone after referral from the potential subject's clinical care team. Written informed consent will be obtained whenever possible. In cases where COVID-19 isolation status or visitor policies preclude an in-person consent process, verbal consent will be obtained utilizing a witnessed consent. This will be then documented in both the electronic health record and the data collection system. An explanation of the trial and discussion of the possible risks and discomforts will be given by the Investigators and/or study staff. Only those potential subjects who fulfill all eligibility criteria will be entered into the trial. Informed consent will take place as an ongoing dialogue between the Investigator/study staff and subjects during the entire duration of their participation. Potential subjects that are unable to read the study consent and/or do not have the opportunity to take home and review with family/friends or do not present in the clinic with a family member will have the consent read to them by a study team member in the presence of a non-partial person that will verify the consent has been read in full to the patient. Privacy will be preserved by minimizing the number of study staff in contact with the potential subject. Coercion will be avoided by clearly stating that declining to participate will not affect clinical care. The informed consent and HIPAA authorization will be documented on paper, and completed paper records will be scanned for digital storage on secure university servers.

13. Study Finances

13.1. Funding Source

Program support from PennMedicine, Department of Medicine, and Centers for Disease Control and Prevention (CDC) U54CK000485.

13.2. Conflict of Interest

All University of Pennsylvania Investigators will follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research.

13.3. Subject Stipends or Payments

There are no subject payments or stipends.

14. Publication Plan

The investigator is responsible for authoring a final clinical study report and sharing with the sponsor team. The Clinical Study Report will be issued within 12 months of data lock and the results summary will be posted to clinicaltrials.gov. as required by legal agreement, local law, or regulation. A manuscript will be progressed for publication in the scientific literature if the results provide important scientific or medical knowledge. This study will be published by the principal investigator, Dr. Lautenbach.

15. References

Agrawal M, Aroniadis OC, Brandt LJ, Kelly C, Freeman S, Surawicz C, Broussard E, Stollman N, Giovanelli A, Smith B, Yen E, Trivedi A, Hubble L, Kao D, Borody T, Finlayson S, Ray A, Smith R. The Long-term Efficacy and Safety of Fecal Microbiota Transplant for Recurrent, Severe, and Complicated Clostridium difficile Infection in 146 Elderly Individuals. J Clin Gastroenterol. 2016 May;50(5):403–407. PMID: 26352106

Aroniadis OC, Brandt LJ, Greenberg A, Borody T, Kelly CR, Mellow M, Surawicz C, Cagle L, Neshatian L, Stollman N, Giovanelli A, Ray A, Smith R. Long-term Follow-up Study of Fecal Microbiota Transplantation for Severe and/or Complicated Clostridium difficile Infection: A Multicenter Experience. J Clin Gastroenterol. 2016 May;50(5):398–402. PMID: 26125460

Bojanova DP, Bordenstein SR. Fecal Transplants: What Is Being Transferred? PLoS Biol. 2016 Jul;14(7):e1002503. PMCID: PMC4942072

Cammarota G, Ianiro G, Gasbarrini A. Fecal microbiota transplantation for the treatment of Clostridium difficile infection: a systematic review. J Clin Gastroenterol. 2014 Sep;48(8):693–702. PMID: 24440934

Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, Sokol H, Arkkila P, Pintus C, Hart A, Segal J, Aloi M, Masucci L, Molinaro A, Scaldaferri F, Gasbarrini G, Lopez-Sanroman A, Link A, de Groot P, de Vos WM, Högenauer C, Malfertheiner P, Mattila E, Milosavljević T, Nieuwdorp M, Sanguinetti M, Simren M, Gasbarrini A, European FMT Working Group. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. BMJ Publishing Group; 2017 Apr;66(4):569–580. PMCID: PMC5529972

Fischer M, Sipe B, Cheng Y-W, Phelps E, Rogers N, Sagi S, Bohm M, Xu H, Kassam Z. Fecal microbiota transplant in severe and severe-complicated Clostridium difficile: A promising treatment approach. Gut Microbes. 2017 May 4;8(3):289–302. PMCID: PMC5479393

Fischer M, Sipe BW, Rogers NA, Cook GK, Robb BW, Vuppalanchi R, Rex DK. Faecal microbiota transplantation plus selected use of vancomycin for severe-complicated Clostridium

difficile infection: description of a protocol with high success rate. Aliment Pharmacol Ther. 2015 Aug;42(4):470–476. PMID: 26096320

Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. Am J Gastroenterol. American College of Gastroenterology; 2012 May;107(5):761–767. PMID: 22290405

Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, Chang H-J, Coward S, Goodman KJ, Xu H, Madsen K, Mason A, Wong GK-S, Jovel J, Patterson J, Louie T. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. American Medical Association; 2017 Nov 28;318(20):1985–1993. PMCID: PMC5820695

Kelly BJ, Tebas P. Clinical Practice and Infrastructure Review of Fecal Microbiota Transplantation for Clostridium difficile Infection. Chest. 2018 Jan;153(1):266–277. PMCID: PMC5812771

Kelly CR, Khoruts A, Staley C, Sadowsky MJ, Abd M, Alani M, Bakow B, Curran P, McKenney J, Tisch A, Reinert SE, Machan JT, Brandt LJ. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med. 2016 Nov 1;165(9):609–616. PMCID: PMC5909820

Khan I, Sarker S-J, Hackshaw A. Smaller sample sizes for phase II trials based on exact tests with actual error rates by trading-off their nominal levels of significance and power. Br J Cancer. 2012 Nov 20;107(11):1801–1809. PMCID: PMC3504941

Krajicek E, Fischer M, Allegretti JR, Kelly CR. Nuts and Bolts of Fecal Microbiota Transplantation. Clin Gastroenterol Hepatol. 2019 Jan;17(2):345–352. PMID: 30268564

Lee YJ, Arguello EP, Jenq RR, Littmann E, Kim GJ, Miller LC, Ling L, Figueroa C, Robilotti E, Perales M-A, Barker JN, Giralt S, van den Brink MRM, Pamer EG, Taur Y. Protective Factors in the Intestinal Microbiome Against Clostridium difficile infection in Recipients of Allogeneic Hematopoietic Stem Cell TransplantationProtective Factors Against C. difficile. J Infect Dis [Internet]. 2017 Jan 30; Available from: https://academic.oup.com/jid/article/doi/10.1093/infdis/jix0111/2964582/Protective-Factors-in-the-Intestinal-Microbiome PMID: 28329030

McDonald LC, Gerding DN, Johnson S, Bakken JS, Carroll KC, Coffin SE, Dubberke ER, Garey KW, Gould CV, Kelly C, Loo V, Shaklee Sammons J, Sandora TJ, Wilcox MH. Clinical Practice Guidelines for Clostridium difficile Infection in Adults and Children: 2017 Update by the Infectious Diseases Society of America (IDSA) and Society for Healthcare Epidemiology of America (SHEA). Clin Infect Dis. 2018 Mar 19;66(7):987–994. PMCID: PMC6018983

Schoenfeld D. Statistical considerations for pilot studies. Int J Radiat Oncol Biol Phys. 1980 Mar;6(3):371–374. PMID: 7390914

Seekatz AM, Aas J, Gessert CE, Rubin TA, Saman DM, Bakken JS, Young VB. Recovery of the gut microbiome following fecal microbiota transplantation. MBio. 2014 Jun 17;5(3):e00893–14. PMCID: PMC4068257

van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Bartelsman JFWM, Tijssen JGP, Speelman P, Dijkgraaf MGW, Keller JJ. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med. 2013 Jan 31;368(5):407–415. PMID: 23323867

Weingarden AR, Hamilton MJ, Sadowsky MJ, Khoruts A. Resolution of severe Clostridium difficile infection following sequential fecal microbiota transplantation. J Clin Gastroenterol. 2013 Sep;47(8):735–737. PMCID: PMC3735621

Youngster I, Mahabamunuge J, Systrom HK, Sauk J, Khalili H, Levin J, Kaplan JL, Hohmann EL. Oral, frozen fecal microbiota transplant (FMT) capsules for recurrent Clostridium difficile infection. BMC Med. 2016 Sep 9;14(1):134. PMCID: PMC5016994

Youngster I, Sauk J, Pindar C, Wilson RG, Kaplan JL, Smith MB, Alm EJ, Gevers D, Russell GH, Hohmann EL. Fecal microbiota transplant for relapsing Clostridium difficile infection using a frozen inoculum from unrelated donors: a randomized, open-label, controlled pilot study. Clin Infect Dis. 2014 Jun;58(11):1515–1522. PMCID: PMC4017893

Youngster I, Russell GH, Pindar C, Ziv-Baran T, Sauk J, Hohmann EL. Oral, capsulized, frozen fecal microbiota transplantation for relapsing Clostridium difficile infection. JAMA. 2014 Nov 5;312(17):1772–1778. PMID: 25322359