CLINICAL RESEARCH PROTOCOL

INVESTIGATIONAL PRODUCT(S):	Penn Microbiome Therapy – 002 (Suspension) for Fecal Microbial Transplantation (FMT)
STUDY NUMBER:	IRB#: 834196 NCT04201821
PROTOCOL(S) TITLE:	Fecal Microbial Transplantation (FMT) For the Treatment of Fecal Incontinence in Women
IND NUMBER:	19405
REGULATORY SPONSOR:	University of Pennsylvania
FUNDING SPONSOR:	Harrison Grant - Department of OB GYN University of Pennsylvania
MEDICAL DIRECTOR:	Brendan Kelly, MD Division of Infectious Diseases, Department of Medicine
VERSION NUMBER & DATE:	V1.0, 03 Sep 2019 V2.0, 05 Nov 2019 V3.0, 19 Feb 2020 V4.0, 14 Jun 2021 V5.0, 12 May 2022 V6.0, 19 Sep 2022

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LIST OF ABBREVIATIONS

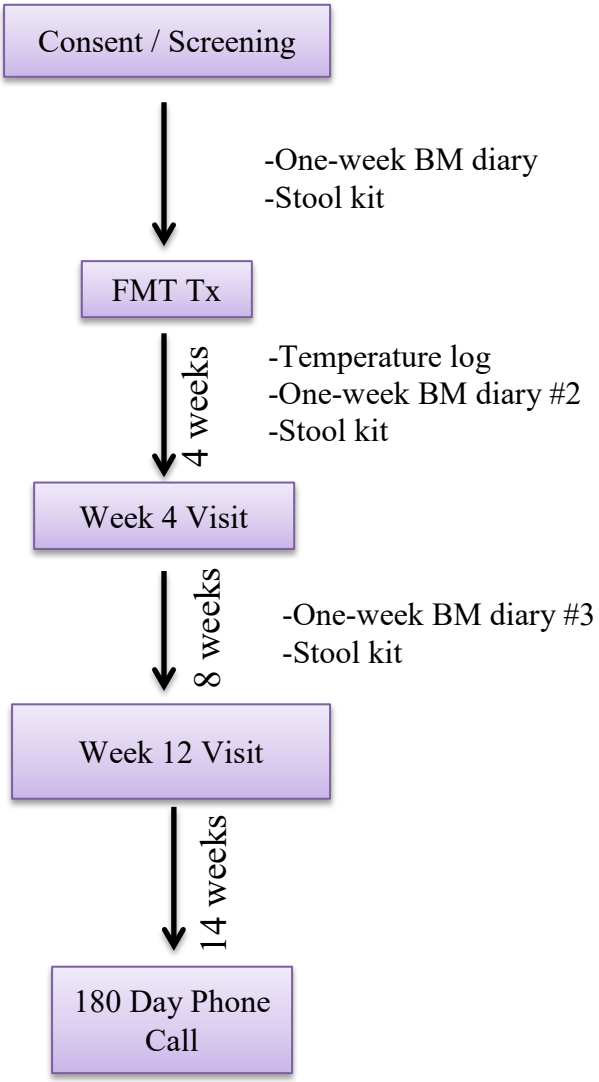
ABL: accidental bowel leakage
AE: adverse event
BM: bowel movement
CDI: *Clostridium difficile* infection
CRF: case report form
CTCAE: common terminology criteria for adverse events
DSMB: Data Safety Monitoring Board
FDA: Food and Drug Administration
FDI-20: Pelvic Floor Disability Index
FI: fecal incontinence
FIQL: Fecal Incontinence quality of life
FMT: Fecal Microbiota Transplantation
GCP: good clinical practices
HIPPA: Health Insurance Portability and Accountability Act of 1996
IBS: irritable bowel syndrome
ICH: International Conference on Harmonisation
IRB: Institutional Review Board
MDRO: multi-drug resistant organism
NG: nasogastric
NGT: nasogastric tube
NIH: National Institutes of Health
OCR: Office of Clinical Research
OHRP: Office for Human Research Protection
PGSC: Patient Global Symptom Control
PHI: protected health information
PI: principal investigator
PMT: Penn Microbiome Therapy
QOL: quality of life
RCT: randomized clinical trial
R-CDI: recurrent *Clostridium dificile* infection
SAE: serious adverse event
SoA: schedule of activities
SUSAR: suspected unexpected serious adverse reaction
UP: unanticipated problems

1 STUDY SUMMARY

1.1 Synopsis

Title:	Fecal Microbial Transplantation (FMT) For the Treatment of Fecal Incontinence in Women
Short Title:	FMT for FI
Study Description:	Open label pilot study assessing FMT to treat fecal incontinence in women $\geq$ 50 years of age.
Objectives:	The primary objective is to determine if fecal microbial transplantation leads to clinical improvement in fecal incontinence.
Primary Endpoint:	Change in the St. Mark's Vaizey score, a measure of fecal incontinence severity, 4 weeks after FMT.
Secondary Endpoints:	Determine whether the effectiveness of microbial engraftment following fecal microbial transplantation is associated with the degree of clinical improvement. Determine whether the baseline microbiota configuration affects engraftment in fecal microbial transplantation for fecal incontinence. Our working hypothesis is that the transplant will be more effective in baseline samples with lower microbial diversity.
Study Population:	15 evaluable women, $\geq$ 50 years of age, who have been diagnosed with fecal incontinence and have failed standard treatment.
Phase:	1/2
Description of Sites/Facilities	Penn Medicine - University of Pennsylvania Health System
Enrolling Participants:	Participants will be enrolled form the Urogynecology clinics at the University of Pennsylvania.
Description of Study Intervention:	Participants will undergo fecal microbial transplantation (FMT) using the Penn Microbiome Therapy – 002 (PMT-002): no more than 152 mL suspension for intragastric or intraduodenal administration from 40g human stool, comprising $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs/dose.
Study Duration:	24 months
Participant Duration:	Approximately 8 months

1.2 Schema


Consent / Screening
-One-week BM diary
-Stool kit
FMT Tx
4 weeks
-Temperature log
-One-week BM diary #2
-Stool kit
Week 4 Visit
8 weeks
-One-week BM diary #3
-Stool kit
Week 12 Visit
14 weeks
180 Day Phone Call

1.3 Schedule of Activities (SoA)

Procedures	Screening/Enrollment	Treatment Visit 1 (within 30 days of screening *)	Follow-Up: 30 & 60 minutes	Phone Contact # 1 (within 7 days of treatment)	Study Visit 2 Week 4 (+/- 7 days)	Study Visit 3 Week 12 (+/- 7 days)	Phone Contact # 2 180 days (+/- 7 days)
Informed Consent	X						
Demographics	X						
Medical History	X						
Concomitant Medication Review	X	X		X	X	X	X
Abbreviated Physical	X						
Stool Collection		X**			X**	X**	
PFIQ-7	X				X	X	
Adaption Index	X				X	X	
Dietary Screener	X						
SF-12	X				X	X	
PFDI-20	X				X	X	
Pregnancy Test (women of childbearing potential)	X	X			X	X	
ABLe Questionnaire	X				X	X	
Radiologic/Imaging Assessment		X					
FIQL	X				X	X	
Bristol Stool Index Questionnaire	X				X	X	
St. Mark's Score	X*				X	X	
PMT Administration		X					
Vital Signs Review			X				
Symptom Review			X				
PGI-I					X	X	X
PGSC					X	X	X
AE/SAE Review		X	X	X	X	X	X
Protocol Deviation Review	X	X		X	X	X	X

Procedures	Screening/Enrollment	Treatment Visit 1 (within 30 days of screening *)	Follow-Up: 30 & 60 minutes	Phone Contact # 1 (within 7 days of treatment)	Study Visit 2 Week 4 (+/- 7 days)	Study Visit 3 Week 12 (+/- 7 days)	Phone Contact # 2 180 days (+/- 7 days)
PAC-SYM	X				X	X	
Temperature Log Completion					X		
One Week BM Diary		X		X	X		

* If time between screening and visit one is greater than 30 days, this test must be repeated prior to administration of FMT.
** Stool Collection will be performed by self-collection procedures and brought by the patient on day of study visit or mailed.

2 INTRODUCTION AND RATIONAL

2.1 Study Rationale

Fecal incontinence, also known as accidental bowel leakage, is a common condition that is an immense burden to older women, caregivers, and the health care system. Traditionally, fecal incontinence has been ascribed to obstetric neuromuscular injury, but preliminary results of a Penn led translational study in the Pelvic Floor Disorders Network show that gut microbiota may be a significant contributor (Arya et al., Neuro Uro, 2018). The overall goal of this study is to gather pilot data in order to conduct a future randomized controlled trial (RCT) for a novel treatment for fecal incontinence in older women utilizing fecal microbial transplantation (FMT). Our hypothesis is that infusion of intestinal microbiota from healthy donors to older women with fecal incontinence will increase microbial diversity, reduce symptom severity, and improve quality of life. We plan to conduct a single arm open label clinical trial of FMT for the treatment of fecal incontinence refractory to conservative management. We will measure the impact of FMT on change in symptom severity and quality of life and stool microbial diversity at 4 and 12 weeks after FMT.

2.2 Background

Childbirth and its associated neuromuscular injury to the anal sphincter muscles have traditionally been considered as the cause of fecal incontinence in women. However, the onset of fecal incontinence is usually after age 50 which is remote from delivery (Robinson, FPMRS, 2013; Rey, Am J Gastro, 2019). Recent large epidemiologic studies suggest that gut motility (diarrhea) and stool consistency (liquid stool) are significant contributing factors that are more important than obstetric factors in the pathogenesis of fecal incontinence (Robinson, FPMRS, 2013; Rey, Am J Gastro, 2019; Bharucha, Gastroenterology, 2010). Since gut motility and stool consistency are modulated by gut microbes, Penn investigators are currently performing a large multi-center translational study using 16S rRNA gene sequencing to investigate stool microbiota and metabolites in women with fecal incontinence and controls. The hypothesis of this study is that fecal incontinence is caused by abnormalities of gut microbial pathways that synthesize butyrate, a metabolite known to modulate gut motility and consistency.

The Penn led study has yielded promising results. *Fecalibacterium prausnitzii*, an important producer of gut butyrate, is lower in women with fecal incontinence than controls. Additionally, functional profiling of the microbial metagenome has revealed that at least four additional pathways may be involved in fecal incontinence (Arya, Neuro Uro, 2018; unpublished data). Thus, gut dysbiosis, involving a variety of microbiota and metabolites, may be important contributors to fecal incontinence and restoring normal fecal microbiota could be a potential novel way of treating fecal incontinence.

Possible options for treating gut dysbiosis include dietary manipulation, probiotics, prebiotics, and FMT. Human feces from healthy donors is diverse and contains many more types of

microbiota than pro- or prebiotics. FMT is also emerging as a promising treatment for inflammatory bowel disease and irritable bowel syndrome (Parfenov, Ter Arkh, 2018; Narula, Inflamm Bowel Dis, 2017; Shi Y, PLoS One, 2016; Holleran, Drugs Today, 2018; Halkjaer, Gut, 2018; Arya, Neuro Uro, 2018). The principal of restoration of dysbiotic gut microbiota through FMT could be applied as a treatment to reduce the severity of fecal incontinence and potentially even remission of disease. Thus, we aim to test the efficacy of FMT for the treatment of fecal incontinence in women.

2.2.1 Assessment for Potential Study Products Drug-Drug Interactions

Potential interaction between the study product and other drug products is currently unknown. As this information is unknown, individuals who are immunocompromised have been excluded from this protocol. Additionally, individuals who have chronic antibiotic use are excluded as investigators are currently unsure if this could potentially be a confounding variable.

2.2.2 Clinical Adverse Event Profile

Based on the published literature for the use of FMT in other disease states, it is expected 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 the investigational products, both as a consequence of the underlying disease being treated, and potentially as an adverse effect of the administered product (Halkjaer, Gut, 2018; Johnsen, Lancet, 2018; Table 1).

To distinguish a causal relationship between these adverse effects (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 one week period surrounding the intervention.

2.2.3 Dosing Rationale

There are no pre-clinical or clinical data for treatment of FI with FMT product to inform optimal dose. Thus, we have relied on the standard dosage used in other disease states. 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 other conditions including Irritable Bowel Disorder and recurrent CDI (Johnsen Lancet 2018; Halkjaer Gut 2018; van Nood NEJM 2013; Youngster JAMA 2014; Kelly CR 2016).

The dose of the PMT investigational product to be employed in this study is based on the use of FMT in prior human studies (See Table 1). A single dose of up to 40g of donated stool, prior to processing into the final form, has been shown to be efficacious in these studies. The PMT products produced from these 40g stool mass contain a range of $1x10^8$ – $1x10^{12}$ anaerobic bacterial CFUs. Given the lack of data for women with FI, we will use the dose that has previously been shown to be efficacious in recurrent CDI.

Table 1: FMT dosing and associated risks.

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

2.3 Risk/Benefit Assessment

2.3.1 Known Potential 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, and death. Other risks include nausea, vomiting, abdominal pain, diarrhea, flatulence, and fever.

As noted above, potential risks of the FMT treatment for FI includes gastrointestinal symptoms (including fever, aspiration, belching, bloating, nausea or vomiting, gastroesophageal reflux, abdominal cramps, abdominal pain, diarrhea, constipation). This is the first attempt to administer FMT for FI in women, however the risks are not expected to be significantly different from that reported in the literature.

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.

2.3.2 Known Potential Benefits

Currently, it is unknown if benefit will be received from participation. It is hypothesized that symptoms of FI will improve after administration of FMT.

2.3.3 Assessment of Potential Risks and Benefits

Gastrointestinal symptoms have been reported following FMT administration in patients with R-CDI (see Table 1). While FMT has not previously been administered for treatment of fecal incontinence, we anticipate that women recruited for this study will be most similar to subjects with irritable bowel syndrome who received FMT. In this patient population, no serious adverse event has been reported following treatment with FMT in several studies (Johnsen, Lancet, 2018; Halkjaer, Gut, 2018). Given the immense burden of FI on quality of life, the potential benefit of improvement in severity of fecal incontinence symptoms vastly outweighs the risk of transient gastrointestinal symptoms.

3 STUDY OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
Determine if fecal microbial transplantation leads to clinical improvement in refractory fecal incontinence in older women.	Number of subjects that show significant improvement at 4 weeks after FMT, and will be maintained at 12 weeks, relative to baseline using the St. Mark's Vaizey score, a measure of fecal incontinence severity.
Primary Safety	
Determine the safety of FMT administration via naso-gastric tube in older women with FI.	Frequency of adverse events, serious adverse events, and adverse events of special interest (including allergic reaction and gastrointestinal symptoms).
Secondary	
Determine if FMT leads to improved quality of life for subjects.	Number of subjects that have an improved quality of life at 4 and 12 weeks, measured by the FIQL scale.
Determine if the effectiveness of microbial engraftment following fecal microbial transplantation is associated with the degree of clinical improvement.	Concentration of microbiota present at baseline vs. at week 4 and at 12 weeks (optional)in subjects that demonstrate a significant improvement at 4 weeks after FMT, relative to baseline using the St. Mark's Vaizey score.
Tertiary	
Determine whether the baseline microbiota of the subject affects engraftment in fecal microbial transplantation for fecal incontinence.	Concentration of microbiota at baseline vs. concentration of microbiota at 4 weeks and at 12 weeks (optional).

4 STUDY PLAN

4.1 Study Design

Traditional treatments of fecal incontinence directed at obstetric neuromuscular injury, such as pelvic floor exercises and sphincteroplasty, have remarkably low efficacy in older women. The proposed study will provide critical feasibility and safety data for conducting a future randomized clinical trial of FMT versus placebo for the treatment of refractory fecal incontinence. We plan a single arm open label clinical trial of FMT for the treatment of refractory fecal incontinence. Older women with fecal incontinence who have failed prior treatment with diet modification and pelvic floor exercises will be invited to participate in the study. Penn Microbiome Therapy – 002 (PMT-002), which is derived from healthy screened donors will be delivered to the duodenum via a nasogastric tube. All subjects will complete validated questionnaires on fecal incontinence and contribute a stool sample for microbiota analysis at baseline and at 4 and 12 weeks after the FMT procedure.

4.2 Scientific Rationale for Study Design

For this first-of-its-kind pilot study, we plan to conduct a pre-post intervention study. No prior studies have assessed the impact of FMT on FI. Thus, we plan to assess the efficacy and safety of this treatment in this population prior to conducting larger placebo or alternate treatment comparison studies.

4.3 Justification for Dose and Route of Administration

There are several routes available for the administration of FMT. For this proof of concept study, FMT will be performed via duodenal infusion of donor stool rather than FMT capsules as a single study showed lower efficacy of capsules in women with irritable bowel syndrome (IBS). Our rationale for choosing FMT via duodenal infusion over colonoscopy is as follows: 1) Duodenal infusion FMT is an office-based procedure performed in an awake patient as compared to FMT via colonoscopy in the operating room under sedation, with a risk of bowel perforation and with much higher associated costs. 2) Duodenal infusion FMT is well tolerated by older adults (van Nood, NEJM, 2013; Pinn, Am J Gastro, 2014; Vrieze, Gastroenterol, 2012). 3) Several studies show that FMT via colonoscopy or duodenal infusion have equivalent efficacy (Ramai, Ann Gastro 2019). As described above, the dosing regimen for FMT in recurrent *Clostridium dificile* (R-CDI) will be used as there have been no studies on optimal dosing of PMT-002 in women with FI.

4.4 End of Study Definition

This clinical trial is considered completed (End of Study) when participants are no longer being examined or the last participant's last study visit has occurred (including long follow up visits or contacts).

A participant is considered to have completed the study if the subject has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 2.3.

5 STUDY POPULATION

5.1 Inclusion Criteria

- Women ≥ 50 years of age with self-reported fecal incontinence defined as:
  - uncontrolled bothersome loss of liquid or solid fecal material that occurs at least weekly over the last 3 months **and**
  - failure of response to conservative management using fiber, diet modification, supervised pelvic floor exercises
- Baseline St. Mark's score of ≥12
- Intolerance, unwillingness, or inadequate response to constipating medications
- Self-reported current negative colon cancer screening based on the 2016 US preventive Services Task Force recommendation (applies to participants age 50-75). N/A if participant is over 75
- Able and willing to sign the informed consent form and agree with study procedures

5.2 Exclusion Criteria

- Known food allergy that could lead to anaphylaxis
- Contraindications to nasogastric tube placement including:
  - Recent midface trauma
  - History of basilar skull fracture
  - Recent ENT surgery
  - Known coagulation abnormalities
  - Esophageal varices and/or esophageal strictures
- Untreated prolapse beyond the hymen
- History of Inflammatory Bowel Disease (does not include IBS)
- Unrepaired rectovaginal fistula/chronic 4th degree laceration
- Full thickness rectal prolapse
- History of congenital anorectal malformation
- History of bowel resection surgery for any indication
- Minor anal procedures within 6 months for treatment of accidental bowel leakage (ABL) (injection of bulking agent or radiofrequency energy) or ligation of hemorrhoids
- Prior pelvic or abdominal radiation
- Diagnosis of cancer of the descending colon or anus

- Clinically significant neurological disorders known to affect anal incontinence
- Chronic antibiotic use (>1 month continuous antibiotic)
- Pregnant or planning to become pregnant during the study duration 1 year
  - N/A if participant is not of childbearing potential
- Childbirth within the last 3 months
- Unwilling to use an acceptable form of birth control method if participant is of childbearing potential
  - N/A if participant is of not of childbearing potential
- Participation in another intervention trail impacting bowel function
- Inability to provide informed consent, complete questionnaires independently or to attend intervention sessions
- History of hepatitis C / HIV infection or other immunocompromised condition
- History of bowel resection surgery or pelvic irradiation
- Unable to speak, read, or write in English at a basic level
- In the opinion of the investigator not suitable for participation in the trial

5.3 Lifestyle Considerations

After administration of the investigational product (PMT-002) there is no way for investigators to stop or retrieve the product from the subject. Additionally, it is unknown how long the product will remain in the subject's body; thus, subjects should be fully informed that withdraw of the investigational product is not possible and be comfortable with this information prior to administration. Furthermore, as this is a first in human trial for the indication of FI unforeseen side effects are expected.

5.4 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently administered the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of inclusion/exclusion criteria for this protocol are not eligible for rescreening.

5.5 Strategies for Recruitment and Retention

Clinical Practices of Investigators

Women presenting to the clinical practices of the Hospital of the University of Pennsylvania Health System Division of OB GYN (Urogynecology) for consultation/complaint or indication for FI (after clinical confirmation of their potential eligibility for admission into the study based on inclusion and exclusion criteria) will be approached to participate by the principal investigator (PI) or providing physician, in addition to the study coordinator. In the recruitment visit, study details including the intervention will be explained. Risks and benefits will be thoroughly discussed and consents given to the patient prior to any study procedures being performed.

Hospital/Local Health Care Referrals

Subjects will be recruited at each site from individual practice(s) of the investigators as noted above as well as faculty/resident clinics. Ongoing contact with practice and faculty members as well as with residents will be made by the investigators and coordinators, reminding them of the inclusion criteria, importance of the study, etc. In addition, the investigators will describe the study to members of other departments in the hospital, primarily family practice, medical endocrinology, and gynecology who also see and treat these patients. Contact with local physicians will be made and/or grand rounds will be given to disseminate information about the study. Letters will be mailed to potential subjects, which provides details of the study and contact information of the study team (to allow for subject to reach out for more information). Penn Datastore may be utilized to provide a list of potential subjects based on clinical practice information.

Referrals from Study Participants

Study participants often refer friends, acquaintances, and colleagues to be potential participants. The investigator will let the patient know that they can refer others to be considered for participation in the study.

Previous Study Participants

The trial will be offered to women who have previously participated in clinical trials offered by the study team, who have consented to be contacted for future participation via their preferred contact method (after confirmation of their potential eligibility for admission into the study based on inclusion and exclusion criteria).

Web sites

The study will be prominently displayed on the local Penn website. Information will also be available at clinicaltrials.gov. Social media and Craigslist will also be utilized, as this has been a proven source of recruitment in the past. Ads placed on social media, specifically Facebook and Instagram, will be one-way ads and the study team will not directly interact with subjects on

social media. The ads will highlight a contact number for the interested individual to call to discuss the study. Social media ads will be monitored by the University of Pennsylvania study coordinator to ensure they are being properly featured and not causing inappropriate comments. This monitoring will occur bi-weekly. If any problems arise with the social media ads they will be immediately removed, however, this is not anticipated as they are one-way ads. This type of advertising has been previously used for studies within the department and has been well received.

Institutional Review Board (IRB) Approval

It is expressly acknowledged that all informational material that could be construed to be advertising will be approved by the central IRB prior to dissemination.

6 STUDY INTERVENTION

6.1 Study Intervention Administration

6.1.1 Study Intervention Description

Fecal microbiota transplantation (FMT) is a treatment that involves administration of minimally manipulated microbial community from stool of a healthy donor into the patient's intestinal tract. Clinically, FMT is performed with the intent of restoring normal function of the gut microbiota (Khoruts, 2016).

6.1.2 Dosing and Administration

A single dose of PMT-002 will be administered via a gastroduodenal tube over 10 to 90 minutes (target 30 minutes). In brief, nasogastric duodenal tube will be placed by a physician and its location will be checked by X-Ray. Once placement is confirmed, the PMT-002 product will be delivered via the NGT using a syringe attached to the end of the tube. Once the product has been delivered, the NGT will be removed. The patient will be observed for 1 hour in the office for potential adverse events.

6.2 Preparation/Handling/Storage/Accountability

6.2.1 Acquisition and accountability

Following the screening and consent visit, the lab director at the PMT manufacturing facility at the University of Pennsylvania will be notified of the date for visit 1 (FMT visit) to ensure the investigational product will be available for that date. On the day of the Study visit, study personnel will obtain the PMT product from the manufacturing facility and transport it to the office where it will be administered. The PMT-002 will be packaged on dry ice from the PMT manufacturing facility. The time of release from frozen storage will be documented and included in PMT product packaging. Upon receipt of the product by the Investigator, packaging will be inspected for integrity, and the 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.

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). Any remaining investigational product will be disposed of at the clinical site.

6.2.2 Formulation, Appearance, Packaging, and Labeling

PMT-002 is composed of donor stool, glycerol, and saline contained in cryobags and labelled (according to FDA regulations 21CFR312.6) for upper GI use. These cryobags will also be labeled with a unique color label to facilitate identification as a PMT-002 drug product.

The potency of each PMT-002 is $1x10^{8}$-10^{12} CFU/dose (1 dose per cryobag), based on Quantitative Anaerobic Culture from Fecal Filtrate. The appearance of the PMT-002 is frozen aqueous solution within the product container is properly labeled, free of defects. Prior to release of the drug product, all serological, stool and multi-drug resistant organism (MDRO) testing will be confirmed to be negative.

6.2.3 Product Storage and Stability

PMT-002 drug product should be stored as detailed on the investigational product labeling, prior to release to site.

The 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-002 should be initiated within 180 total minutes of product release from frozen storage by the manufacturer.

PMT-002 doses will be thawed at room temperature for 30-60 minutes prior to administration.

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

6.2.4 Preparation

Preparation of the investigational product [PMT-002 (Suspension)] will be performed at the PMT manufacturing facility at the University of Pennsylvania. PMT-002 is packaged in EVA

bags suitable for storage at < -70°C (target -80°C). The investigational products will be packaged in single doses.

6.3 Measures to Minimize Bias: Randomization and Blinding

Neither subjects nor investigators will be blinded.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request and without impact to their regular medical care, but they will receive no further treatment with this investigational product.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance.
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF). Subjects who sign the informed consent form and do not receive FMT will be replaced.

7.2 Lost To Follow-Up

A participant will be considered lost to follow-up if they fail to return for greater than 2 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 2 weeks and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENT AND PROCEDURES

8.1 Efficacy Assessments

8.1.1 Screening Visit

Potential candidates will be evaluated for eligibility per sections 6.1 and 6.2 and written informed consent will be obtained. Consented participants will be required to complete the following forms during their Baseline visit:

- Consent
- Eligibility Check List (includes St. Mark's Vaizey questionnaire)
- Pregnancy Test (if childbearing age)

If eligible, the following will be completed:

- Dietary Screener
- Questionnaires (Demographics, Medical History, SF-12, PFDI-20, ABLe Questionnaire, FIQL, Bristol Stool Index, PFIQ-7, Adaptation Index, PACSYM)
- Abbreviated Physical Exam
- Medication Log

Subjects will also be required to take the following home to complete prior to their treatment visit.

- One-week BM diary
- Stool Collection Kit (see stand-alone collection instructions)

8.1.2 Visit 1: Treatment

At this visit, the study coordinator will collect the one-week BM diary and stool sample from the subject. The PI or another trained physician will meet with the patient. Vital signs will be done to document temperature, blood pressure, heart rate, and oxygen saturation. A nasogastric tube will be placed and secured. The subject will then be escorted to the radiology suite by the study coordinator. A chest x-ray will be performed to ensure adequate placement of the nasogastric tube. The PI or trained physician will confirm adequate placement of the tube with the attending radiologist. The subject will then be escorted back to the office suite by the study coordinator. The PI or trained physician will then administer the treatment. The PMT-002 product will be drawn into a syringe and the syringe will be connected to the nasogastric tube (NGT) and the product will be pushed into the tube until all of the FMT product is administered. The NGT will then be removed by the PI or trained physician. The subject will remain in the office for one hour following the administration of the PMT product.

8.1.2.1 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, 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. The following vital sign changes will be recorded if observed: (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).

During the monitoring period, information will be solicited from the patient regarding the following adverse events associated with FMT including aspiration, belching, bloating, nausea, vomiting, gastroesophogeal reflux, abdominal cramps, abdominal pain, diarrhea, and constipation.

Subjects will be sent home with:

- Second one-week BM diary. The diary will include a card for the collection of all solicited adverse events.
- Thermometer

- Temperature and Adverse Events log form
- Stool Collection Kit

8.1.3 Phone Contact 1 (1 week post treatment)

The study coordinator will call to review the temperature log and assess for any adverse events. The study coordinator will inform the PI immediately if any subject reports an oral temperature of >100.4. The PI or other trained physician will phone triage patients reporting elevated temperatures and bring them in for a study visit / direct them to the emergency room if necessary. If a subject is asked to come in for a visit, the visit will follow the procedures described in section 9.1.8.

8.1.4 Visit 2 (4 weeks post treatment)

Subjects will come to the office for a visit 4 weeks (± 7 days) after administration of the FMT treatment or be conducted by telemedicine or telephone.

During their first follow-up visit, the subjects will return to the clinical site in person or via mail:

- Second one-week bowel movement (BM) diary
- Temperature and Adverse Events log form
- Stool specimen

During the visit, the following forms will be completed:

- Medication Log
- Adaption Index
- SF-12
- PFDI-20 (Pelvic Floor Disability Index)
- ABLe Questionnaire
- FIQL (Fecal Incontinence quality of life)
- Bristol Stool Index
- St. Mark's Score
- PGSC (Patient Global Symptom Control)

- Global Impression Improvement
- PACSYM
- PFIQ-7
- AE/SAEs

Subjects will be sent home with a final one-week BM diary that they will return during their 12-week visit. The diary will include a card for the collection of all solicited adverse events. Subjects will also be sent home with a stool collection kit to be returned at the 12-week visit or be returned by mail if a telemedicine or telephone visit (optional).

8.1.5 Visit 3 (12 weeks post treatment)

Participants will return to the office for visit 3, twelve weeks (± 7 days) after the FMT treatment. The clinical research coordinator will collect final one-week BM diary as well as the stool sample (if available). This visit may occur via telemedicine or telephone, and samples provided via mail or drop off at the clinical site. The following questionnaires will be completed:

- Medication Log
- Adaption Index
- PFIQ-7
- SF-12
- PFDI-20
- ABLe Questionnaire
- FIQL
- Bristol Stool Index
- St. Mark's Score
- PGSC
- Global Impression Improvement
- PACSYM
- AE/SAEs

8.1.6 Phone Contact 2 (End-of-study at 180 days)

The study will conclude after subjects are contacted by telephone or in-person at 180 days (+/-10 days) after final FMT for a final interview focused on potential adverse events.

8.1.7 Early Termination Visits

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

8.1.8 Unscheduled Visits

Data collected from subjects who contact the study team (by telephone 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.

8.2 Safety and Other Assessments

For all women of childbearing potential, a urine pregnancy test will be performed prior to FMT and at all subsequent study visits. If a subject's urine pregnancy test is positive prior to treatment with FMT, the subject will be removed from the study. It is recommended that women not become pregnant as it is unknown how this treatment will effect pregnancies. If a subject does become pregnant on study, the pregnancy will be followed.

To monitor for signs of infection subjects will be required to keep a temperature log for 7 days following FMT (a digital thermometer will be provided). Subjects will be instructed to call the study doctor if their temperature rises above 100.4 degrees or drops below 96 degrees. The study doctor will phone triage patients reporting elevated temperatures and bring them in for a study visit or direct them to the emergency room if necessary.

Subjects will also be provided with a card for the collection of all solicited adverse events in their study diary. The card will be reviewed with the diary for any adverse events.

8.3 Adverse Events and Serious Adverse Events

8.3.1 Definition of Adverse Events (AE)

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.

8.3.2 Definition of Serious Adverse Events (SAE)

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.

All fatal or life-threatening related SAEs or suspected unanticipated serious adverse events (SUSARs) should be reported to the Food and Drug Administration (FDA) within 7 calendar days of knowledge of the event and that non-life threatening SAEs/SUSARs should be reported to the FDA as soon as possible but no later than 15 calendar days after knowledge of the event (21 CFR 312.32).

8.3.3 Classification of an Adverse Event

8.3.3.1 Severity of Event

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Grade 1: Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.

- Grade 2: Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

- Grade 3: Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

- Grade 4: Life-threatening

- Grade 5: Death

8.3.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be considered.

- Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals.

- Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (de-challenge). Re-challenge information is not required to fulfill this definition.

- Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. Note: there must be an alternative, definitive etiology documented by the clinician.

8.3.3.3 Expectedness

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 the investigational products, both as a consequence of the underlying disease being treated, and potentially as an adverse effect of the administered product.

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)
- sepsis or shock (temperature, heart rate, or blood pressure change as already described)
- bowel injury (abdominal pain change)
- aspiration (respiratory rate or oxygen saturation change)
- gastrointestinal symptoms (belching, bloating, nausea, vomiting, GERD, abdominal cramps, abdominal pain, diarrhea, and constipation)

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)
- gastrointestinal symptoms (aspiration, belching, bloating, nausea, vomiting, GERD, abdominal cramps, abdominal pain, diarrhea, and constipation)

In the subsequent, 28-, 48-, 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)
- metabolic changes (polydipsia, polyuria, weight gain, or weight loss)

8.3.4 Adverse Events of Special Interest

Any gastrointestinal symptom or allergic reaction adverse event grade 3 or higher will be classified as an adverse event of special interest. These events may be related to the use of the FMT product and should be recorded as an adverse event of special interest and reported to the sponsor. Reporting of Adverse Events of Special Interest should follow the reporting pathway described for Serious Adverse Events.

8.3.5 Time Period and Frequency for Event Assessment and Follow-Up

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 or participant contact (phone call/email). Participants will be monitored by medical histories, physical examinations, and

baseline status. 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. Additionally, the subject study diary will include a card for the collection of all solicited adverse events. 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 an 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. Expectedness to study treatment or process – [Unexpected – if the event, severity, and/or frequency is not described in the investigator brochure (if applicable) or protocol].

5. Action taken with respect to study or investigational treatment or process (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable).

6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy).

7. 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.

8.3.6 Adverse Event Reporting

Reporting period

Adverse events will be reported from the time of informed consent until study completion (180 days).

Investigator reporting: notifying the study Sponsor

Every SAE, regardless of suspected causality (e.g., relationship to study product(s) or study procedure(s) or disease progression) must be reported to the Sponsor within **24 hours** of learning of its occurrence.

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

Send the SAE report to the Sponsor.

New information regarding the SAE will be reported as it becomes available and in the same manner that the initial SAE (i.e. SAE form). The investigator must follow the event to resolution or until the event is deemed and documented irreversible, whichever is longer.

Investigator Reporting: Local Reporting Requirements

The investigator will report AEs and SAEs to the IRB of record and other local regulatory groups per the local requirements.

8.3.7 Serious Adverse Event (SAE) Reporting

The study clinician will immediately report to the Sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse events (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the Sponsor.

New information regarding the SAE will be reported as it becomes available and in the same manner that the initial SAE (i.e. SAE form). All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study Sponsor and should be provided as soon as possible.

The SAE reports to the Sponsor should be sent to the pharmacovigilance coordinator.

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 the IRB of record and other local regulatory groups per the local requirements.

8.3.8 Stopping/Halting Rules

A study wide pause of PMT administration will be instituted if one of the following occur:

- Death attributable to FMT
- Suspected transmission of infection from donor to patient
- If two (2) or more subjects experience the same Grade 3 or higher AE
- If two (2) or more subjects experience an SAE related to the study product
- There is an increase in frequency of long-term adverse events (e.g., IBD, metabolic disease) in > two (2) FMT treatment subjects

8.3.9 Unanticipated Problems

8.3.9.1 Definition of Unanticipated Problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and

- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.3.9.2 Unanticipated Problem Reporting

Unanticipated problems (UPs) should be reported by the investigator to the reviewing IRB and OCR.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypotheses

OBJECTIVES	**Hypothesis**
9.2 Primary	
Determine if fecal microbial transplantation leads to clinical improvement in refractory fecal incontinence in older women.	Our working hypothesis is that the St. Mark's Vaizey score, a measure of fecal incontinence severity, will significantly improve at 4 weeks after FMT, and will be maintained at 12 weeks, relative to baseline. A secondary outcome will be quality of life at 4 and 12 weeks.
Primary Safety	
Determine the safety of FMT administration via NG tube in older women with FI.	Our hypothesis is that there will be low occurrence of serious adverse events following administration of FMT via NG tube.
Secondary	
Determine whether the effectiveness of microbial engraftment following fecal microbial transplantation is associated with the degree of clinical improvement.	Our working hypothesis is that more transplanted strains will be present at 4 weeks in subjects with a higher response score.
Tertiary	
Determine whether the baseline microbiota configuration affects engraftment in fecal microbial transplantation for fecal incontinence.	Our working hypothesis is that the transplant will be more effective in baseline samples with lower microbial diversity.

9.3 Sample Size Determination

Initial open label clinical trials of FMT for irritable bowel syndrome were performed with 10-15 patients. (Holvoet, Gut 2017; Pinn ,Am J Gastro, 2014; Mazzawi, PLoS One, 2018) In the proposed pilot study, we will examine 15 evaluable women with refractory fecal incontinence to gather essential feasibility and outcomes data to conduct a future RCT of FMT. In a prior study of 13 adults with irritable bowel syndrome, significant differences in signals for several bacteria (*Bacteroides*, *Desulfitispora*, *Bifidobacteria*) were noted between 8 responders and 5 non-

responders to FMT (response rate 60%). (Mazzawi PLoS One, 2018) If the response rate is near 60% in our pilot study, we will be able to detect a difference in bacterial engraftment of 1.59 standard deviations. Our power will be decreased if the response rate is either very low (<3) or very high (>12). With 15 subjects in a single arm study, the response rate will be determined to within 10% (interquartile range).

9.4 Statistical Analyses

9.4.1 General Approach

Data on safety outcomes will be summarized using descriptive statistics. Feasibility of conducting a clinical trial of FMT will be calculated by measuring the feasibility of conducting a clinical trial of FMT will be calculated by percentage of screened patients who enroll in the study (Number enrolled / Number screened x 100). Fecal incontinence severity symptom and quality of life (QOL) scores at baseline, 4 and 12 weeks after FMT treatment will be compared using Friedman test (repeated measures ANOVA for non-parametric data) Responder will be defined as 5 point or greater improvement in St. Mark's score from baseline to 4 weeks following FMT.

9.4.2 Analysis of Stool Samples

Sequence reads from shotgun metagenomic sequencing will be subjected to quality control, taxonomic assignment, gene function analysis, and metagenomic assembly using the Sunbeam metagenomics pipeline, which was co-written by CHOP Microbiome Center researchers.

The FMT inoculum will be sequenced alongside fecal samples collected from subjects before and after the FMT procedure. DNA sequence reads from the inoculum will be assembled into contigs (overlapping DNA segments) using MEGAHIT (Li Bioinformatics, 2015). The metagenomic contig sequences represent segments of bacterial genomes from the microbiota. We will identify protein coding genes in the contig sequences, and use this gene set as unique identifiers for the genomes in the inoculum. Reads from patients before and after FMT will be aligned to the inoculum marker gene sequences. Gene sequences which are present in patients before the transplant will be removed from the set of inoculum marker genes. The percentage of total non-human reads aligning to the inoculum marker gene set will be used as a measure of bacterial engraftment following FMT.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Informed Consent Process

10.1.1.1 Consent/Assent and Other Informational Documents Provided To Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be conducted and documented in the source document (including the date and time), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.1.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated by the Sponsor or the PI at any site if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) Sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the IRB, and Sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the Sponsor, IRB and/or Food and Drug Administration (FDA).

In terminating the study, the Sponsor and the Principal Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

10.1.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the Sponsor and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the Sponsor.

All research activities will be conducted in as private a setting as possible.

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).

The study monitor, other authorized representatives of the Sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or Sponsor requirements.

10.1.4 Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the University of Pennsylvania. After the study is completed, the de-identified data will be stored for future use by other researchers with IRB approval to do so. Biological specimens (stool samples) will be preserved in frozen storage for future analysis utilizing the same guidelines.

10.1.5 Safety Oversight

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet at least semiannually to assess safety and efficacy data of the study. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB.

10.1.6 Clinical Monitoring

A monitoring plan that satisfies the International Council on Harmonisation (ICH) Good Clinical Practices (GCP) guidelines for clinical monitoring will be used. 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 study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit. The monitor, a member of the Women's Health Clinical Research Center, will lead this effort, and report findings to the PI and the DSMB when necessary. The monitor will have full knowledge of the study protocol, Manuals of Procedures, and is familiar with the database system, REDCap (Research Electronic Data Capture) and is trained to review patient charts. The monitor along with the Project Manager/Lead Study Coordinator at the site will be responsible for training and supervising other personnel.

10.1.7 Quality Assurance and Quality Control

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.

10.1.8 Data Handling and Record Keeping

10.1.8.1 Data Collection and Management Responsibilities

Data will be collected prospectively by designated research personnel at the study site, supervised by the PI. Original source documents will be kept in the study subject folder. Well-designed data collection forms (CRFs) will be developed to minimize data collection and

recording errors. CRFs will be completed, with copies and will be printed and stored in binders in a secure location 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. Administrative forms will be designed, such as visit procedure checklists, to assist the research staff in complying with protocol procedures. All data are being collected solely for the purpose of research and do not become part of the subject's medical record.

10.1.8.2 Study 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. Final records will be retained per institutional guidelines.

10.1.8.3 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.

10.1.9 Protocol Exceptions and Deviations

10.1.9.1 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.

10.1.9.2 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 24 – 48 hours of PI knowledge and to local regulatory review committees per institutional guidelines.

The PI and the study team should document all scenarios where the protocol is not followed and provide, in particular:

- Who deviated from the protocol;
- What was the deviation;
- When did the deviation occur;
- How did the deviation happen;
- What is the impact of the deviation;
- A root cause analysis of why the deviation occurred.

If the assessment is determined to be of limited impact (minor deviation), the documentation for this assessment and the outcome should be reported to the Sponsor at the time of annual report. Reporting to the IRB should follow specific local requirements.

If the assessment results in a determination that any of the following are potentially affected, the deviation would be considered of significant impact:

- having the potential to adversely affect subject safety; OR
- increases risks to participants; OR
- adversely affects the integrity of the data; OR
- violates the rights and welfare of participants; OR
- affects the subject's willingness to participate in research;
- there is a potential for an overall impact on the research that should be shared with the IRB for consideration and development of next best steps to address it.

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.

These scenarios should be reported to the Sponsor within 24 – 48 hours of discovery. Reporting to IRB should follow local requirements.

10.1.10 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial.

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