Fecal Microbiota Transplantation for the Treatment of Obesity: An Investigation of the microbial contribution to pathogenesis of disease

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I. BACKGROUND AND SIGNIFICANCE:

Obesity is a major health burden affecting over one third of Americans and is associated with chronic diseases such as diabetes mellitus, cardiovascular disease, nonalcoholic fatty liver disease (NAFLD) and cancer [1-3]. Pharmacologic options and lifestyle modifications, including caloric restriction, exercise, and behavioral modifications have provided only modest weight loss benefit [4,5]. Surgery, while shown to result in significant weight loss and resolution of co-morbid metabolic illness, is invasive and accompanied by significant morbidity and mortality risk [6]. There is an urgent need to develop novel treatment strategies targeting the underlying pathology leading to obesity and obesity-related diseases.

Associations between the gut microbiota and obesity have been identified. Germ-free mice are protected from diet –induced obesity, insulin resistance, and NAFLD when fed western-type diets [7]. Additionally, both obese humans and mice have been found to have an altered gut flora composition compared to lean counterparts [8]; specifically, a decrease in the *Bacteroidetes/Firmicutes* ratio [9,10].

Potential mechanisms of bacteria mediated weight gain/loss have been linked to short-chain fatty acids (SFCAs), which are a known byproduct of intestinal bacteria and are used as an energy source by colonic epithelial cells. Carbohydrates are fermented to SCFAs such as acetate, propionate, and butyrate by colonic bacteria, specifically Clostridial clusters IV and XIVa, including Eubacterium, Roseburia, Faecalibacterium, and Coprococcus [11]. Additionally, SCFAs modulate the release of gut hormones from enteroendocrine cells such as glucagon-like peptide 1 (GLP-1), through binding of SCFAs to the G-protein-coupled receptors GPR41 and GPR43 [12-14].

The main mechanism of action of GLP-1 is to increase glucose-mediated insulin release, delaying gastric emptying, and inhibiting glucagon release. GLP-1 agonists have been shown to promote satiety [15], leading to weight loss. GLP-1 is secreted within minutes of food intake by L-cells, located in the distal ileum and colon. Oral (but not IV) glucose administration causes GLP-1 secretion [16]. Additionally**,** GLP-1 readily crosses the blood brain barrier where it is thought to regulate ingestive behavior in the hypothalamus, which subsequently has direct effects on gastric emptying and resultant satiety [15]. GLP-1 is secreted 15 to 30 minutes after food intake and is there measured at multiple time points post-prandial [17]. Additionally, GLP-1 has been shown to increase following other weight loss interventions such as gastric bypass, with significant changes seen as early as 6 weeks [17].

This connection has led to attempts at manipulations of the gut microbiome in order to modulate this process [18]. In a recent trial, obese diabetic patients received a fecal microbiota transplantation (FMT) from either lean donors or autologous stool [19]. Those in the treatment group were noted to have improved peripheral and hepatic insulin sensitivity. FMT involves the transfer of donor stool to a recipient. It has become widely accepted as a treatment for recurrent Clostridium difficile infection (CDI), with a cumulative cure rate of >90% and minimal adverse events [20]. These preliminary studies suggest that FMT using lean donor stool may be

used to treat obesity and its sequela. **We hypothesize that for obese patients, induction and maintenance FMT utilizing lean donor stool will correct a dysbiosis that has led to a decrease in circulating GLP-1 which acts as a surrogate marker for weight loss.**

II. SPECIFIC AIMS

Specific Aim 1: Assess the safety of FMT in patients with obesity.
Hypothesis: Induction FMT followed by weekly maintenance capsule FMT will be safe and well tolerated by obese patients**.**

Specific Aim 2: Assess GLP-1 as a therapeutic biomarker for clinical response to fecal microbiota transplantation
Hypothesis: Fecal Microbiota Transplantation will lead to an increase in SCFA which will lead to an increase in the metabolic regulator GLP-1.

Specific Aim 3: Determine the impact of fecal microbiota transplantation on the intestinal microbiome of patients with obesity via 16s ribosomal RNA sequencing.
Hypothesis: Fecal microbiota transplantation will result in a sustained repopulation of the patient's microbiome that corresponds to the bacteria from the donor stool.

III. Methods and Study Design

This is a randomized controlled pilot study to assess the safety and to measure the microbiological and clinical impacts of FMT in patients with obesity. We will continue to prospectively enroll and screen adult patients until 20 patients have completed all study visits. We anticipate total enrollment to be 50 patients to account for screen fails and dropouts. Eligible patients are those who are obese (BMI of 35 kg/m^2 or greater). Patients will be randomized 1:1 to receive FMT or placebo. The participants will receive either an FMT from a lean healthy donor using 30 induction FMT capsules or placebo capsules. The participants will continue to receive a monthly maintenance dose of 12 FMT oral capsules or placebo at week 4 and week 8. Serum for hormone levels will be collected at baseline, weeks 1, 4, 6, 8 and 12**.** Stool for microbial assessment will be collected at baseline and weeks 1, 4, 6, 8, 12, 26, and 52. A Food Frequency Questionnaire will be taken by the patient at week 0. Donor Stool will be obtained from OpenBiome. OpenBiome is a nonprofit 501(c)(3) organization that provides hospitals with screened, filtered, and frozen material ready for clinical use (See Attachments for OpenBiome Quality and Safety Program as well as donor screening examples).

Primary Safety Endpoint:
Safety Endpoints: Number and nature of adverse events at week 1, 6, 12 and 26.

Primary Clinical Endpoint:
The primary clinical end point is an increase in the AUC for GLP-1 after a mixed meal test from baseline and 12.

Secondary Endpoints:

- Microbial Endpoints: Recipients fecal microbial diversity at week 6 and 12 compared to baseline
- Clinical Endpoints: Decrease in BMI and waste circumference at week 6 and 12 compared to baseline.

Trial Treatment:
The treatment for this trial will be administration of an induction dose of 30 FMT capsules ingested orally. Treatment will occur after screening.

Subsequent FMT administration will be maintenance capsules of frozen FMT material ingested orally every 4 weeks (weeks 4 and 8). All ingestions will be undertaken in the clinic under supervision. A follow-up phone call will be undertaken 4 hours after each ingestion visit to screen for adverse events. At 48 hours and 24 hours prior to and the day of each capsule dose (regardless of group) the patient will take a single dose of 20mg of omeprazole.

Dosage Form

1. Initial dose - filtered solution of donor stool in 30 gelatin capsules at Day 0 (16500µl total).
2. Maintenance dose – twelve capsules of fecal material taken every 4 weeks (550µl of fecal material in each size 00 gelatin capsule)

Dosing Regimen
Single induction dose of 30 capsules (16500µl)
Monthly dose of 12 capsules of fecal material (total 6600µl of fecal material at week 4 and week 8)

Capsule preparations are delivered to clinical investigators in double containment shipping vessels packed with dry ice, as with non-encapsulated placebo preparations. Packaging is labeled with the same information included on non-encapsulated preparations, with the addition of a clause instructing clinicians to administer the material to patients within 90 minutes of removal from frozen storage. Patient instructions listed in Appendix 6.

Packaging
Encapsulated fecal material in freezer safe bottles will be stored in Styrofoam boxes from the Microbiome Health Research Institute at Massachusetts Institute of Technology, as described in their Drug Master File (15543).

The Placebo Treatment:
Those in the placebo arm will undergo procedures as in the active arm. They will be given 30 placebo capsules at time of induction (Day 0) and this will be followed by 12 placebo capsules each month**.**

Placebo Oral Capsules

Placebo capsules are prepared to support randomized, placebo-controlled studies of FMT. These capsules are prepared according to the specifications described below and utilized in studies as indicated by the study IND.

1. An autoclaved solution consisting of 80% cocoa butter, 20% glycerol and brown food coloring, without the addition of human stool, is used as a placebo control.
2. The autoclaved placebo solution is transferred to a reservoir.
3. Capsules are generated as outlined in 6-9 of section V above.
4. Each capsule is visually inspected for integrity and contamination of the outer surface. Capsules passing inspection are placed into an HDPE plastic bottle for immediate storage at -80ºC.
5. Placebos are quarantined until they have passed bioburden testing, as outlined in section XII below.
6. Doses are transferred into the final pharmaceutical bottle packaging. The dosage size depends upon the requirements of an individual clinical trial's specifications. Clinical trial specifications vary with the trial protocol, objectives, and the indication being targeted.

Labeling

See Drug Master File (15543).

IV. STUDY PROCEDURES

An outline of the study timeline and measurements is shown in Appendix 1

Overview. This is a randomized controlled pilot study to assess the safety of FMT in obese patients. Additionally, the study will measure the microbiological and clinical impacts of FMT in patients with obesity. We will continue to prospectively enroll adult who are obese (BMI of 35 kg/m^2 or greater) after providing written informed consent until 20 have completed all study visits. We anticipate total enrollment to be 50 patients to account for screen fails and dropouts. The study participants will be randomized 1:1 to either the treatment arm or the placebo arm. The treatment arm will receive an FMT induction followed by monthly (weeks 4 and 8) maintenance oral capsules for 12 weeks. The placebo group will receive placebo capsules at the time of their induction followed by monthly intake of oral placebo capsules for 12 weeks. The participants will not receive dietary instruction and will be asked to continue with their regular diet. Information about their dietary habits will be collected at each study visit. Donor Stool and placebo material will be obtained from OpenBiome. OpenBiome, with whom we have previously collaborated with, is a nonprofit 501(c)(3) organization that provides hospitals with screened, filtered, and frozen material ready for clinical use.

A. Recipient Procedures

Week -2: Screening – potential subjects will undergo the following screening procedures one to two weeks prior to FMT to determine if they meet the recipient selection criteria;

1. Repeat of Medical record review to confirm diagnosis and treatment history if questions remain after initial screen. This will include either review of partner's available data or data provided from home provider (When applicable)
2. Symptom assessment (Symptom Diary Card) and physical exam by study physician
3. Anthropometric measurements: BMI, Iliac measurements, waist-to-hip ratio
4. Baseline blood work to assess for sequela of obesity: insulin, LFTs, CBC, and HgA1C and fasting serum lipids and blood glucose
5. Stool sample to exclude current infections (bacterial culture, ova & parasites, Clostridum difficule toxin) and a sample for high-throughput 16S rRNA sequencing and metabolomics.
6. Urine Pregnancy Test for females of child-bearing age: Women of child-bearing potential will be counseled by study clinicians that those randomized to the treatment arm will be requested to avoid pregnancy by hormonal or barrier methods from randomization for 4 months (duration of FMT therapy and one month lag afterwards).
7. Diet recall and question about specific dietary restrictions or preferences. The patients will be asked to maintain their standard diets throughout the study.
8. Baseline levels of ghrelin, Leptin, PYY and adiponectin will be measured
9. A mixed meal tolerance test will also be performed. GLP-1, leptin and PYY measurements will be taken at time 0, 15, 30, 45, and 60 minutes.
10. Pre FMT screening labs including HIV, Hepatitis B and C and Syphillis
11. Serum will also be collected for metabolomic profiling
12: Test swallow of an empty capsule
13. Indirect Calorimetry – see Appendix 7
14. Patients will be sent home with 9 doses of a proton pump inhibitor to be taken 48 hours 24 hours prior to and the day of each capsule administration (3 times total).

Week 0: (Treatment and Control Arms): Randomization: Eligible patients will be randomized 1:1 according to a computer-generated randomization list. The randomization will be held centrally at the Brigham and Women's Hospital Research Pharmacy. The treatment location will be masked to the patient and investigators performing data analysis. The health care worker administering FMT or placebo will not be aware of the treatment being administered. Treatment will be administered based on the result of randomization.

- Food Frequency Questionnaire
- Stool transplant (or Placebo) via capsule induction
- A proton pump inhibitor will be taken daily at 48 hours and 24 hours prior to and day of capsule consumption.

Week 1: (Treatment and Control Arms): Follow up and adverse event screening. Additionally:

- BMI, Iliac measurements, hip to waist ratio
- Indirect Calorimetry
- Stool for microbiome analysis and metabolomics
- CBC, LFTs, FBG, HgA1C, lipids, glucose, metabolomics
- diet recall
- Symptom Diary Card and Adverse Event screening

Weeks 2-12: (Treatment and Control Arms)

Symptom diary Cards will be filled out weekly for adverse event monitoring

Week 4, 8 (Treatment and Control Arms): Monitored Capsule Consumption and Follow-up study visits for assessment of:

- Ingestion of 12 FMT capsules or inactive placebo.
- A proton pump inhibitor will be taken daily for the 48 hours 24 hours prior to and day of capsule consumption.
- BMI, Iliac measurements, hip to waist ratio
- Stool for microbiome analysis and metabolomics
- CBC, LFTs, FBG, HgA1C, lipids, glucose, metabolomics
- diet recall
- Symptom diary Card and Adverse Event screening

Weeks 6, 12 : Follow-up study visits for assessment of:

- BMI, Iliac measurements, hip to waist ratio
- Stool for microbiome analysis and metabolomics
- CBC, LFTs, FBG, HgA1C, lipids, glucose, metabolomics, ghrelin and leptin, PYY and adiponectin
- Mixed meal tolerance test followed by serum for GLP-1, Leptin and PYY at time 0, 15, 30, 45, and 60
- diet recall
- Indirect Calorimetry
- Symptom Diary Card and Adverse Event screening

Week 26 and 52:

- Safety assessment and Symptom Diary Cards via phone interview by a physician to screen for adverse events.
- Stool sample for microbiome analysis to be mailed in
- Ask for the patients current weight

Stool collection instructions are outlined in Appendix 2.
Patient obligations in the study will end at week 52 however the patient's medical record may be followed prospectively for evaluation of weight loss and weight loss surrogates as well as metabolic parameters done for clinical care through year 5 post-enrollment as needed. This may include review of partner's available records or records from home provider. (when applicable)

Stopping Criteria

Adverse events will be specifically monitored at week 1, 4, 6, 8, 12, 26, and 52 after FMT by direct patient interviews, or by reporting from clinical sites of adverse events at any time in-between. A data safety and monitoring board (DSMB, Appendix 5) will review all patient data to ensure optimal patient safety and precautions for subjects with obesity treated with FMT. A severity grade for adverse events (Appendix 6) and subject symptom diary cards will be used (Appendix 7). The DSMB will meet a minimum of 2 times: after treatment of the first 10 subjects, and after treatment of all 20 subjects. Study will be stopped under the following circumstances;

- Any unexpected serious adverse events occur that the DSMB determines as at least possibly related to FMT or detection of new pathogenic intestinal infection in stool samples in any patient treated.

Any subject who develops a serious adverse event that is judge to be probably, possibly, or definitely related to FMT therapy will receive no further FMT treatment. They will remain in the trial only for adverse event follow-up.

Accountability Procedures for the Investigational Product

The location, volume and number of all FMT donor solutions will be maintained in a log by the research team. The principal investigator will be responsible for accurate record and tracking of all FMT solutions. Logging of FMT solution used for administration will be performed using a two-person process.

Data to be Recorded Directly on the CRFs

Data will be obtained from patients directly, and their electronic health record as follows; Demographics , age, gender, co-morbidities, medication use, and laboratory values.

Donor Procedures (OpenBiome):

Prior to enrollment, donors (age >18), receive informed consent with oversight from MIT's IRB and COUHES. Donors are interviewed by a healthcare professional to determine whether they meet the following:

Clinical Exclusion criteria:

1. **Infectious risk factors:**
    a. Known HIV, Hepatitis B or C infections or exposure within previous 12 months
    b. High risk sexual behaviors
    c. Use of illicit drugs
    d. Tattoo or body piercing within previous 6 months
    e. Incarceration or history of incarceration
    f. Known current communicable disease
    g. Risk factors for variant Creutzfeldt-Jakob disease
    h. Travel within previous 3 months to countries where risk of infectious diarrhea is elevated
2. Gastrointestinal and systemic comorbidities:
    a. History of IBD, irritable bowel syndrome, chronic constipation or chronic diarrhea
    b. History of gastrointestinal malignancy or known polyposis
    c. Antibiotic use within the previous 3 months
    d. Immunosuppressive state or use of immunosuppressive medications
    e. History of major gastrointestinal surgery (e.g., gastric bypass)
    f. Metabolic syndrome ; history of NAFLD, hypertension, dyslipidemia, CAD, CHF
    g. Systemic autoimmunity, e.g., multiple sclerosis, connective tissue disease
    h. Atopic diseases including asthma and eczema, eosinophilic disorders of the gastrointestinal tract
    i. Chronic pain syndromes, e.g., chronic fatigue syndrome, fibromyalgia
    j. Specifically for this study: BMI < 23; waist circumference < 35 inches for women, < 40 inches for men; fat mass < 20%; FBG <95 mg/dL; HbA1C<5.7; LDL < 100 mg/dL; TG<100 mg/dL; HDL> 60 mg/dL; ALT < 20 U/L for women, < 30 U/L for men.

Donor Testing

Prospective donors that do not meet any of the exclusion criteria outlined above will then be subjected to a battery of serological and stool-based assays to determine whether common infectious agents are present. All tests will be outsourced to third party .
Laboratory Improvement Amendments (CLIA) certified testing facilities. As a condition for participation in this program, donors are required to submit written authorization for the disclosure of the results of these tests to the Microbiome Health Research Institute, in compliance with the Health Insurance Portability and Accountability Act (HIPAA). We will redact all personal identifying information from each report when we receive it. We will share copies of these raw diagnostic reports with our clinical partners. In the event that material from multiple donors is provided in a single shipment, results will be provided for all samples along with a file indicating which samples correspond to which donor screens. Documentation will be provided for the battery of tests prior to enrollment of a donor and for tests performed at the end of the collection window. Positive results for any of the following assays will be treated as exclusion criteria for all materials.

Serologic testing:

a. HIV antibody, type 1 and 2
b. Hepatitis A (IgM)
c. Hepatitis B panel (HBsAg & HBc [IgM])
d. Hepatitis C (HCV antibody)
e. Treponema pallidum screening cascade (EIA with reflex to RPR)
f. Liver function panel
g. Complete blood count
h. C-reactive protein assay
i. HTLV 1 and 2 antibodies
h. measurement of FBG, HgA1C, ALT, lipid profile if not done previously.

Stool testing:

a. EIA assay for Clostridium difficile toxins A and B
b. Culture-based assays for common enteric pathogens (including Salmonella, Shigella, E. coli, Campylobacter and Vibrio)
c. Fecal Giardia antigen EIA
d. Fecal Cryptosporidium antigen EIA
e. Ova and parasites exam
f. Tri-chrome stain for Isospora
g. Cyclospora smear test
h. H. pylori EIA
f. measurement of SCFA and 16S rRNA microbiome interrogation

Collection Window and Donor Monitoring

Donors that meet the above criteria are enrolled to provide material for FMT. Donor material is collected for up to 60 days following the initial screening. During this collection window donors must not violate any of the risk factors identified in Part A above. During a collection window, all material is quarantined until the donor has passed a second battery of serological and stool tests as described in Part B above. Material will be released for clinical use only after the donor has successfully completed both sets of assays (before and after the collection window).

In the event that a donor is recruited to provide additional material beyond an initial 60-day collection window, additional testing will be performed at 60 day intervals. Repeat donors that have not been tested within 60 days will be treated as new donors subject to the same screenings described in Parts A and B above. This ensures that all donors (even long-term participants) are subject to regular health evaluation.
In the event that the donor passes a loose stool or has other symptoms of disease, donors will immediately meet with a healthcare professional to evaluate their continued suitability for participation in our program. If the clinician determines that the donor is not healthy, the donor will be un-enrolled from the program. In the event of such a diagnosis, all material contributed during the preceding collection window will be destroyed. In addition to this qualitative exclusion by clinician's discretion, we will also treat three or more loose stools passed in a 24-hour period as an absolute exclusion criterion, and all material from the affected donor's collection window will be destroyed, regardless of clinical evaluation. Although less frequent passage of loose stool is not medically indicated as a disease and is common among healthy individuals, in the interests of caution we will also destroy material collected near any loose stool movement. All material collected from within 48 hours of two loose stools will be destroyed. All material collected from within 24 hours of a single loose stool will similarly be destroyed.

Production and Process Controls

1. The donor deposits stool in a commode, seals the lid, and places the collection container in a plastic bag to serve as secondary containment. Donors receive training to ensure that samples are not contaminated during the collection process.
2. After passage, the sealed sample collection container is immediately transferred from the donor to a qualified technician to process the sample.
3. Samples are transferred to a UV-sterilized biosafety hood as quickly as possible, not to exceed 60 minutes from the time of collection.
4. Within the biosafety hood, the stool is transferred to a sterile filter bag. The filter bag fits around the collection commode entirely, so there is no risk of material escaping during this transfer process. All stool material is added to the same side of the membrane in the filter bag.
5. An autoclaved dilutant consisting of 12.5% glycerol and a normal saline buffer (0.90% w/v NaCl in water) is added to the filter bag.
6. The sample solution sealed inside the filter bag is then introduced to a homogenizer blender for 60 seconds to mix the materials.
7. Samples are then aliquoted into sterile bottles using sterile, disposable serological pipettes.
8. The bottles are then capped, sealed and frozen immediately at -80°C. Any samples not fully processed and frozen within 120 minutes of passage will be destroyed.
9. Samples are sealed with a tamper evident shrink band as an additional level of containment and to ensure samples are not contaminated or tampered with during storage and distribution.
10. Samples will be delivered to clinicians on dry ice, in double-containment vessels, with temperature indicators to ensure that samples have not thawed during transportation.
11. Production of stool capsules and placebo
12. Preparation of inactive slurry

V. Subject Selection and Withdrawal.

Donors: OpenBiome donors are rigorously assessed and monitored.

1. **Donor candidates** are screened with comprehensive evaluation of medical histories, behavioral risks, and current health status.
2. **Laboratory Screening:** Donor candidates are screened for 20 stool and serological tests at a CLIA-certified laboratory. Less than 20% of those screened become qualified donors
3. **Continuous Requalification:** Qualified donors are under medical monitoring through the entire donation window and are fully rescreened every 60 days
4. **Quarantine Procedure:** Prior to release, donated material is quarantined for 60 days in between two full panel screens at a CLIA certified laboratory. After passing a first battery of tests, a donor may donate specimens for a 60-day window. All material made from these specimens is held in quarantine until a second battery of tests is administered. The material from this 60-day window is release only if and when the donor passes this second battery of tests.

5. **Additional metabolic criteria for donors:** The donor used for this study must additionally meet the following inclusion criteria:
    - **a.** BMI <21 kg/m^2 or total body fat % < 20%
    - **b.** HDL > 60 mg/dL for women; > 50 for men; LDL< 100 mg/dL; TG< 100 mg/dL
    - **c.** FBG < 95 mg/dL
    - **d.** waist circumference for men < 40 inches; women <35 inches
    - **e.** ALT < 20 U/L for women; < 30 U/L for men.

Patients:

Patient enrollment will be done via two methods:
1. Patient initiated communication with investigator via the clinicaltrials.gov website. (This may be E-mail or phone calls depending on patient preference)
2. Referral of appropriate patients from the GI or Primary Care clinic at Brigham and Women's Hospital (BWH. All study procedures will take place at Brigham and Women's Hospital.

Selection of subjects. Obese (BMI 35 kg/m^2 or greater) subjects who meet the inclusion criteria will be enrolled in the trial, after providing written informed consent. The study participants will be randomized 1:1 to either the treatment arm or the placebo arm. The treatment arm will receive an FMT capsule induction followed by monthly maintenance oral capsules for 12 weeks. The placebo group will receive placebo capsules at the time of their induction followed by monthly intake of oral placebo capsules for 12 weeks. The participants will not receive dietary instruction and will be asked to continue with their regular diet. Information about their dietary habits will be collected.

Inclusion criteria:

1. Age 18 years or older
2. Obesity defined as a BMI of 35 kg/m^2 or greater

Exclusion criteria:

1. Triglycerides > 500 mg/dL

2. Use of antibiotics <8 weeks prior to participation
3. Use of probiotics <4 weeks prior to participation
4. Alcohol use of greater than 20g/daily or suspicion of alcohol abuse and dependence
5. Substance abuse, current
6. LFTS greater then 3x the ULN
7. Cirrhosis.
8. DM type 2 that is insulin dependent, treated with GLP1-agonists, or poorly controlled on oral medications (HbA1C > 10%)
9. Use of any weight loss medication or participation in a weight loss study or program such as Weight Watchers in the past 3 months
10. History of recent weight change (weight loss or weight gain in the two months preceding trial enrollment). This is defined as a gain or loss of 10 or more pounds in the preceding 2 months.
11. Patients who are pregnant or breastfeeding
12. Patients who are unable to give informed consent
13. Patients who have previously undergone FMT
14. Patients who have a confirmed active malignancy or cancer
15. Patients who are immunocompromised including HIV infection.
16. Participation in a clinical trial in the preceding 30 days or simultaneously during this trial
17. Previous gastric or small intestinal surgery that alters gut anatomy such as fundoplication, gastric resection, gastric bypass, small bowel resection, and ileocectomy
18. Other comorbidities including: systemic lupus, inflammatory bowel disease, or Chronic kidney disease as defined by a GFR <60mL/min/1.73m2 44 or rheumatoid arthritis
19. History of rheumatic heart disease, endocarditis, or valvular disease due to risk of bacteremia from colonoscopy
20. Any condition, based on clinical judgment that may make study participation unsafe
21. History of severe food allergies (e.g. anaphylaxis or anaphylactoid reaction)
22. Use of immune modulators including methotrexate, mycophenolate mofetil, tacrolimus, cyclosporine, thalidomide, Interleukin-10, or Interleukin-11 or prednisone within the past 2 months
23. Treatment with infliximab, adalimumab, certolizumab, natalizumab, vedolizumab or thalidomide within the prior 2 months
24. Congenital or acquired immunodeficiencies
25. Patients with allergies to ingredients Generally Recognized As Safe (GRAS): glycerol, sodium chloride, hypromellose, gellan gum, titanium dioxide, cocoa butter
26. Dysphagia: oropharyngeal, esophageal, functional, neuromuscular (e.g. stroke, multiple sclerosis, ALS)
27. History of aspiration
28. History of severe gastroparesis

For patients outside of the Partner's system, medical diagnoses that are identified by the patient and relevant to inclusion and exclusion will be confirmed with their treating physician

Subject Withdrawal Criteria: Patients – patients will be withdrawn under the following circumstances:

1. Patient withdraws their consent for follow-up visits or contacts.
2. Patient is unable to successfully perform capsule test swallow.
3. These above patients will only be followed-up for week 2 adverse event screening, as they will not have received FMT. These subjects will be replaced, as they will not be considered "treated"

VI. SUBJECT ENROLLMENT

Recruitment Procedures:

BWH gastroenterologists and Primary Care Physicians, including attending physicians and fellows, will be informed of the study's aims and inclusion criteria. These doctors who have firsthand knowledge of the patient's medical history must give approval for his/her patient to be contacted for research purposes and will inform the principal investigator of patients who meet the study criteria and who may be good candidates for the study. The referring physician will introduce the study to the potential patient and request the patient's permission to be approached by study staff.

After the treating physician has described the research study to the patient and has given permission to be contacted, a member of the study staff will be available for any follow-up questions. Investigators and study coordinators will reinforce with any potential subjects that participation is voluntary, that they do not have to participate, and the decision not to participate will not affect their care now or in the future. The patient will be given a copy of the informed consent, and encouraged to discuss the study with family, friends, their PCP, and/or others. Study staff will follow up to see if there are any questions or concerns that have not been addressed. These follow-up contacts will reoccur until the patient has either (a) been enrolled or (b) turned down participation in the study.

To minimize the "undue influence" that the principal investigator may have on study subjects, Dr. Allegretti's patients (whom she feels are eligible) will be initially approached by another physician or nurse practitioner in the practice about the study. Subjects will be approached by a study staff member (investigators, research assistant or coordinators) when they have given their permission to be contacted. They will be given both oral information about the study and they will have the opportunity to ask questions of the study staff at the time of recruitment. Patients will also have an opportunity to discuss participation with their primary physicians, including Dr. Allegretti, as well once they have received all study information.

We will not utilize advertisement material or other informational materials for patients beyond the consent form. Patients will be given as much time as they need to decide. They will be given a copy of the consent form to take home, read, and consider, and they will be encouraged to discuss participation with family members and health care providers.

The consent form will be signed that day or at a future visit to the clinic. Once signed, the subject will undergo further screening to determine eligibility. No study related procedures will be performed until the informed consent form has been signed.

If they decide to participate, both study and controls will be asked to maintain their standard diet.

If patients reach out to us via the provided E-mail or phone number located at the clinicaltrials.gov website, we will pre-screen them using questions from the provided E-mail/phone script. If the patient's responses do not indicate any exclusion criteria being met, then we will contact their primary care provider via email or phone call (with patient permission) to confirm medical diagnosis. Once patient's medical and treatment history are thoroughly vetted with the primary care physician, we will ask the physician to further discuss the study with the patient to minimize "undue influence" and ensure appropriateness for participation. When we are given the approval of the physician to enroll the patient in the study, we will then schedule the standard screening visit to re-review medical history (inclusion and exclusion criteria) and perform informed consent. (See below) If the patient has no medical problems and has no treating physician due to lack of medical problems, the patient will be eligible to come in for a screening visit.

Consent Procedures:

The treating physician will introduce the study to the potential patient and request the patient's permission to be approached by study staff. With the treating physician's permission, either a physician investigator or study coordinator will describe the research study in detail, including participation and risks and alternative courses of treatment, and answer any questions or concerns that the patient may have. Patients will be given as much time as they need to consider participation before signing the consent form.

In order to avoid coercion, study staff will reinforce that participation is voluntary and that their decision will not affect the medical care that they receive now or in the future. If patients seek more time to consider participation, they will be given a copy of the consent form and encouraged to discuss the study with family, friends, PCP, or others. Study staff will follow-up to see if any questions or concerns have not been addressed. A physician investigator will obtain informed consent signatures.

VII. Assessment of Safety

Patient safety monitoring schedule - although there have been no reported serious adverse events associated with fecal microbiota transplantation (FMT) in patients with recurrent refractory Clostridium difficile, we will have a special data safety and monitoring board (DSMB) to ensure optimal patient safety and precautions for subjects with obesity treated with FMT. Adverse events will be specifically monitored at 1, 4, 6, 8, and 12, weeks after FMT by direct patient interviews, or by reporting from clinical sites of adverse events at any time in-between. Adverse events will also be assessed via telephone calls at weeks 26 and 52. The DSMB will meet a minimum of 2 times: after treatment of the first 10 subjects, and after treatment of all 20 subjects. A copy of the DSMB charter is located in Appendix 3.

For this trial, an adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation patient administered a biologic product; such an occurrence does not necessarily have to have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product whether or not considered related to the medicinal product. A serious adverse event (SAE) is defined as any untoward medical occurrence that results in death, is life threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, is an important medical event that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes previously mentioned. Adverse events will be determined by a toxicity grading scale (Appendix 4).

Patients will be asked to document their symptoms using the symptom diary card (Appendix 5) as a memory aide. Subjects will be asked to complete a symptom diary card at baseline, prior to FMT, and on the day of treatment to assess their baseline. They will then be asked to complete a symptom diary card each day for 1 week post-FMT and then once a week thereafter for 12 weeks and then during follow-up at weeks 26 and 52. The symptom diary card will help the patient monitor and keep track of adverse events and will be reviewed by the Principal Investigator.

In addition the DSMB may convene additional meetings if necessary to ensure the ongoing monitoring and safety of the subjects treated with FMT. The internal DSMB will include the following individuals: Dr. Walter Chan (BWH Gastroenterology, Chair),
Dr. Marvin Ryou (Gastroenterology – BWH) and Dr. Andrew Courtwright (Pulmonology/Ethics-BWH). All events will be reported directly to the DSMB and the Institutional Review Board (IRB) in accordance with Harvard University policy: Investigators will promptly report to the IRB all unanticipated problems involving risks to human subjects or others under Title 21 of the Code of Federal Regulations (21 CFR) part 56 (Institutional Review Boards), part 312 (Investigational New Drug Application), and part 812 (Investigational Device Exemptions).Harvard Medical School policy is consistent with guidance set forth by the Office for Human Research Protections (OHRP) (presented January 15, 2007) http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm and the FDA (presented January 14, 2009)
http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126572.pdf when determining what related events require review by the Institutional Review Board. Any serious AEs also will be evaluated by the DSMB for review and determination of whether the trial should continue.

All subjects will be followed for an additional six months following the end of their treatment. Subjects that did not experience a AEs or SAEs will receive a telephone call to monitor their current symptoms and quality of life. For subjects that did experience a AE or SAE that was found to be connected to the FMT treatment, we ask that they return to our medical center for an exam with their gastroenterologist. All treatment and outcome of AEs will be documented on the CRF and summarized in the CSR. All AEs will be followed to resolution or stabilization by the study physician.

VIII. Biostatistical Analysis:

This is an exploratory study as relatively little is known about the microbiome of patients with obesity. Thus, the main objective of our study is to assess the safety of FMT in obese patients. Secondarily we will be able to characterize variability in the microbiome of obese patients to aid

in estimating effect sizes for designing larger studies powered to detect associations between microbiome data and GLP-1 levels and possible weight loss/BMI.

IX. Risks and Discomforts:

Fecal Microbial Therapy:

Known Risks of FMT

- Altered bowel pattern (diarrhea, constipation)
- Cramping
- Belching

Potential Risks of FMT

- Transmission of pathogenic bacteria, viruses, fungi
- Transmission of allergens
- Alteration in intestinal metabolism

Privacy and Confidentiality:

This study involves the collection of personal health information. Accidental release of personal health information is a risk of participation in this study. Measures will be taken to protect the confidentiality of all subjects' information. These measures include keeping all information collected about the subjects' confidential, keeping information in locked rooms, and having physicians who are directly involved with a subject's clinical care involved in the study.

Venipuncture: Risks of having blood drawn include pain, bruising, or infection.

Pregnancy: The risks to fetuses and women who are pregnant are unknown.

X. Potential Benefits:

Subjects may not receive any benefits from taking part in this study. However this research and the results of this study may benefit others in the future.

The potential benefits include:

- Restoration of fecal diversity
- Increase in GLP-1 hormones
- Weight loss

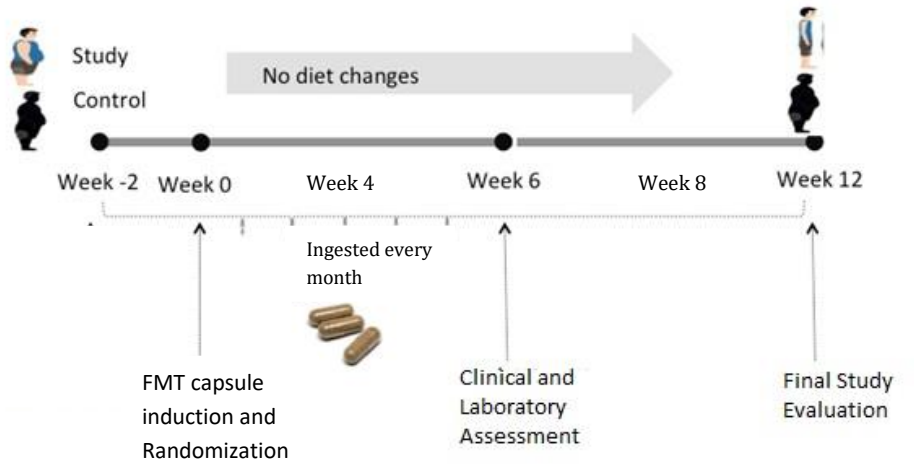
As this is a pilot study, it is difficult to quantify the expected benefits.

XI. Monitoring and Quality Assurance:

The Principal Investigator, Dr. Jessica Allegretti, will assure the validity and integrity of the data and adherence to the IRB-approved protocol.

Study staff will review completed CRF's before each visit to ensure completeness of previous entries. Entries that need clarification will be reviewed by the PI, and the subject and/or treating gastroenterologist will be consulted if needed.

Appendix 1:



Appendix 2.

Stool collection Instructions

Important note about sample collection and shipping:

Collect and ship your stool samples by FedEx according to the Samples Collections Schedule that your research coordinator explained to you. Please ship within 24 hours of collection. Please contact your research coordinator, Madeline Carrellas (617)732-9223 if you have any questions.

This kit contains the supplies needed for 1 stool sample collection and 1 return shipment.

Part 1 — Preparation for one collection

1. Each kit contains the following stool collection materials (see photo):
- 1 Blue Bowl + Seat to collect your stool
- 1 Tube with a screw cap top (a Para-Pak vial). This tube has a built-in spoon for scooping and contains a liquid that is a preservative solution
- 1 Pair of gloves for hygienic collection
- 1 Plastic dropper [optional use]

2. Wash your hands with soap and water, then rinse.

3. Urinate into toilet BEFORE stool collection.

Part 2 — Collection Seat Placement

1. Lift toilet seat.

2. Place Blue Bowl and Seat at the back of the toilet bowl for collection.

3. Lower toilet seat and ensure stability of the blue collection bowl.

4. Deposit stool directly into the blue collection bowl.

DO NOT urinate into the blue stool collection bowl.

Part 3 — Collecting the Stool Sample

1. Unscrew and remove the lid of the clean collection tube provided.

2. Using the spoon attached to the lid, scoop one pea-sized amount of your stool into the tube. Please DO NOT provide more stool than the pea-sized amount requested.

For liquid stool the plastic dropper can be used. Please provide ONE dropper full of material.

3. Close and tighten the lid firmly.

4. Shake the closed tube by inverting it a few times to completely immerse the stool sample in the liquid, and then tap the bottom of the closed tube on a table top to ensure that the sample is at the bottom of the tube.

5. Dispose of the blue bowl, the seat, and the gloves in the trash. Wash your hands with soap and water.

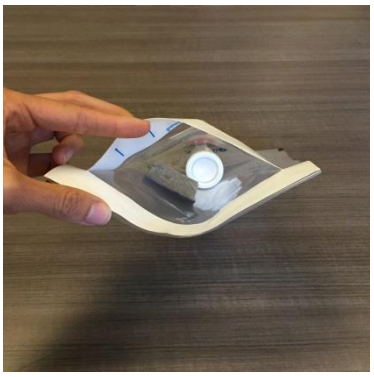
Stool shipping Instructions

Part 4 — Labeling, Packaging and Shipping the Specimen

1. Each kit contains the following shipping materials:
- FedEx – UN3373 pack with pre-filled shipping label
- An easy fold return shipper (cardboard box)
- A small plastic bag with biohazard symbol
- A strip of absorbent material (gray tissue)

2. Please record the date and the time of the collection ONLY as previously explained to you by your research coordinator. *DO NOT record your name or any personal information.* To protect your privacy, this has been replaced with a patient barcode.

3. Place the tube inside the small plastic bag with biohazard symbol on it. Please keep the absorbent material (gray tissue) inside this plastic bag.



4. Seal the plastic bag by removing the white tab and exposing the adhesive. Firmly press flap to completely seal the bag. Place this plastic bag, with the vial inside, in the small cardboard box and close the box.

5. Place the cardboard box inside the plastic pre-labeled FedEx UN3373 pak.

6. Follow the instructions below to either schedule a FedEx pickup or to drop off the package.

FedEx Shipping Instructions:
Biological Samples

Instruction for pick-up schedule (preferred option):
1) Call 1-800-GO-FEDEX (1-800-463-3339) to schedule a home pickup within 24 hours of collection.
2) Your call will be answered by an automated system and will give you several options. Follow the options and verbally state "schedule a pick up".
3) The system will ask you to enter or verbalize your 9 digit account number. The FedEx account number is 686124762.
4) When asked if you want an express or ground delivery, please state "express".
5) Depending on what time of day you call, the system may ask if you the package is ready for pick up now. Please respond "NO" if it is to be picked up the next day. If it needs to be picked up that day, please respond "YES".
6) You will be asked for your zip code followed by the number of packages, and if any of them are over 150 pounds – give the zip code of your home address and state that you have 1 package that does not weigh over 150 pounds.
7) You will be required to provide the address where the package will be picked up.
8) The system will then confirm the pickup time and location. After confirming the pickup is scheduled properly, the system will hang up.
9) At any time during the call you may be directed to a customer service representative to confirm the details of your pick up.

Instruction for drop-off (alternative option):
1) Call 1-800-GO-FEDEX (1-800-463-3339) to determine the appropriate FedEx dropbox location.
2) Verify with FedEx that the FedEx location of your choice can accept UN3373 pack and drop the package off within 24 hours of collection.

If you have any questions about the stool collection procedures, please do not hesitate to contact your research coordinator at 617-732-9223

Thank you for your participation in this study!

In-clinic Stool collection Instructions

Each stool collection kit contains:

- 1 Blue Bowl + Seat to collect your stool.
- 2 Tubes with a screw cap top (called Para-Pak vial). These tubes have a built-in spoon for scooping:
    1 empty tube, without any liquid (marked by blue dot stickers)
    1 tube containing a preservative solution (no sticker)
- 1 Pair of gloves

Part 1 - Collection Seat Placement

1. Lift toilet seat.
2. Place Blue Bowl and Seat at the back of the toilet bowl for collection.
3. Lower toilet seat and ensure stability of the blue collection bowl.
4. Deposit stool directly into the blue collection bowl.

DO NOT urinate into the blue stool collection bowl.

Part 2 - Collecting the Stool Samples

1. Collection tube with blue dot sticker (no liquid)
    a. Unscrew and remove the lid of the clean collection tube with blue dot sticker.
    b. Using the spoon attached to the lid, scoop one pea-sized amount of your stool into the empty tube. Please DO NOT provide more stool than the pea-sized requested.
    c. Close and tighten the lid firmly.

2. Collection tube with preservative solution
    a. Unscrew and remove the lid of the other collection tube (without blue sticker).
    b. Using the spoon attached to the lid, scoop one pea-sized amount of your stool into the empty tube. Please DO NOT provide more stool than the pea-sized requested.
    c. Close and tighten the lid firmly.
    d. Shake the closed tube by inverting it a few times to completely immerse the stool sample in the liquid, and then tap the bottom of the closed tube on a table top to ensure that the sample is at the bottom of the tube.
3. Dispose of the bowl, the seat, and the gloves in the trash. Wash your hands with soap and water.
4. Return both your collection tubes to the research coordinator.

Appendix 3.

Charter, Data and Safety Monitoring Board for

Fecal Microbiota Transplantation for the Treatment of Obesity: An Investigation of the microbial contribution to pathogenesis of disease

January 2016

1. Introduction

This Charter is for the Data and Safety Monitoring Board (DSMB) for the study "**Fecal Microbiota Transplantation for the Treatment of Obesity: An Investigation of the microbial contribution to pathogenesis of disease**."

The DSMB will consist of a team of clinical researchers who are unaffiliated with this project. These individuals will not be investigators in this study and will be experienced in conducting and interpreting clinical trials; they will review the efficacy and safety endpoints at the below stated time points. There will be 3 members on the DSMB consisting of at least two gastroenterologists and one Ethics specialist. The members of the DSMB are:

Dr. Walter Chan (Gastroenterology – BWH, Chair)
Dr. Marvin Ryou (Gastroenterology – BWH)
Dr. Andrew Courtwright (Pulmonary/Ethics-BWH)

The Charter is intended to be a living document. The DSMB may wish to review it at regular intervals to determine whether any changes in procedure are needed.

2. Responsibilities of the DSMB

The DSMB is responsible for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures, and for monitoring the overall conduct of the study.

The DSMB is an independent group advisory and is required to provide recommendations about starting, continuing, and stopping the study. In addition, the DSMB is asked to make recommendations, as appropriate, to the about:

- Selection, recruitment, and retention of participants
- Adherence to protocol requirements
- Completeness, quality, and analysis of measurements
- Amendments to the study protocol and consent forms
- Participant safety
- Notification of and referral for abnormal findings

3. Scheduling, Timing, and Organization of Meetings

Data and Safety Monitoring Meetings

The DSMB will meet a minimum of 2 times: after treatment of the first 10 subjects, and after treatment of all 20 subjects. In addition the DSMB may convene additional meetings if necessary

to ensure the ongoing monitoring and safety of the subjects treated with FMT. Any serious AEs also will be evaluated by the DSMB for review and determination of whether the trial should continue. An example of the DSMB meeting minutes are at the end of this document. The study will not proceed at each of these time points until the DSMB gives approval to continue.

Safety Reporting

In accordance with applicable policies of the BWH/PHRC Institutional Review Board (IRB), the investigator-sponsor will report, to the IRB, any observed or volunteered Unanticipated Problem that is determined to be 1) unexpected); 2) related or at least possibly related to study participation; and 3) suggests that the research places subjects or others at a risk of unknown harm or addition/increased frequency of harms (including physical, psychological, economic, legal, or social harm) than was previously known or recognized. Unanticipated problems may be adverse events, protocol deviations, noncompliance or other types of problems, but MUST meet all of the criteria listed above. Unanticipated problem reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable unanticipated problems will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator-sponsor's receipt of the respective information. For Internal Fatal/Life-Threatening Unanticipated Problems, the PI should notify the IRB Chair by phone immediately and consider voluntarily halting subject enrollment.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator will report the unanticipated problem to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

4. Grading and Attribution Methods for Adverse Events

Grading Scale

0 No adverse event or within normal limits
1 Mild adverse event – did not require treatment
2 Moderate adverse event – resolved with treatment
3 Severe adverse event – resulted in inability to carry on normal activities and required professional medical attention
4 Life threatening or disabling adverse event
5 Fatal adverse event

Attribution Scale

Definite: The adverse event is clearly related to the study drug
Probable: The adverse event is likely related to the study drug
Possible: The adverse event may be related to the study drug
Unlikely: The adverse event is doubtfully related to the study drug
Unrelated: The adverse event is clearly not related to the study drug

Data and Safety Monitoring Meeting Minutes

Date:

Title of Protocol/IRB Number:

Principal Investigator/Designee:

Recommendations:

□ **Continue the trial without modification**

□ **Accrual:**
- □ Recommend study be closed because of slow accrual
- □ Continue to monitor study, but consider closure because of slow accrual

□ **Recommend study is amended/changed:**
- □ For patient safety reasons
  - □ Rate of adverse events
  - □ Early stopping of inferior therapy
- □ To extend accrual because of an event rate slower than expected

□ **Other:** _____

Signature/Principal Investigator or Designee:

_____

DSMB meetings will be held at the Brigham and Women's Hospital. The purpose of the first meeting is to review and discuss this charter and process.

The DSMB also will review adverse event data, other safety data, quality and completeness of study data, and enrollment data at each meeting to ensure proper trial conduct. At intervals, as noted above, the DSMB will also review formal interim analyses of the primary end point.

5. Reports of DSMB Deliberations

- Initial summary: The Director or designee will review this summary and approve or disapprove the recommendation(s), or request additional information. The recommendations will then be sent to the DCC, and the clinical investigators.

- Action plan: If the DSMB's recommendations require significant changes or follow-up, the BWH IRB staff will prepare an action plan outlining the steps required to implement the recommendations.

- Formal minutes: The DSMB Chair is responsible within 30 days of the meeting or call to present minutes to the IRB. These minutes are subject to FOIA requests and are prepared accordingly to summarize the key points of the discussion and debate, requests for additional information, response of the investigators to previous

recommendations, and the recommendations from the current meeting. These minutes will be reviewed by IRB staff, key study personnel before being forwarded to the DSMB Chair for final review and approval. The DSMB Chair may sign the minutes or indicate approval electronically via email. Then, the minutes are sent to the BWH IRB for approval. Subsequently, the minutes are sent back to the IRB and the relevant investigators, and included in the materials for the subsequent DSMB meeting to be approved by voice vote at that meeting. Once they have been voted and approved by the Board, they are considered Final.

Appendix 4.
Toxicity Grading Scale for Adverse Events

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ESTIMATING SEVERITY GRADE				
Clinical adverse event NOT identified elsewhere in this DAIDS AE Grading Table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent
SYSTEMIC				
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria medical intervention indicated OR Mild angioedema with no medical intervention	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social &	Symptoms causing greater than minimal interference with	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing or minimal interference with usual social & functional	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions
Fever (nonaxillary)	37.7 – 38.6°C	38.7 – 39.3°C	39.4 – 40.5°C	> 40.5°C
Pain (indicate body site) DO NOT use for pain due to injection (See Injection Site Reactions: Injection site pain) See also	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than emergency room visit) indicated

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding or total
INFECTION				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social &	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR	Life-threatening consequences (e.g., septic shock)
INJECTION SITE REACTIONS				
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social &	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for
Injection site reaction (localized)				
Adult ≥ 18 years	Erythema OR Induration of 5x5 cm – 9x9 cm ($25\ cm^2 – 81\ cm^2$)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR	Necrosis (involving dermis and deeper tissue)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Pruritis associated with injection See also Skin: Pruritis (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA
SKIN – DERMATOLOGICAL				
Alopecia	Thinning detectable by study participant (or by caregiver for young children and disabled adults)	Thinning or patchy hair loss detectable by health care provider	Complete hair loss	NA
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Hyperpigmentation	Slight or localized	Marked or generalized	NA	NA
Hypopigmentation	Slight or localized	Marked or generalized	NA	NA
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
CARDIOVASCULAR				
Cardiac arrhythmia (general) (By ECG or physical exam)	Asymptomatic AND No intervention indicated	Asymptomatic AND Non-urgent medical intervention indicated	Symptomatic, non-lifethreatening AND Non-urgent medical intervention indicated	Life-threatening arrhythmia OR Urgent intervention indicated
Cardiac-ischemia/infarction	NA	NA	Symptomatic ischemia (stable	Unstable angina OR Acute myocar

			angina) OR Testing consistent with ischemia	

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hemorrhage (significant acute blood loss)	NA	Symptomatic AND No transfusion indicated	Symptomatic AND Transfusion of ≤ 2 units packed RBCs (for children ≤ 10 cc/kg) indicated	Life-threatening hypotension OR Transfusion of > 2 units packed RBCs (for children > 10
Hypertension				
Adult ≥ 18 years (with repeat testing at same visit)	140 – 159 mmHg systolic OR 90 – 99 mmHg	160 – 179 mmHg systolic OR 100 – 109 mmHg diastolic	**≥** 180 mmHg systolic OR **≥** 110 mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood
Pericardial effusion	Asymptomatic, small effusion requiring no intervention	Asymptomatic, moderate or larger effusion requiring no	Effusion with non-life threatening physiologic consequences OR	Life-threatening consequences (e.g., tamponade) OR Urgent intervention
Prolonged PR interval				
Adult ≥ 18 years	PR interval 0.21 – 0.25 sec	PR interval > 0.25 sec	Type II 2nd degree AV block OR	Complete AV block

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Prolonged QTc				
Adult ≥ 18 years	Asymptomatic, QTc interval 0.45 – 0.47 sec OR Increase interval	Asymptomatic, QTc interval 0.48 – 0.49 sec OR Increase in	Asymptomatic, QTc interval ≥ 0.50 sec OR Increase in interval	Life-threatening consequences, e.g. Torsade de pointes or other associated

Thrombosis/embolism	NA	Deep vein thrombosis AND No intervention indicated (e.g., anticoagulation,	Deep vein thrombosis AND Intervention indicated (e.g., anticoagulation,	Embolic event (e.g., pulmonary embolism, life-threatening thrombus)
Vasovagal episode (associated with a procedure of any	Present without loss of consciousness	Present with transient loss of consciousness	NA	NA
Ventricular dysfunction (congestive	NA	Asymptomatic diagnostic finding AND intervention	New onset with symptoms OR Worsening	Life-threatening congestive heart failure
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total
Comment: Please note that, while the grading scale provided for Unintentional Weight Loss may be used as a guideline when grading anorexia, this is not a requirement and should not be used as a substitute for clinical				
Ascites	Asymptomatic	Symptomatic AND Intervention indicated (e.g., diuretics or	Symptomatic despite intervention	Life-threatening consequences

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Cholecystitis	NA	Symptomatic AND Medical intervention indicated	Radiologic, endoscopic, or operative intervention indicated	Life-threatening consequences (e.g., sepsis or perforation)
Constipation	NA	Persistent constipation requiring regular use of dietary	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Diarrhea				
Adult ≥ 18 years	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement	Life-threatening consequences (e.g., hypotensive shock)

Dysphagia-Odynophagia	Symptomatic but able to eat usual diet	Symptoms causing altered dietary intake without	Symptoms causing severely altered dietary intake with	Life-threatening reduction in oral intake
Mucositis/stomatitis (clinical exam) Indicate site (e.g., larynx, oral) See Genitourinary for See also Dysphagia-Odynophagia and	Erythema of the mucosa	Patchy pseudomembranes or ulcerations	Confluent pseudomembranes or ulcerations OR Mucosal bleeding with minor trauma	Tissue necrosis OR Diffuse spontaneous mucosal bleeding OR Life-threatening
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive	Life-threatening consequences (e.g., hypotensive shock)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Pancreatitis	NA	Symptomatic AND Hospitalization not indicated (other	Symptomatic AND Hospitalization indicated (other than	Life-threatening consequences (e.g., circulatory
Proctitis (functional-symptomatic) Also see Mucositis/stomatitis for clinical exam	Rectal discomfort AND No intervention indicated	Symptoms causing greater than minimal interference with social & functional activities OR Medical intervention indicated	Symptoms causing inability to perform social & functional activities OR Operative intervention indicated	Life-threatening consequences (e.g., perforation)
Vomiting	Transient or intermittent vomiting with no or minimal	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR	Life-threatening consequences (e.g., hypotensive shock)
NEUROLOGIC				
Alteration in personality-behavior or in mood (e.g., agitation, anxiety, depression, mania, psychosis)	Alteration causing no or minimal interference with usual social & functional activities	Alteration causing greater than minimal interference with social & functional activities	Alteration causing inability to perform social & functional activities	Behavior potentially harmful to self or (e.g., suicidal and homicidal ideation or attempt, acute psychosis) OR Causing inability to perform basic self-care

Altered Mental Status For Dementia, see Cognitive and behavioral/attentional disturbance (including dementia	Changes causing no or minimal interference with usual social & functional activities	Mild lethargy or somnolence causing greater than minimal interference with usual social & functional activities	Confusion, memory impairment, lethargy, or somnolence causing inability to perform usual social & functional activities	Delirium OR obtundation, OR coma
Ataxia	Asymptomatic ataxia detectable on exam OR Minimal ataxia causing no or minimal	Symptomatic ataxia causing greater than minimal interference with usual social &	Symptomatic ataxia causing inability to perform usual social & functional activities	Disabling ataxia causing inability to perform basic self-care functions

PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Cognitive and behavioral/attentional disturbance (including dementia and attention deficit	Disability causing no or minimal interference with usual social & functional activities OR	Disability causing greater than minimal interference with usual social & functional activities OR	Disability causing inability to perform usual social & functional activities OR Specialized	Disability causing inability to perform basic self-care functions OR Institutionalization indicated
CNS ischemia (acute)	NA	NA	Transient ischemic attack	Cerebral vascular accident (CVA, stroke) with
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with	Difficulty sleeping causing inability to perform usual social & functional	Disabling insomnia causing inability to perform basic self-care functions
Neuromuscular weakness (including myopathy & neuropathy)	Asymptomatic with decreased strength on exam OR Minimal muscle weakness causing no or minimal	Muscle weakness causing greater than minimal interference with usual social & functional	Muscle weakness causing inability to perform usual social & functional activities	Disabling muscle weakness causing inability to perform basic self-care functions OR Respiratory muscle

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Neurosensory alteration (including paresthesia and painful neuropathy)	Asymptomatic with sensory alteration on exam or minimal paresthesia causing no or minimal interference with usual social & functional	Sensory alteration or paresthesia causing greater than minimal interference with usual social & functional activities	Sensory alteration or paresthesia causing inability to perform social & functional activities	Disabling sensory alteration or causing inability to perform basic self-care functions
Seizure: (new onset) **– Adult ≥ 18 years** See also Seizure: (known pre-existing seizure disorder)	NA	1 seizure	2 – 4 seizures	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Seizure: (known pre- existing seizure disorder) **– Adult ≥ 18 years** For worsening of existing epilepsy the grades should be based on an increase from	NA	Increased frequency of pre-existing seizures (non-repetitive) without change in seizure character OR Infrequent break-through seizures while on stable	Change in seizure character from baseline either in duration or quality (e.g., severity or focality)	Seizures of any kind which are prolonged, repetitive (e.g., status epilepticus), or difficult to control (e.g., refractory epilepsy)
Syncope (not associated with a procedure)	NA	Present	NA	NA
Vertigo	Vertigo causing no or minimal interference with usual social &	Vertigo causing greater than minimal interference with	Vertigo causing inability to perform usual social & functional activities	Disabling vertigo causing inability to perform basic self-care functions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
RESPIRATORY				
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 – 80%	FEV1 or peak flow 50 – 69%	FEV1 or peak flow 25 – 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress				
Adult ≥ 18 years	Dyspnea on exertion with no or minimal interference with	Dyspnea on exertion causing greater than minimal	Dyspnea at rest causing inability to perform usual social & functional activities	Respiratory failure with ventilatory support indicated
MUSCULOSKELETAL				
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social &	Joint pain causing greater than minimal interference with	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social &	Stiffness or joint swelling causing greater than minimal interference with	Stiffness or joint swelling causing inability to perform usual social & functional	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Bone Mineral Loss				
Adult ≥ 18 years	BMD t-score -2.5 to -1.0	BMD t-score < -2.5	Pathological fracture (including loss of vertebral	Pathologic fracture causing life-threatening
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with	Muscle pain causing greater than minimal interference with	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Osteonecrosis	NA	Asymptomatic with radiographic findings AND No operative	Symptomatic bone pain with radiographic findings OR Operative	Disabling bone pain with radiographic findings causing inability to perform
GENITOURINARY				
Cervicitis (symptoms) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV infection)	Symptoms causing or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with social & functional activities	Symptoms causing inability to perform social & functional activities	Symptoms causing inability to perform self-care functions
Cervicitis (clinical exam) (For use in studies evaluating topical study agents) For other cervicitis see Infection: Infection (any other than HIV	Minimal cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption	Moderate cervical abnormalities on examination (erythema, mucopurulent discharge, or friability) OR Epithelial disruption of 25 – 49%	Severe cervical abnormalities on examination (mucopurulent discharge, or friability) OR Epithelial disruption 50 – 75% total surface	Epithelial disruption > 75% total surface
Inter-menstrual bleeding (IMB)	Spotting observed by participant OR Minimal blood observed during clinical or examination	Inter-menstrual bleeding not greater duration or amount than usual menstrual cycle	Inter-menstrual greater in duration or amount than usual menstrual cycle	Hemorrhage with life-threatening OR Operative intervention indicated
Urinary tract obstruction (e.g., stone)	NA	Signs or symptoms of urinary tract obstruction without hydronephrosis or renal dysfunction	Signs or symptoms of urinary tract with hydronephrosis or renal dysfunction	Obstruction causing threatening consequences

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Vulvovaginitis (symptoms) (Use in studies evaluating topical study agents) For other vulvovaginitis see	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions
Vulvovaginitis (clinical exam) (Use in studies evaluating topical study agents) For other vulvovaginitis see	Minimal vaginal abnormalities on examination OR Epithelial disruption < 25% of total surface	Moderate vaginal abnormalities on examination OR Epithelial disruption of 25 - 49% total surface	Severe vaginal abnormalities on examination OR Epithelial disruption 50 - 75% total surface	Vaginal perforation OR Epithelial disruption > 75% total surface
OCULAR/VISUAL				
Uveitis	Asymptomatic but detectable on exam	Symptomatic anterior uveitis OR Medical	Posterior or pan-uveitis OR Operative intervention	Disabling visual loss in affected eye(s)
Visual changes (from baseline)	Visual changes causing no or minimal interference with usual social &	Visual changes causing greater than minimal interference with usual social &	Visual changes causing inability to perform usual social & functional activities	Disabling visual loss in affected eye(s)
ENDOCRINE/METABOLIC				
Abnormal fat accumulation (e.g., back of neck, breasts, abdomen)	Detectable by study participant (or by caregiver for young children and disabled	Detectable on exam by health care provider	Disfiguring OR Obvious changes on casual visual inspection	NA
Diabetes mellitus	NA	New onset without need to initiate medication OR Modification of current medications	New onset with initiation of medication indicated OR Diabetes uncontrolled despite	Life-threatening consequences (e.g., ketoacidosis, hyperosmolar non- ketotic

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Gynecomastia	Detectable by study participant or caregiver (for young children and	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA
Hyperthyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment	Life-threatening consequences (e.g., thyroid storm)
Hypothyroidism	Asymptomatic	Symptomatic causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities OR Uncontrolled despite treatment	Life-threatening consequences (e.g., myxedema coma)
Lipoatrophy (e.g., fat loss from the face, extremities,	Detectable by study participant (or by caregiver for young children and	Detectable on physical exam by health care provider	Disfiguring OR Obvious on casual visual inspection	NA

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
HEMATOLOGY ***Standard International Units are listed in italics***				
Absolute CD4+ count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	300 – 400/mm^3 *300 – 400/µL*	200 – 299/mm^3 *200 – 299/µL*	100 – 199/mm^3 *100 – 199/µL*	< 100/mm^3 *< 100/µL*
Absolute lymphocyte count **– Adult and Pediatric > 13 years** (HIV NEGATIVE ONLY)	600 – 650/mm^3 *0.600 x 10^9 – 0.650 x 10^9/L*	500 – 599/mm^3 *0.500 x 10^9 – 0.599 x 10^9/L*	350 – 499/mm^3 *0.350 x 10^9 – 0.499 x 10^9/L*	< 350/mm^3 *< 0.350 x 10^9/L*
Comment: Values in children ≤ 13 years are not given for the two parameters above because the absolute counts				
Absolute neutrophil count (ANC)				
Adult and Pediatric, > 7 days	1,000 – 1,300/mm^3 *1.000 x 10^9 – 1.300 x 10^9/L*	750 – 999/mm^3 *0.750 x 10^9 – 0.999 x 10^9/L*	500 – 749/mm^3 *0.500 x 10^9 – 0.749 x 10^9/L*	< 500/mm^3 *< 0.500 x 10^9/L*
Comment: Parameter changed from "Infant, < 1 day" to "Infant, ≤1 day"				
Fibrinogen, decreased	100 – 200 mg/dL *1.00 – 2.00 g/L* OR 0.75 – 0.99 x LLN	75 – 99 mg/dL *0.75 – 0.99 g/L* OR 0.50 – 0.74 x LLN	50 – 74 mg/dL *0.50 – 0.74 g/L* OR 0.25 – 0.49 x LLN	< 50 mg/dL *< 0.50 g/L* OR < 0.25 x LLN OR Associated with gross bleeding

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE THREATENING**
Hemoglobin (Hgb) **Comment:** The Hgb values in mmol/L have changed because the conversion factor used to convert g/dL to mmol/L has been changed from 0.155 to 0.6206 (the most commonly used conversion factor). For grading Hgb results obtained by an analytic method with a conversion factor other than 0.6206, the result must be converted to g/dL using the appropriate conversion factor for that lab.				
Adult and Pediatric ≥ 57 days (HIV <u>POSITIVE</u> ONLY)	8.5 – 10.0 g/dL *5.24 – 6.23 mmol/L*	7.5 – 8.4 g/dL *4.62–5.23 mmol/L*	6.50 – 7.4 g/dL *4.03–4.61 mmol/L*	< 6.5 g/dL *< 4.03 mmol/L*
Adult and Pediatric ≥ 57 days (HIV <u>NEGATIVE</u> ONLY)	10.0 – 10.9 g/dL *6.18 – 6.79 mmol/L* OR Any decrease 2.5 – 3.4 g/dL	9.0 – 9.9 g/dL *5.55 - 6.17 mmol/L* OR Any decrease 3.5 – 4.4 g/dL	7.0 – 8.9 g/dL *4.34 - 5.54 mmol/L* OR Any decrease ≥ 4.5 g/dL	< 7.0 g/dL *< 4.34 mmol/L*
Comment: The decrease is a decrease from baseline				
International Normalized Ratio of prothrombin time (INR)	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 x ULN	> 3.0 x ULN
Methemoglobin	5.0 – 10.0%	10.1 – 15.0%	15.1 – 20.0%	> 20.0%
Prothrombin Time (PT)	1.1 – 1.25 x ULN	1.26 – 1.50 x ULN	1.51 – 3.00 x ULN	> 3.00 x ULN
Partial Thromboplastin Time (PTT)	1.1 – 1.66 x ULN	1.67 – 2.33 x ULN	2.34 – 3.00 x ULN	> 3.00 x ULN
Platelets, decreased	100,000 – 124,999/mm^3 *100.000 x 10^9 – 124.999 x 10^9/L*	50,000 – 99,999/mm^3 *50.000 x 10^9 – 99.999 x 10^9/L*	25,000 – 49,999/mm^3 *25.000 x 10^9 – 49.999 x 10^9/L*	< 25,000/mm^3 *< 25.000 x 10^9/L*
WBC, decreased	2,000 – 2,500/mm^3 *2.000 x 10^9 – 2.500 x 10^9/L*	1,500 – 1,999/mm^3 *1.500 x 10^9 – 1.999 x 10^9/L*	1,000 – 1,499/mm^3 *1.000 x 10^9 – 1.499 x 10^9/L*	< 1,000/mm^3 *< 1.000 x 10^9/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
CHEMISTRIES ***Standard International Units are listed in italics***				
Acidosis	NA	pH < normal, but ≥ 7.3	pH < 7.3 without life- threatening consequences	pH < 7.3 with life- threatening consequences
Albumin, serum, low	3.0 g/dL – < LLN *30 g/L – < LLN*	2.0 – 2.9 g/dL *20 – 29 g/L*	< 2.0 g/dL *< 20 g/L*	NA
Alkaline Phosphatase	1.25 – 2.5 x ULN†	2.6 – 5.0 x ULN†	5.1 – 10.0 x ULN†	> 10.0 x ULN†
Alkalosis	NA	pH > normal, but ≤ 7.5	pH > 7.5 without life- threatening consequences	pH > 7.5 with life- threatening consequences
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Bicarbonate, serum, low	16.0 mEq/L – < LLN *16.0 mmol/L – < LLN*	11.0 – 15.9 mEq/L *11.0 – 15.9 mmol/L*	8.0 – 10.9 mEq/L *8.0 – 10.9 mmol/L*	< 8.0 mEq/L *< 8.0 mmol/L*
Comment: Some laboratories will report this value as Bicarbonate (HCO_3) and others as Total Carbon Dioxide (CO_2). These are the same tests; values should be graded according to the ranges for Bicarbonate as listed				
Bilirubin (Total)				
Adult and Pediatric > 14 days	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
Calcium, serum, high				
Adult and Pediatric ≥ 7 days	10.6 – 11.5 mg/dL *2.65 – 2.88 mmol/L*	11.6 – 12.5 mg/dL *2.89 – 3.13 mmol/L*	12.6 – 13.5 mg/dL *3.14 – 3.38 mmol/L*	> 13.5 mg/dL *> 3.38 mmol/L*
Calcium, serum, low				
Adult and Pediatric ≥ 7 days	7.8 – 8.4 mg/dL *1.95 – 2.10 mmol/L*	7.0 – 7.7 mg/dL *1.75 – 1.94 mmol/L*	6.1 – 6.9 mg/dL *1.53 – 1.74 mmol/L*	< 6.1 mg/dL *< 1.53 mmol/L*
Comment: Do not adjust Calcium, serum, low or Calcium, serum, high for albumin				

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Cardiac troponin I (cTnI)	NA	NA	NA	Levels consistent with myocardial infarction or unstable angina as
Cardiac troponin T (cTnT)	NA	NA	NA	≥ 0.20 ng/mL OR Levels consistent with myocardial infarction or unstable angina as
Cholesterol (fasting)				
Adult ≥ 18 years	200 – 239 mg/dL *5.18 – 6.19 mmol/L*	240 – 300 mg/dL *6.20 – 7.77 mmol/L*	> 300 mg/dL *> 7.77 mmol/L*	NA
Creatine Kinase	3.0 – 5.9 x ULN†	6.0 – 9.9 x ULN†	10.0 – 19.9 x ULN†	≥ 20.0 x ULN†
Creatinine	1.1 – 1.3 x ULN†	1.4 – 1.8 x ULN†	1.9 – 3.4 x ULN†	≥ 3.5 x ULN†

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Glucose, serum, high				
Nonfasting	116 – 160 mg/dL *6.44 – 8.88 mmol/L*	161 – 250 mg/dL *8.89 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75*	> 500 mg/dL *> 27.75 mmol/L*
Fasting	110 – 125 mg/dL *6.11 – 6.94 mmol/L*	126 – 250 mg/dL *6.95 – 13.88 mmol/L*	251 – 500 mg/dL *13.89 – 27.75*	> 500 mg/dL *> 27.75 mmol/L*
Glucose, serum, low				
Adult and Pediatric ≥ 1 month	55 – 64 mg/dL *3.05 – 3.55 mmol/L*	40 – 54 mg/dL *2.22 – 3.06 mmol/L*	30 – 39 mg/dL *1.67 – 2.23 mmol/L*	< 30 mg/dL *< 1.67 mmol/L*
Lactate	ULN - < 2.0 x ULN without acidosis	≥ 2.0 x ULN without acidosis	Increased lactate with pH < 7.3 without life-	Increased lactate with pH < 7.3 with life- threatening

Comment: Added ULN to Grade 1 parameter				
LDL cholesterol (fasting)				
Adult ≥ 18 years	130 – 159 mg/dL *3.37 – 4.12 mmol/L*	160 – 190 mg/dL *4.13 – 4.90 mmol/L*	≥ 190 mg/dL *≥ 4.91 mmol/L*	NA
Lipase	1.1 – 1.5 x ULN	1.6 – 3.0 x ULN	3.1 – 5.0 x ULN	> 5.0 x ULN
Magnesium, serum, low	1.2 – 1.4 mEq/L *0.60 – 0.70 mmol/L*	0.9 – 1.1 mEq/L *0.45 – 0.59 mmol/L*	0.6 – 0.8 mEq/L *0.30 – 0.44 mmol/L*	< 0.60 mEq/L *< 0.30 mmol/L*
Pancreatic amylase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Phosphate, serum, low				
Adult and Pediatric > 14 years	2.5 mg/dL – < LLN *0.81 mmol/L – < LLN*	2.0 – 2.4 mg/dL *0.65 – 0.80 mmol/L*	1.0 – 1.9 mg/dL *0.32 – 0.64 mmol/L*	< 1.00 mg/dL *< 0.32 mmol/L*
Potassium, serum, high	5.6 – 6.0 mEq/L *5.6 – 6.0 mmol/L*	6.1 – 6.5 mEq/L *6.1 – 6.5 mmol/L*	6.6 – 7.0 mEq/L *6.6 – 7.0 mmol/L*	> 7.0 mEq/L *> 7.0 mmol/L*
Potassium, serum, low	3.0 – 3.4 mEq/L *3.0 – 3.4 mmol/L*	2.5 – 2.9 mEq/L *2.5 – 2.9 mmol/L*	2.0 – 2.4 mEq/L *2.0 – 2.4 mmol/L*	< 2.0 mEq/L *< 2.0 mmol/L*
Sodium, serum, high	146 – 150 mEq/L *146 – 150 mmol/L*	151 – 154 mEq/L *151 – 154 mmol/L*	155 – 159 mEq/L *155 – 159 mmol/L*	≥ 160 mEq/L *≥ 160 mmol/L*
Sodium, serum, low	130 – 135 mEq/L *130 – 135 mmol/L*	125 – 129 mEq/L *125 – 129 mmol/L*	121 – 124 mEq/L *121 – 124 mmol/L*	≤ 120 mEq/L *≤ 120 mmol/L*
Triglycerides (fasting)	NA	500 – 750 mg/dL *5.65 – 8.48 mmol/L*	751 – 1,200 mg/dL *8.49 – 13.56 mmol/L*	> 1,200 mg/dL *> 13.56 mmol/L*

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Uric acid	7.5 – 10.0 mg/dL *0.45 – 0.59 mmol/L*	10.1 – 12.0 mg/dL *0.60 – 0.71 mmol/L*	12.1 – 15.0 mg/dL *0.72 – 0.89 mmol/L*	> 15.0 mg/dL *> 0.89 mmol/L*
URINALYSIS ***Standard International Units are listed in italics***				
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Proteinuria, 24 hour collection				
Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h	2,000 – 3,500 mg/24 h	> 3,500 mg/24 h *> 3.500 g/d*

Appendix 5.
Fecal Microbiota Transplantation - Record of Side Effects

This diary is one way researchers will get information from you regarding any possible problems or side effects in this study.

- **What you are going to do is simple.** Just keep a record of any unpleasant thing that happens to you while you are in the study, before, during, and after we have completed the stool transplant. We even want you to record things that do not seem to be part of the stool therapy, at all.
- **When do you start? When do you end?** You will record one entry 1-week prior and on the day of the transplant. You will then complete one entry per day for the first week following the treatment and then once a week thereafter for 12-weeks.
- **What do you look for? What do you report?** Any symptom or problem whether or not it may be from the medicine, stool therapy. This could include: fever, abdominal pain, a big belly, lots of gas, diarrhea, nosebleeds, and anything else you know is not quite right.
- **What will you do?** In the first 7 days after the transplant, you will report some of the specific things that have bothered you by checking the boxes in the diary (see below). You can also write any other problems that you may have had during that time. **Additionally, you will record your temperature once for each day for the first 7 days after the transplant, unless you feel hot. If you feel hot, please take your temperature again.** Please make sure to record the highest temperature taken that day if you take it more than once.

  Continue to record any problem up to 6 months after the transplant.

How will you record it? Like this…

EVENT	DATE OF ONSET	INTENSITY	ACTION TAKEN	MEDICATION	DATE RESOLVED
Fever	3/1/12	3	Missed 2 days of school	Tylenol-200mg	3/3/12
Sore throat	3/5/12	1	None	None	3/6/12

OTHER SYMPTOMS
Record each symptom at its ***worst*** level for each day.
For example, a sore throat that starts at 'Grade 1" but increases to 'Grade 2' should be recorded as 'Grade 2".

Examples of Grades:
Grade 1 – Mild: I noticed the symptom. It did not keep me from doing my normal activities.
Grade 2 – Moderate: I noticed the symptom and it kept me from doing some of my normal activities.
Grade 3 – Severe: I really noticed the symptom and it kept me from doing activities that I wanted or needed to do.
Grade 4 – Very severe: The symptom made me unable to perform basic self-care functions such as washing myself **OR** medical or surgical intervention was needed to prevent serious consequences.

Subject ID:_____

Date:____/____/_____ Check here is no side effects present: ☐
Highest temperature of the day:_____°F Total Number of Stools:_____

Check if symptom present	Event	Date of Onset	Intensity	Action taken	Medications	Date Resolved
☐	Fever					
☐	Abdominal Pain/					
☐	Diarrhea					
☐	Nausea/Vomiting					
☐	Blood in Stool					
☐	Other 1					
☐	Other 2					
☐	Other 3					

Grade 1 – Mild: I noticed the symptom. It did not keep me from doing my normal activities.
Grade 2 – Moderate: I noticed the symptom and it kept me from doing some of my normal activities.
Grade 3 – Severe: I really noticed the symptom and it kept me from doing activities that I wanted or needed to do.
Grade 4 – Very severe: The symptom made me unable to perform basic self-care functions such as washing myself **OR** medical or surgical intervention was needed to prevent serious consequences.

Appendix 6.

Sensormedics Vmax Encore 29(VIASYS Respiratory Care Inc.,Yorba Linda, CA)

Indirect Calorimetry Measurements information use in protocols.

During a metabolic study we are measuring Resting Energy Expenditure (REE) or the number of calories being burned at rest per day. Depending on body size, a healthy adult may burn from 1000 to 3000 Kcal per day just to maintain normal body functions. Traditionally, predicted equations were used to estimate REE based on age, height, and weight. Unfortunately these equations do not account for stress factors that could be affecting the patient (fever, burns, disease, medications, etc.).

Indirect Calorimetry calculates heat production by measuring gas exchange; in other words, oxygen consumption and carbon dioxide production. During resting conditions food sources are broken down to produce energy (heat or KCALS). The oxygen consumed and the carbon dioxide produced are measured to provide an indirect assessment of caloric expenditure. REE provides the total energy expenditure in 24 hours and is calculated from the gas exchange data (VO2, VCO2) collected by the Vmax. The parameter RQ will assist in determining subject's caloric needs. RQ provides information on what the subject is utilizing for energy; carbohydrates, fats, or proteins.

$$RQ = VCO2/VO2$$

Carbohydrates .90 - 1.00

Fats .70 - .78

Proteins .80

Combination Fats/Carbohydrates .80 - .82

RQ greater than 1.00 – 1.20 may indicate overfeeding (Lipogenesis)

RQ less than .70 may indicate underfeeding (Ketogenesis)

NOTE: Hyperventilation can cause the RQ to exceed 1.00

Testing Procedure:

Energy expenditure (EE), respiratory quotient (RQ) and substrate utilization will be calculated from measurements of oxygen (VO2) and carbon dioxide (VCO2) in inspired and expired air .The fasted subject (12 hours prior to testing) is placed in a supine position in a quiet environment where subject will be undisturbed. Subject is also fully rested and has not performed an exercise prior to testing. The Sensormedics Vmax Encore equipment

(VIASYS Respiratory Care Inc.,Yorba Linda, CA) is use to collect measurements at specified study times.

A clear plastic canopy is placed over the subject head and torso with the pump turned on. FECO2 is monitored the pump is adjusted and stabilized within desired range of 0.75-0.85. Once FECO2 is within range, test will begin and data will be collected for 30 minutes. During the study subject should refrain from sleeping. The goal of the test is to obtain Resting Energy Expenditure (REE), therefore the first five minutes are not accounted for in final analysis, to allow patient to meet a steady state condition. Once test is completed the canopy is removed from subject and pump is turned off. In order to accurate info on RQ substrate utilization for the subject, a 24 hour Urinary N2 test must be collected prior to Vmax test. Data from 24 hour urinary N2 test will be entered into computer to be analyzed with results from the Vmax indirect calorimetry test to obtain RQ substrate unitization and REE.

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