A PHASE II RANDOMIZED TRIAL TO EVALUATE THE SAFETY AND EFFICACY OF FECAL MICROBIOTA TRANSPLANTATION USING THE PENN MICROBIOME THERAPY PRODUCTS FOR SEVERE OR SEVERE-COMPLICATED/FULMINANT *CLOSTRIDIUM DIFFICILE* INFECTION

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Funding	Program support from Penn Medicine, Department of Medicine and Centers for Disease Control and Prevention [redacted]
Investigational Products	Penn Microbiome Therapy (PMT) -001, 002, 003
IRB Number	832962
IND Number	18904
ClinicalTrials.gov Number	NCT03970200

Table of Contents

Table of Tables

List of Abbreviations

AE: Adverse event
AESI: Adverse event of special interest
AFT: accelerated failure time
ANC: Absolute neutrophil count
BMI: body mass index
CCH: Chester County Hospital
CDI: *Clostridium difficile* infection
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
mL: milliliters
m-ITT: modified intention to treat
PAH: Pennsylvania Hospital
PHI: protected health information (PHI)
PMPMC: PennMedicine Princeton Medical Center (PMPMC)
PMT: Penn Microbiome Therapy
PPI: proton pump inhibitor
PPMC: Penn-Presbyterian Medical Center
R-CDI: Recurrent *Clostridium difficile* infection
SAE: Serious Adverse Event
S/SC/F-CDI: severe or severe-complicated/fulminant *Clostridium difficile* infection
SIRS: systemic inflammatory response syndrome
SUSAR: Suspected unexpected serious adverse reaction
WBC: white blood cells

Study Summary

Title	A Phase II, Randomized Trial to Evaluate the Safety and Efficacy of Fecal Microbiota Transplantation using the Penn Microbiome Therapy Products for Severe or Severe-Complicated/Fulminant *Clostridium difficile* Infection
Short Title	PMT for Severe-CDI
IRB Number	832962
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 severe and severe-complicated/fulminant *Clostridium difficile* infection (S/SC/F-CDI) by comparing the clinical outcomes of three treatment strategies: (1) no investigational product (standard of care only); (2) upper gastrointestinal administration FMT in additional to standard of care; (3) lower gastrointestinal FMT in addition to standard of care. **Secondary:** To evaluate the safety of the PMT suite of investigational products for use in treating S/SC/F-CDI. **Exploratory:** To characterize (1) the baseline gut bacterial community composition and host immune phenotype associated with S/SC/F-CDI, (2) the change in gut bacterial community composition and host immune phenotype associated with each of the above treatment strategies, and (3) to determine the relationship between baseline gut bacterial community composition, host immune phenotype, and treatment outcome in patients with S/SC/F-CDI.
Number of Subjects	Target enrollment: 90 subjects

Main Inclusion and Exclusion Criteria

Inclusion Criteria:

1. One or more episodes of CDI with symptoms including bowel movements altered in frequency or consistency from baseline.
2. Stool test positive for *Clostridium difficile* by EIA by FDA-cleared assay within 7 days prior to enrollment.
3. Age ≥ 18 years.
4. Meeting criteria for S/SC/F-CDI within 72 hours of 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).
5. Receiving antibiotic treatment for S/SC/F-CDI per current IDSA guidelines (i.e., vancomycin or fidaxomicin).

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. Receipt of FMT or enrollment in a clinical trial for FMT within the last 3 months.
7. COVID-19 infection, as defined by a positive nucleic acid or antigen test within the prior 14 days and symptoms consistent with COVID-19 infection

Investigational Product	1. Penn Microbiome Therapy – 001 (PMT-001): no more than 152mL 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 152mL 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	Standard of care antibiotics as prescribed by treating clinician. (Must be concordant with current IDSA guidelines to be eligible for enrollment).
Statistical Methodology	Primary outcome will be time to resolution of severe symptoms. We will compare both FMT groups (i.e., upper and lower gastrointestinal administration) in aggregate versus the standard of care group via competing risk regression model.
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). Though there have been only small, observational studies and case series of FMT for patients with severe and severe complicated/fulminant CDI, these suggest that FMT may also be of benefit in severe and severe complicated/fulminant CDI (S/SC/F-CDI) as well (Weingarden 2013; Agarwal 2015; Aroniadis 2015; Fischer 2015; Fischer 2017). We will perform a randomized controlled trial to evaluate the safety and efficacy of Penn Microbiome Therapy (PMT) products for FMT in subjects who meet criteria for S/SC/F-CDI. The PMT products comprise three different formulations of FMT for oral, enteric (via tube), or rectal delivery. The S/SC/F-CDI trial will compare the efficacy of PMT with standard of care antibiotic therapy to standard of care antibiotic therapy alone. We will also assess the safety of PMT in this study population. Finally, we will characterize key microbiome and host immune phenotype features associated with S/SC/F-CDI disease and recovery in an exploratory fashion. The planned study will inform treatment recommendations for S/SC/F-CDI and lay the groundwork for novel CDI risk stratification and S/SC/F-CDI prevention strategies.

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 alone are the recommended therapy for S/SC/F-CDI (McDonald 2018), but observational studies suggest a benefit for FMT in the S/SC/F-CDI population as well (Weingarden 2013; Agarwal 2015; Aroniadis 2015; Fischer 2015; Fischer 2017).

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.
  - 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). Observational studies have suggested that FMT may have similar efficacy for S/SC/F-CDI (Weingarden 2013; Agarwal 2015; Aroniadis 2015; Fischer 2015; Fischer 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	S/SC/F-CDI	case series	50g stool	250mL suspension	colonoscopy	(not reported)
Agrawal et al	2015	S/SC/F-CDI	case series	~30-60g stool	150-500mL suspension	upper endoscopy, lower endoscopy, enema	diarrhea, constipation, abdominal pain, ileus
Aroniadis et al	2015	S/SC/F-CDI	case series	(not reported)	suspension	upper endoscopy, lower endoscopy, enema, colonoscopy	diarrhea, abdominal pain
Fischer et al	2015	S/SC/F-CDI	case series	50-100g stool	300mL suspension	sigmoidoscopy or colonoscopy	treatment failure and death
Fischer et al	2017	S/SC/F-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). Protocols for the treatment of S/SC/F-CDI have typically used similar FMT doses as protocols for the treatment of R-CDI, but have allowed for repeat FMT administration at short intervals if clinically indicated (Weingarden 2013; Agarwal 2015; Aroniadis 2015; Fischer 2015; Fischer 2017). See Table 1 for a summary of doses described in the literature.

The S/SC/F-CDI trial protocol allows for repeat PMT dosing at 3-day intervals, up to a maximum of three doses, if subjects fail to meet pre-established criteria for clinical improvement. The repeat administration strategy is based on the observation that some patients with S/SC/F-CDI fail to improve with initial FMT administration but do improve after repeat administration (Weingarden 2013; Agarwal 2015; Aroniadis 2015; Fischer 2015; Fischer 2017).

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 severe and severe-complicated/fulminant *Clostridium difficile* infection (S/SC/F-CDI) by comparing the clinical outcomes of three treatment strategies: (1) no investigational product (standard of care only); (2) upper gastrointestinal administration FMT in additional to standard of care; (3) lower gastrointestinal FMT in addition to standard of care.

2.2. Secondary Objective

To evaluate the safety of the PMT suite of investigational products for use in treating S/SC/F-CDI.

2.3. Exploratory Objective

To characterize (1) the baseline gut bacterial community composition and host immune phenotype associated with S/SC/F-CDI, (2) the change in gut bacterial community composition and host immune phenotype associated with each of the above treatment strategies, and (3) to determine the relationship between baseline gut bacterial community composition, host immune phenotype, and treatment outcome in patients with S/SC/F-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 severe or severe complicated/fulminant CDI. If eligible, subjects will participate in the informed consent process. Following informed consent, subjects

will be randomized to one of three arms: (1) standard of care antibiotics (no intervention), (2) PMT-upper GI administration plus standard of care antibiotics, or (3) PMT-lower GI administration plus standard of care antibiotics. Subjects will then be eligible to receive up to two additional administrations of PMT (upper or lower GI) at 3-day (+/- 1 day) intervals if they do not meet pre-defined criteria for clinical improvement (see Section 3.2.1 below). Subjects will be followed until 180 days after their last PMT dose.

3.1.1. Screening Phase

Potential subjects will be identified by active surveillance for inpatients at participating hospitals with positive *C. difficile* EIA stool testing. A positive stool EIA test for *C. difficile* will prompt review of the electronic health record to determine whether a potential subject may meet inclusion/exclusion criteria. If a potential subject may meet inclusion/exclusion criteria, the study team will contact the subject's inpatient care team to ascertain whether the care team has any objection to the study team approaching the potential subject. If the potential subject's care team has no objection, 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. A record of all screened subjects will be maintained to evaluate the frequency of identified exclusions or care-team objections. The study will also be open to patients transferred to a participating hospital who had positive (FDA cleared) *C. difficile* EIA stool testing at an outside hospital prior to transfer, so long as a record of the positive test can be obtained. Care teams at the participating hospitals may refer such patients for participation.

3.1.2. Study Intervention Phase

All S/SC/F-CDI trial interventions will be performed inpatient at the listed study site hospitals. During the intervention period, following informed consent and randomization, subjects in either the upper GI (PMT-002 or PMT-003) or lower GI (PMT-001) intervention arm will receive single-dose PMT administration, with PMT formulation and route of administration (suspension for enema, suspension for enteric delivery, or oral capsules) determined per randomization and stratification scheme (Section 3.1.6). Administration of the product will take place over 10 to 90 minutes (target 30 minutes). Subjects randomized to PMT will be eligible to receive up to two additional administrations of PMT (by clinically appropriate route of administration) at 3-day (+/- 1 day) intervals, if they fail to meet criteria for clinical improvement (Section 3.2.1). Antibiotics prescribed as part of routine clinical care for CDI will not be altered by 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 monitoring: Daily for 3 Days after Administration/Enrollment

The subject will be monitored every calendar day for three days after each PMT administration in order to assess their eligibility for repeat up to 2 additional doses of PMT at 3 days after the first administration and 3 days after a second administration (if given), as well as to assess for adverse events. If the subject continues to meet criteria for severe or severe-complicated disease within the prior 24 hours at 3 days after the initial administration and then 3 days after the second administration (if given), the subject will be eligible for a repeat dose of PMT (same formulation as per initial randomization). If PMT administration is unable to be performed at 3 or 6 days following the first administration, then administration within 1 calendar day before or after those days will be permitted. Assessment for eligibility for an additional dose will be performed on the day of repeat administration. For subjects in the non-PMT arm, monitoring will be performed daily for three days after enrollment for adverse event assessment.

3.1.5. Post-PMT Administration Follow-Up

Following a subject's final PMT administration or enrollment for those in the non-PMT arm, subjects enter the follow up phase. During this phase, the following will occur: (1) 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 or post-enrollment for the non-PMT arm; (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/post-enrollment (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 or after enrollment for non-PMT arm; (4) subjects will be contacted by telephone or in-person at 30 days (+/- 3 days) after final FMT or after enrollment for non-PMT arm for an interview focused on potential adverse events; (5) subjects will be contacted by telephone or in-person at 90 days (+/- 7 days) after final FMT or after enrollment for non-PMT arm for an interview focused on potential adverse events; (6) subjects will be contacted by telephone or in-person at 180 days (+/- 7 days) after final FMT or after enrollment for non-PMT arm 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). 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 or after enrollment for non-PMT arm. 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.

3.1.6. Allocation to Interventional Group

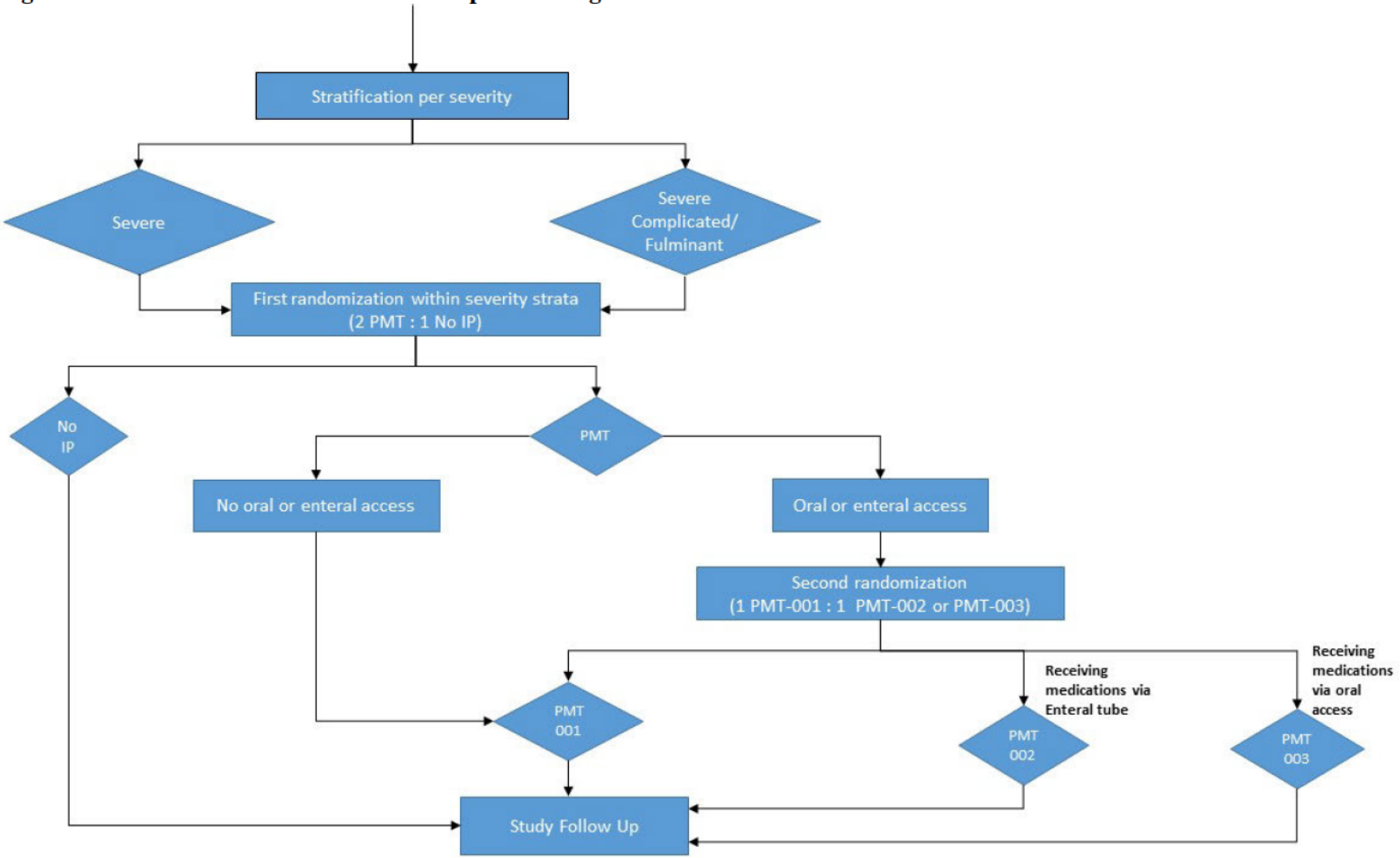
Stratified randomization will be performed based on severity category (severe versus severe complicated) in a 2:1 fashion (two to the FMT intervention (PMT-001, 002, or 003) and one to the standard of care control group). The randomization schedule will be prepared using a random-size block strategy 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.

If subjects are known to have enteral access (either actively receiving capsule medications and/or nutrition by mouth or via pre-existing enteric tube, as prescribed by the clinical care team), they will undergo a second randomization in a 1:1 fashion between allocation to FMT by enema (i.e., PMT-001) or FMT by upper enteral administration (i.e., PMT-002 or PMT-003). Subjects who are randomized to FMT but who lack enteral access or who are deemed to have ileus (i.e., enteral medications and nutrition held) by the clinical care team will receive PMT-001 enema. Subjects who are randomized to FMT but have bowel discontinuity will only be eligible for the intervention arm that coincides with their presumed side of CDI (e.g. if CDI in residual rectum/colon, only eligible for PMT-001). Prior to enrollment of the first subject, a randomization table will be produced for the second (route of administration) randomization using R statistical software.

Subjects randomized to receive FMT, and then to receive FMT via upper delivery will have the decision made between PMT-002/PMT-003 according to how other medications and nutrition are being delivered (i.e., those receiving nutrition and medications via enteric tube will receive PMT-002 suspension, and those eating food and receiving oral medications will receive PMT-003 capsules). Cross-over between PMT-002 and PMT-003 will be permitted if repeat FMT administration is indicated, but a subject's mode of nutrition and medication administration has changed. Cross-over between PMT-001 and PMT-002/003 will be permitted if repeat FMT administration is indicated, but a subject has lost enteral access and is unable to tolerate oral medications.

For a graphical representation of the allocation to the interventional groups, refer to Figure 1.

Figure 1: Allocation to Interventional Group Flow Diagram



3.2. Study Endpoints

3.2.1. Primary Study Endpoints

The primary outcome is resolution of symptoms associated with S/SC/F-CDI, listed below. The primary outcome will be satisfied when the subject is discharged from the hospital (not to hospice or palliative care) or, while the subject remains hospitalized, when the following criteria are met for 72 hours:

- If radiology study or studies performed, ileus/dilation/megacolon either not noted or noted as resolved (electronic medical record search words: "ileus", "dilated", "dilation", or "megacolon")
- Ileus/megacolon either noted as resolved by any provider documentation or not noted (electronic medical record search words: "ileus", "megacolon")
- WBC<15,000 cells/uL
- Serum creatinine decreased, unchanged, or increased by ≤0.2 mg/dL over 72 hours (if not receiving continuous renal replacement therapy (CRRT) or hemodialysis (HD))
- Lactate ≤2.2 mmol/L (if measured by clinical care team)
- No vasopressors used (including epinephrine, norepinephrine, phenylephrine, or vasopressin)
- Temperature <38.5 °C and ≥35.6°C
- < 8 bowel movements per day and < 600 mL unformed stool (if volume recorded)
- Meeting fewer than 3 systemic inflammatory response syndrome (SIRS) criteria (i.e., no more than two of the following: 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)

Discharge to hospice, need for bowel surgery (colectomy, diverting ileostomy), and death will count as failures. Subjects who undergo bowel surgery after initial PMT administration will not be eligible for additional administrations.

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
- Repeat 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.

Monitoring post-PMT administration will occur in three phases:

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 or Post-Enrollment monitoring: Daily for 3 Days

Safety endpoints for all routes of administration will include new ileus, worsened leukocytosis, new/worsened acute kidney injury, new/worsened serum lactate elevation, new/worsened fever, or worsened diarrhea. The definitions of each adverse event are given in Section 3.2.1. The adverse events commonly encountered in prior FMT studies were given in Table 1.

3.2.3.3. Post-PMT or Post-Enrollment 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, 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. One or more episodes of CDI with symptoms including bowel movements altered in frequency or consistency from baseline.
2. Stool test positive for *Clostridium difficile* by EIA by FDA-cleared assay within 7 days prior to enrollment.
3. Age ≥ 18 years
4. Meets any one of the listed criteria for severe or severe-complicated/fulminant disease (see Table 2 below) within 72 hours of enrollment.
5. Receiving antibiotic treatment for S/SC/F-CDI per current IDSA guidelines.

Enrollment criteria details:

1. Must either meet ≥1 criteria in severe category or in severe complicated category to be enrolled.
2. If the subject meets criteria in both categories, stratify to the higher severity category (severe complicated).
3. Detailed enrollment criteria definitions (for Table 2):
    a. WBC ≥15,000 cells/uL – if any value in the time period meets this definition.
    b. Hypotension with systolic blood pressure sustained < 90mmHg for three or more hours or requiring vasopressors (epinephrine, norepinephrine, phenylephrine, or vasopressin).

c. Acute kidney injury – increase in serum creatinine level by ≥50% or new dialysis initiation.
    i. If a baseline serum creatinine value is not available, acute kidney injury will be defined as a serum creatinine >1.5 mg/dL.
d. Temperature ≥38.5 °C or <35.6°C - one value needed in time period.
e. Ileus, bowel dilation or megacolon.
    i. Ileus: If noted in any provider documentation or problem list (search words "ileus") OR
    ii. If the words "dilated" "dilation" or "ileus" are noted in a radiology report on intestines/colon, or if "megacolon" noted.
f. Lactate >2.2 mmol/L - if any value in the time period meets this definition.
g. SIRS criteria:
    i. Heart rate > 90 beats per minute
    ii. Respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg
    iii. Temperature >38ºC or <36ºC
    iv. WBC > 12,000 cells/uL, <4,000 cells/uL, or >10% immature (band) forms

Use Table 2 below to decide if the subject meets criteria for enrollment and their stratification category (severe versus severe-complicated).

Table 2: Enrollment and stratification criteria

	WBC ≥15,000 cells/uL	**Acute kidney injury**	**Hypo-tension**	**Ileus, bowel dilation, or megacolon**	**Temp ≥38.5 °C or <35.6°C**	**Use of vaso-pressors/ hypotension**	**Lactate >2.2 mmol/L**	**≥3 SIRS criteria**
WBC ≥15,000 cells/uL	S	S	SC	SC	SC	SC	SC	SC
Acute kidney injury	S	S	SC	SC	SC	SC	SC	SC
Ileus or abdominal distension	SC	SC	SC	SC	SC	SC	SC	SC
Temp ≥38.5 °C or <35.6°C	SC	SC	SC	SC	SC	SC	SC	SC
Use of vasopressors/ hypotension	SC	SC	SC	SC	SC	SC	SC	SC
Lactate >2.2 mmol/L	SC	SC	SC	SC	SC	SC	SC	SC
≥3 SIRS criteria	SC	SC	SC	SC	SC	SC	SC	SC

S = Severe category
SC = Severe-complicated category
WBC = white blood cell count
SIRS = systemic inflammatory response syndrome

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. Receipt of FMT or enrollment in a clinical trial for FMT within the last 3 months.
7. COVID-19 infection, as defined by a positive nucleic acid or antigen test within the prior 14 days and symptoms consistent with COVID-19 infection

4.3. Subject Recruitment

Potential subjects will be identified by active surveillance for inpatients with positive *C. difficile* EIA stool testing at six participating PennMedicine University of Pennsylvania Health System hospitals (see Section 4.5). A positive stool EIA test for *C. difficile* will prompt review of the electronic health record to determine whether a potential subject may meet inclusion/exclusion criteria. If a potential subject may meet inclusion/exclusion criteria, 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. A record of all screened subjects will be maintained to evaluate the frequency of identified exclusions or care-team objections. The study will also be open to patients transferred to a participating hospital who had positive *C. difficile* EIA stool testing at an outside hospital prior to transfer, so long as a record of the positive test can be obtained. Care teams at the participating hospitals may refer such patients for participation.

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 90 subjects, with 30 randomized to each arm.

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 severe or severe complicated/fulminant CDI. If eligible, subjects will participate in the informed consent process. Following informed consent, subjects will be randomized to one of three arms: (1) standard of care antibiotics (no intervention), (2) PMT-upper GI administration plus standard of care antibiotics, or (3) PMT-lower GI administration plus standard of care antibiotics. In the intervention arms, subjects will then be eligible to receive up to two additional administrations of PMT (upper or lower GI) at 3-day intervals if they do not meet pre-defined criteria for clinical improvement for 24 hours (see Section 2.1). If PMT administration is unable to be performed on the scheduled days (3-day intervals), administration within 1 calendar day before or after those days will be permitted. Subjects will be assessed for eligibility for an additional dose on the day of repeat administration. Subjects will be followed until 180 days after their last PMT dose.

During the intervention period, following informed consent and randomization (Section 3.1.2), subjects in either intervention arm (not the standard of care control arm) will receive single-dose PMT administration, with PMT formulation determined per randomization. Administration of the product is described in Section 5.7 below. Subjects will be eligible to receive up to two additional administrations of PMT (per same randomization) at 3 days and 6 days (+/-1 day) after the initial dose if they fail to meet criteria for clinical improvement (Section 3.2.1) and as long as they do not have bowel surgery. Antibiotics prescribed as part of routine clinical care for CDI will not be altered by 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. 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. The bag may be massaged during thawing. 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 3.1.5). 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 3.

6.1. Screening and Enrollment

6.1.1. Surveillance Test Review

Potential subjects will be identified by active surveillance for inpatients at participating hospitals with positive *C. difficile* EIA stool testing.

6.1.2. Medical Record Review for Inclusion/Exclusion Criteria

A positive stool EIA test for *C. difficile* 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 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. 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. Enteral Access Check

The enrolled subject's enteral access will be reviewed to determine whether the subject qualifies for randomization between upper and lower gastrointestinal delivery of investigational product.

6.1.5. Randomization

Following enrollment, randomization will be performed as per Section 3.1.6.

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 subject is assigned will be prepared and received as described above (Section 5.3).

6.2.2. Administration

During the intervention period, following informed consent and randomization, subjects in either intervention arm (not the standard of care control arm) will receive single-dose PMT administration, with the PMT formulation determined per randomization. Administration of the product (PMT-001, PMT-002, or PMT-003) will take place over 10 to 90 minutes (target 30 minutes). Subjects will be eligible to receive up to two additional administrations of PMT at 3-day intervals, if they fail to meet criteria for clinical improvement. Antibiotics prescribed as part of routine clinical care for CDI will not be altered by the intervention.

6.2.3. Post-Intervention 60 minutes

Subjects will be monitored for a period of 60 minutes following completion of 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).

6.2.4. Post-Intervention 3 Days

The subject will be monitored every calendar day for three days after each PMT administration in order to assess their eligibility for repeat up to 2 additional doses of PMT at 3 days after the first administration and 3 days after a second administration (if given) as well as to assess for adverse events. If the subject continues to meet criteria for severe or severe-complicated disease within the prior 24 hours at 3 after the initial administration and 3 days after a second administration (if given) (see Section 3.2.1) and if the subject has not undergone bowel surgery, the subject will be eligible for an additional dose of PMT (formulation per repeat assessment of subject's mode of nutrition and medication – i.e., change in formulation is allowed). If PMT administration is unable to be performed on the scheduled days, administration within 1 calendar day before or after those days will be permitted. Subjects will be assessed for eligibility for an additional dose on the day of repeat administration. Subjects in the non-PMT arm will be monitored every calendar day for three days after enrollment to assess for adverse events.

6.3. Study Follow-up Phase

6.3.1. Follow-up 7 days

Following a subject's final PMT administration or following enrollment in the non-PMT arm, 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 or post-enrollment in non-PMT arm. In preparation for discharge, subjects will be given a diary for tracking daily temperature, number of bowel movements, and any episodes of emesis for 7 days post last FMT or post-enrollment (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 or post-enrollment for non-PMT arm; 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, daily swabs of available stool and peri-rectal swabs will be taken for microbiome analysis for 7 days post-FMT or post-enrollment for non-PMT arm. If blood that was collected for clinical purposes would otherwise be discarded, it will be captured for immune response phenotyping. Stool specimens will be obtained by placing flocked nylon swabs in stool if available (e.g. in bag of fecal management system). In addition, peri-rectal swabs will be obtained if possible. Blood will be collected from residual material available in EDTA ("purple top") vacutainer tubes already collected in the clinical laboratory. If subjects have been discharged from the hospital, no swabs will be collected. If no residual blood is available, or if subjects have been discharged from the hospital, no blood will be collected. Stool, peri-rectal and 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 or post-enrollment for non-PMT arm 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).

6.3.3. Follow-up 90 days

Subjects will be contacted by telephone or in-person at 90 days (+/- 7 days) after final FMT or post-enrollment for non-PMT arm 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).

6.3.4. 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 or post-enrollment for non-PMT arm 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 90 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.

6.7. 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 3: Schedule of study procedures

Study Phase	Screening & Enrollment	Intervention	Follow-Up: 30 & 60 minutes	Follow-Up: First 3 Days	Follow-Up: 7 days	Follow-Up: 30 days	Follow-Up: 90 days	Follow-Up: 180 days
Study Days	**1**	**1* (Retreatment Day 4 / 7*)**	**1* (Retreatment Day 4 / 7*)**	**Final Intervention +1, +2, + 3**	**Final Intervention + 7**	**Final Intervention + 30**	**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	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
Microbiome Swab from Otherwise Discarded Stool				X	X			
Immune Phenotypic Analysis on Otherwise Discarded Whole Blood				X	X			

*If PMT administration is unable to be performed on days 1, 4 and/or 7, administration and 30/60-minute follow up within 1 calendar day before or after those days will be permitted (for the first administration, administration within 1 calendar day after enrollment will be permitted). If the first administration occurs on day 2, then the second and third administration dates (if eligible) will be days 5 and 8 (+/- 1 calendar day).

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 3-day, 7-day, 30-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 (Section 6.2.3). 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 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, stool microbiome analysis will be performed on stool samples and peri-rectal swabs. Likewise, 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. Stool specimens will be obtained by placing flocked nylon swabs in available stool. Peri-rectal swabs will be obtained if possible. 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 S/SC/F-CDI symptoms) will be satisfied when the criteria listed in Section 3.2.1 are met for 72 hours or when the subject is discharged from the hospital (not to hospice or palliative care). The criteria will be assessed via 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 and peri-rectal swabs obtained as described in Section 7.6. 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 daily for 3 days and at 7 days, 30 days, 90 days, and 180 days as outlined in the schedule of study procedures (Table 3).
- Examination of safety-related secondary outcomes, including: bacteremia, mortality, hospital admission, colectomy or diverting ileostomy.

8. Statistical Plan

8.1. Primary Endpoint

The primary outcome is time to resolution of symptoms associated with S/SC/F-CDI. The primary outcome will be satisfied when the subject is discharged from the hospital (not to hospice or palliative care) or, while the subject remains hospitalized, when the criteria listed in Section 3.2.1 are satisfied for 72 hours.

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 enrollment), colectomy or diverting ileostomy within 30 days after enrollment, cumulative days of hospitalization from enrollment until 30 days after enrollment, cumulative days in intensive care unit from enrollment until 30 days after enrollment, bacteremia from enrollment until 30 days after enrollment, readmission within 60 days of discharge from index hospitalization.

8.3. Exploratory Endpoints

Exploratory endpoints will include: (1) the baseline gut bacterial community composition and host immune phenotype associated with S/SC/F-CDI, (2) the change in gut bacterial community composition and host immune phenotype associated with each of the above treatment strategies, and (3) the relationship between baseline gut bacterial community composition, host immune phenotype, and treatment outcome in patients with S/SC/F-CDI.

8.4. Sample Size and Power Determination

Given the anticipated enrollment of 90 subjects, and the planned primary survival analysis comparing time to resolution of symptoms associated with S/SC/F-CDI across FMT (aggregate) and no-FMT groups, we anticipate the following statistical power to detect a reduction in time to resolution of symptoms in the FMT (aggregate) group, based on a log-rank test:

S/SC/F-CDI Duration without FMT (days)	Percent Time Reduction with FMT (Duration in days)	Type 1 Error	Power (by bootstrap simulation)
2	50% (1)	0.05	0.88
		0.1	0.93
		0.2	0.97
4	25% (3)	0.05	0.27
		0.1	0.39
		0.2	0.53
	50% (2)	0.05	0.88
		0.1	0.93
		0.2	0.97
	75% (1)	0.05	0.99
		0.1	0.99
		0.2	0.99
7	528.6% (5)	0.05	0.35
		0.1	0.47
		0.2	0.61
	42.9% (4)	0.05	0.73
		0.1	0.82
		0.2	0.90
	57.1% (3)	0.05	0.96
		0.1	0.98
		0.2	0.99

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 97% power to detect a reduction from 4 to 2 days (50% reduction) in the FMT group if the baseline duration of severe symptoms is between 4 days and 90% power to detect a reduction from 7 to 4 days (42.9% reduction) in the FMT group if the baseline duration of severe symptoms is between 7 days, both based on a type I error rate of 20%. The power calculations were performed using PASS 16 (Power Analysis and Sample Size Software (2018). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass.).

8.5. Statistical Methods

8.5.1. 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.

8.5.2. Efficacy Analysis

A modified intention-to-treat (m-ITT) analysis will be performed for the primary outcome of time to CDI resolution (endpoint outlined above). In the m-ITT analysis, subjects who die following randomization but before receiving their first FMT will be excluded. A competing risks survival model will be used to compare the FMT (aggregate) and no-FMT groups. A p-value (one-sided) of <0.2 will be considered significant.

8.5.3. Interim Analysis

No interim analysis is planned.

8.5.4. 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.

8.5.5. Exploratory Analyses

To characterize (1) the baseline gut bacterial community composition and host immune phenotype associated with S/SC/F-CDI, we will apply 16S ribosomal RNA (rRNA) gene sequencing to initial (pre-FMT) stool specimens, 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. To characterize (2) the change in gut bacterial community composition and host immune phenotype associated with each of the above treatment strategies, we will apply the same assays to post-FMT stool, peri-rectal, and serum/blood specimens. To determine (3) the relationship between baseline gut bacterial community composition, host immune phenotype, and treatment outcome in patients with S/SC/F-CDI, we will perform regression modeling with bacterial community composition, cytokine/chemokine levels, and granulocyte/mononuclear cell counts as predictor variables.

8.6. Subject Population(s) for Analysis

For the primary analysis, a modified intention-to-treat (mITT) population will be used, in which subjects who die following randomization but before receiving their first FMT will be excluded. Secondary intention-to-treat and 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-, 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 reactions 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 drugs (SUSAR) will be reported to the FDA as soon as possible but no later than 15 calendar days after 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 will be 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, 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.

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