FULL TITLE: Fecal microbiota transplantation to reduce immune activation in ART-treated people living with HIV with low CD4/CD8 ratio: The Gutsy study

Protocol No. CTNPT 038

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GCP Statement

This clinical study will be conducted in accordance with applicable Health Canada regulations, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines on current Good Clinical Practice (GCP), and the Declaration of Helsinki.

Confidentiality Statement

This clinical study protocol contains information which is of a confidential, trade-secret or proprietary nature. The protocol is for the use of Dr. Jean-Pierre Routy and his designated representatives participating in the investigational trial. It is not to be disclosed to any other person or party without the prior written approval of Dr. Jean-Pierre Routy.

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INVESTIGATOR AGREEMENT

Protocol Title:	Fecal microbiota transplantation to reduce immune activation in ART-treated people living with HIV with low CD4/CD8 ratio: The Gutsy study
Protocol No.:	CTNPT 038
Version No.:	2.0
Date:	15 January 2024

This clinical study will be conducted in accordance with applicable Health Canada regulations, ICH guidelines on current GCP, and the Declaration of Helsinki.

I confirm that I have read and understand this protocol and I agree to conduct this clinical study in accordance with the design and specific provisions of the protocol, with the exception of a change intended to eliminate an immediate hazard to participants. Any deviation from the study protocol will be documented in the case report form.

I agree to promptly report to the applicable ethics boards any changes in the research activity and all unanticipated problems involving risks to human participants or others. Additionally, I will not make any changes in the research without prior ethics and sponsor approval, except where necessary to ensure the safety of study participants.

Name	Signature	Date (dd-mmm-yyyy)

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ABBREVIATIONS AND DEFINITIONS

Acronym/Abbreviation	Definition
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine transaminase
ART	Antiretroviral Therapy
AST	Aspartate transaminase
CBC	Complete Blood Count
CCR	C-C Chemokine Receptor
CD	Cluster of Differentiation
CHUM	Centre hospitalier de l'université de Montréal
CIHR	Canadian Institutes of Health Research
CMV	Cytomegalovirus
CRE	ESBL producing Enterobacteriaceae
CRP	C-Reactive Protein
CTA	Clinical Trial Application
CTN	CIHR Canadian HIV Trials Network
CVIS	Chronic Viral Illness Service
DNA	Deoxyribonucleic acid
eCRF	Electronic Case Report Form
EIA	Enzyme Immune Assay
ELISA	Enzyme-linked Immunosorbent Assay
ESBL	Extended Spectrum Beta-Lactamase
FDA	Food and Drug Administration
FMT	Fecal Microbiota Transplantation
HBV	Hepatitis B Virus
HC	Health Canada
hCG	human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus
HLA-DR	Human leukocyte antigen-DR
hs-CRP	High-sensitivity C-reactive protein

Acronym/Abbreviation	Definition
IBD	Irritable bowel disease
ICJME	International Committee of Medical Journal Editors
I-FABP	Intestinal-Fatty Acid Binding Protein
IFN-γ	Interferon-gamma
IHMS	International Human Microbiota Standard
IL	Interleukin
IP-10	Interferon-gamma induced protein-10
LBP	Lipopolysaccharide Binding Protein
LDL	Low Density Lipoprotein
LNHPD	Licensed Natural Health Product Database
LPS	Lipopolysaccharide
mAbs	monoclonal antibodies
MRSA	Methicillin-Resistant Staphylococcus Aureus
MUHC	McGill University Health Centre
NAFLD	Non-Alcoholic Fatty Liver Disease
NK	Natural Killer (Cell)
PBMC	Peripheral Blood Mononuclear Cell
PD-1	Programmed Cell Death – 1
PLWH	People Living with HIV
qPCR	Quantitative Polymerase Chain Reaction
REB	Research Ethics Board
REG3α	Regenerative Islet-derived protein 3 α
SAE	Serious Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SIV	Simian Immunodeficiency Virus
SJHC	St. Joseph's Health Care London Hospital
sST2	Soluble Suppression of Tumorigenicity 2
Th17	T helper cell 17 (population of T cells)
TILDA	Tat/Rev Induced Limiting Dilution Assay
TNF-α	Tumor Necrosis Factor-alpha

PROTOCOL SYNOPSIS

Full Title	Fecal microbiota transplantation to reduce immune activation in ART-treated people living with HIV with low CD4/CD8 ratio: The Gutsy study
Short Title	The Gutsy Pilot Study
Protocol and Version No.	CTNPT 038, Version 2.0
Clinical Phase	Phase II
Study Duration	Enrollment period: March 2024 – April 2025
	Study period: March 2024 – April 2026
Sponsor-Investigator	Dr. Jean-Pierre Routy
Number of Centres	1
Study Design	Single-blind, randomized, placebo-controlled, interventional study
Primary Objective	To evaluate changes in plasma levels of the marker of gut permeability REG3α before and after FMT compared to placebo
Secondary Objectives	To assess, before and after FMT, compared to the placebo arm: - Safety and tolerability of FMT - Changes in plasma levels of gut damage markers - Changes in plasma levels of pro-inflammatory markers and anti-inflammatory markers - Changes in T and myeloid cell activation in blood
Exploratory Objectives	To assess, before and after FMT, compared to the placebo arm: - Changes in microbiota composition and diversity in stools - Changes in HIV reservoir size in blood and gut mucosa by PCR
Sub-study Objectives	To assess, before and after FMT, compared to the placebo arm: - The gut mucosa architecture in a subset of participants agreeing to have colon biopsies before and 12 weeks after the first FMT/placebo. - Changes in inflammatory cytokines by qPCR as well as T and myeloid cell activation in colon biopsies - Changes in HIV reservoir size in colon CD4 T-cells
Sample Size	N = 20
Randomization	1:1
Study Population	• Male or female, ≥18 years of age • Diagnosed HIV infection • Treated with ART for at least 3 years, on the same stable ART regimen for at least three months • Viral load suppressed to <50 copies/mL for at least 3 years (viral blips below 200 copies/ml are allowed) • CD4 count >200 and CD4/CD8 ratio <1
Investigational Products Description	FMT capsules containing 80-100g of healthy donor stool processed and encapsulated in gelatin capsules
Control	Placebo capsules containing microcrystalline cellulose, for equivalence in weight and color.
Administration and Dosing	At Day 0 and Day 22: Participants will take orally 30 to 40 FMT capsules or placebo capsules.
Outcome Measures	**Primary:** Changes in plasma REG3α levels will be measured as markers of gut permeability

	Secondary: - Safety and tolerability of FMT measured by evaluating adverse events, hematology, and serum chemistries over the course of the study. These evaluations will include HIV viral load, glucose levels, a lipid profile and plasma levels of hsCRP and D-dimer. - Changes in plasma levels of gut damage markers including I-FABP and LPS. - Changes in Plasma levels of pro-inflammatory markers ((1-3)-β-D-Glucan, IL-1β, IL-6, IL-8. IP-10, IL-17A and F, IL-22) and anti-inflammatory markers (IL-10, IL-37) will be measured by Multiplex or ELISA. - Changes in T and myeloid cell activation in blood, as assessed by flow cytometry expression of HLA-DR and CD38, and CD83 and CD86 respectively.
	Exploratory: - Changes in microbiota composition and diversity in stools, as assessed using 16s rDNA sequencing. - Changes in HIV reservoir size in blood and gut mucosa by PCR.
	Sub-study: - Composition and gut mucosa architecture in the colon sub-study at Baseline 2 (V3, day 0) versus after FMT or placebo treatment (V6, Week 12). Gut architecture will be monitored by immunostaining of the epithelial tight junctions (Claudin-3/Occludin)1. Myeloperoxidase staining will allow for quantification of inflammatory myeloid cells in the gut (monocytes and T cells). - Changes in inflammatory cytokines by qPCR as well as T and myeloid cell activation in colon biopsies, as assessed by flow cytometry expression of HLA-DR and CD38, and CD83 and CD86 respectively. - Changes in HIV reservoir size in colon CD4 T-cells as assessed by qPCR.
Statistical Analysis	Analyzed parameters will be compared to baseline for each participant and fold changes will be compared between the treated and the placebo group by t-test or non-parametric analysis such as Wilcoxon rank sum test as appropriate. 95% confidence interval of the estimated difference will be computed. Microbiota 16S rRNA diversity will be interpreted using high dimensional class comparisons via linear discriminant analysis of effect size (LEfSe) and volcano plot, as well as DESeq2 analyses.

STUDY FLOW CHART

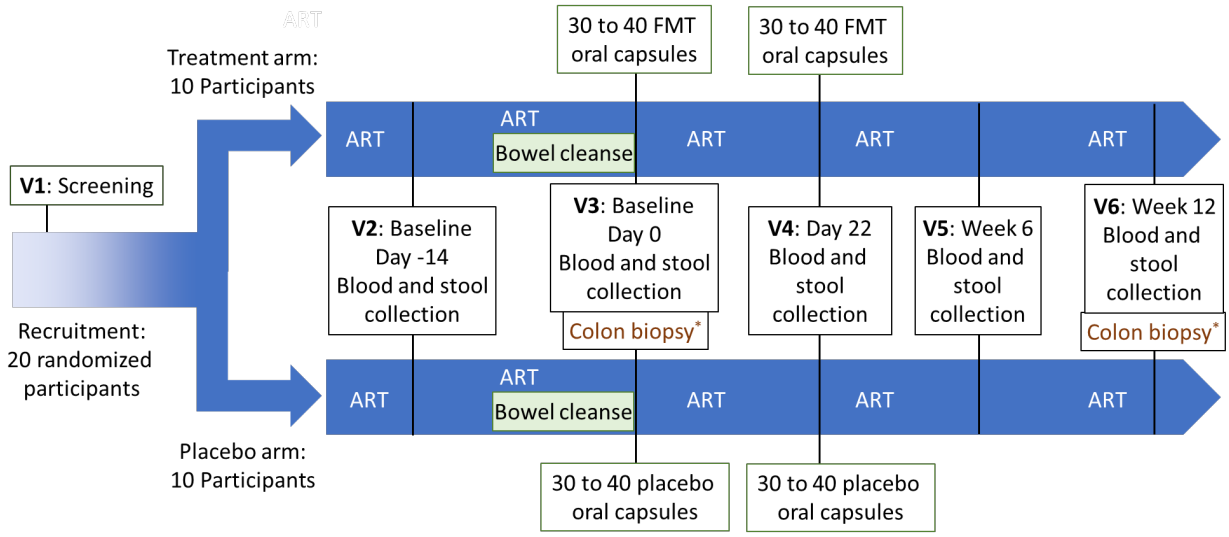


Figure 1: Flow Chart. Visit 1, the Screening visit, will take place up to 7 weeks prior to the Baseline 1 visit (Day -14 ±7 days, Visit 2). At the Screening visit, the informed consent document will be explained to the participant and will be signed prior to any screening and study activities. Two baseline visits will be conducted, at Day -14 and Day 0. All study visits will be relative to Baseline 2 (Day 0, Visit 3). Data collected at these two baseline visits will be directly compared to determine intra-patient variability. A bowel cleanse will be given to participants prior to the treatment visit (Visit 3). After the collection of blood and stool samples, two FMT treatment or placebo (30 to 40 capsules each) will be given to participants at Day 0 (Visit 3) and Day 22 (Visit 4). Blood and stool will be collected post-treatment at Week 6 and Week 12 (Visit 5 and Visit 6). See Section 9.1 Schedule of Events (Table 3) for more details.

* Optional gut biopsies will be taken for the sub-study at the indicated time points. The consent form for the optional gut biopsy will also be explained at the screening visit, but consent for this will not be necessary to be part of the main study.

1. INTRODUCTION, BACKGROUND, AND STUDY RATIONALE

1.1 Background

Antiretroviral therapy (ART) decreases Human Immunodeficiency Virus (HIV) plasma viral load below the limit of detection, leading to major improvements in the health of people living with HIV (PLWH). However, this people remain at risk for developing inflammatory non-AIDS events such as cardiovascular diseases, fatty liver, neurocognition dysfunction and cancer[1,2]. These non-AIDS events are associated with persistent immune activation and are more frequent when ART initiation is delayed, and increase with aging, co-infections like cytomegalovirus (CMV) and viral hepatitis as well as microbial translocation. It has been observed in HIV infection and colitis (or

inflammatory bowel disease (IBD)) that abnormal composition of the gut microbiota, alteration of the gut barrier, T-helper (Th) 17 cell dysfunction and microbial translocation lead to systemic inflammation and immune activation contributing to non-AIDS events[3-9]. Long-term ART only partially reduces inflammation and poorly replenishes Th17 function, highlighting the importance of research focusing on improving the gut microbiota and the epithelial barrier. Furthermore, persistent HIV infection in long-lived memory CD4+ (Clusters of Differentiation 4) T cells and likely macrophages also contribute to inflammation and microbial translocation, creating a vicious cycle nurturing inflammation. Importantly, the size of the HIV reservoir has been linked to the level of inflammation and immune activation measured in CD8+ T cells and macrophages[3,10]. It remains unknown whether the reduction of inflammation can lead to a decrease in the size of the HIV reservoir.

Gut damage and immune activation

HIV infection is characterized by functional impairment and increased CD4+ T cell death. If HIV is not fully cleared, persistent antigens and inflammatory signals impair antigen-specific responses. This overall activation leads to exhaustion of the immune system including non-specific T cells. This is particularly relevant as HIV replicates preferentially in the Th17 CD4+ T cells from the gut, inducing cell death and mucosa damage[11-13]. Microbial products penetrate the damaged gut barrier and pass into the systemic circulation. Such bacterial translocation contributes to systemic immune activation, disease progression, and development of non-AIDS events[14-17]. Markers of bacterial translocation, including lipopolysaccharide (LPS), LPS binding protein (LBP), and soluble CD14 (sCD14) have been correlated with immune activation and disease progression[3,11,18,19]. While bacterial translocation is thought to be a major cause of immune activation, we have shown that circulating glucan, a marker of fungal translocation, contributes to the immune activation in an LPS-independent manner[20].

Although ART suppresses HIV replication to undetectable levels, microbial translocation and Th17 damage remains, contributing to a persistent immune activation. Monocytes/ macrophages, Dendritic cells, and Natural Killer (NK) cells detect microbial products in the mesenteric lymph nodes and in systemic circulation and secrete pro-inflammatory cytokines (interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF)-α). These cytokines drive CD4+ T cell activation, leading to elevated expression of the HIV co-receptor C-C Chemokine receptor (CCR) 5 and the gut homing receptor CCR6[21]. CD4+ T cells expressing CCR6 have been shown by our group to be preferentially infected[12,13,22].

Gut microbiota, dysbiosis and immune regulation

The gut microbiota composition and metabolites play an important role in inflammation in obesity, diabetes, cancer and HIV infection. Its role includes food and metabolite processing, microbial regulation and immune regulation[23,24]. PLWH, compared to uninfected controls, present with a relative decrease of Firmicutes and more abundant Proteobacteria in their gut microbiota. *Lactobacilli* presence in stools is associated with higher CD4+ T cell count and a lower systemic immune activation. Dysbiosis, an altered gut microbial composition, combined with microbial translocation has been linked to non-

AIDS events in HIV-infected individuals and influences CD4+ T cell recovery on ART as reported by our group and others[3,14,18,25,26].

1.2 Summary of Previous Data with Fecal Microbiota Transplant (FMT)

In PLWH, the mucosal immune system is disturbed by HIV infection. Th17 and Th22 cells, important components of mucosal immunity, are rapidly depleted following HIV or simian immunodeficiency virus (SIV) infection, contributing to a reduced barrier integrity, microbial translocation, and systemic immune activation.[27-29] In a pilot study, Hensley-McBain *et al*. reported that FMT significantly increases the number of peripheral Th17 and Th22 cells and reduced CD4 T-cell activation in the gut in SIV-infected macaques receiving ART. Moreover, the transplant was well-tolerated and no side effects were observed.[30]

A pilot study in ART-suppressed individuals who received one-time FMT from stool bank via colonoscopy reported no serious adverse effects during the 24 weeks of follow-up.[31] Microbial engraftment occurred but was partial, and limited to specific bacterial taxa including an increase of Faecali bacterium[31], which has been shown to exert anti-inflammatory effects in murine experimental colitis.[28] The authors considered that the limited effects of FMT might be related to the single dose of FMT. Serrano-Villar *et al.* reported that repeated oral low dose FMT in capsules was one way to safely introduce incremental compositional changes into the gut microbiota in ART-treated PLWH.[32] Compared to placebo, FMT significantly decreased the gut damage marker I-FABP four weeks after initiating FMT. Furthermore, mild engraftment of the donor's microbiota persisted until week 36 after initiating FMT.[32]

Safety should be a main focus of any intervention in a clinical trial setting. Although concern exists on the safety of FMT administration, even more so in immunosuppressed recipients, PLWH with low CD4 T-cell count were shown to have the most profound modification of their gut microbiota and therefore would benefit greatly from FMT.[25] As reviewed by Shogbesan *et al.*,[33] FMT is successful in the treatment of recurrent *C. difficile* in immunocompromised patients including organ transplant recipients and PLWH. Encouragingly, FMT showed similar rates of adverse events in immunocompromised participants compared to immunocompetent ones including PLWH with CD4 counts lower than 200 cells/mm^3[3,34-37]. Additionally, Schunemann *et al.* showed that FMT increased CD4 counts in an individual with HIV.[38] To better assess the efficacy and safety of FMT, well-designed randomized controlled clinical trials are ongoing. However, large studies assessing the influence of FMT in PLWH are still needed.

1.3 Potential Risks and Benefits to Human Participants

1.3.1 Potential Risks Associated with Fecal microbiota transplant

Previous trials reported mild adverse events attributed to FMT including diarrhea, cramping, belching, abdominal pain, bloating, and transient fever. Despite a large body

of experience, transmission of unrecognized infectious diseases with our Health Canada prescribed donor screening guidelines is felt to be extremely rare and has not been described. The FMT process in this study will be delivered by oral capsule and may be uncomfortable for some participants.

Dr. Silverman's group, which will be supplying the FMT and placebo capsules, has extensive experience with the screening process for donor infusate[39]. Although FMT infusate has been screened for bacteria, viruses, fungi, and parasites, there is a risk of transmitting known and unknown infectious organisms from donor stool. Post-FMT bacteremia, sepsis, and fatal events very rarely occur.

In our experience, the most common complaints associated with FMT include loose stools, abdominal pain, and dizziness, largely consistent with the previously published data[40]. Observed AEs were mostly mild. Even in cases involving clinical cure of *C. difficile*, initial post-FMT stool consistency may be loose and take weeks for the bowel pattern to normalize[41]. It is however felt that these symptoms are usually the result of long-term complications of *C. difficile* and resultant irritable bowel rather than complications of FMT itself. Reporting on long-term adverse effects of FMT for indications other than *C. difficile* is still limited. However, there is a possibility of as-yet-unknown risks associated with FMT given the complex interplay between transplanted microbiota and the recipient bowel[42-44]. However, this risk is likely low and mitigated by the potential benefit of transplantation in patients with known resistant atherosclerosis. Possible allergy and/or anaphylaxis to antigens in donor stool; theoretical risk of developing disease possibly related to donor gut microbiota including but not limited to: obesity, metabolic syndrome, cardiovascular disease, autoimmune conditions, allergic/atopic disorders, neurologic disorders, psychiatric conditions, and malignancy.

1.3.2 Potential Risks to Blood Draws

The risks of drawing blood from a vein include: discomfort (often momentary) at the blood draw site; possible bruising, redness, swelling, and/or bleeding at the site; feeling of lightheadedness when blood is drawn; and rarely an infection at the site where the blood was drawn (cleaning of the site prior to blood draw should prevent this from happening).

1.3.3 Potential risks and/or discomforts to Colon mucosal biopsies

Participants at the McGill University Health Centre (MUHC), Montréal site will be invited to participate in an optional sub-study to collect colon mucosal biopsy. They will be asked to sign a separate consent form if they wish to take part in the sub-study.

The day before each colon mucosal biopsy, the consenting participant will be required to take measures to clear his/ her bowels for the examination by drinking a picosulfate based solution and magnesium citrate as enunciated in the colonoscopy information preparation sheet. The information sheet addendum will be given to each participant referring side effects, risks, contraindications, and the medication interactions for the two magnesium-based products used to clean their bowels.

During the colon mucosal biopsy procedure, the participant may feel pressure in the rectum similar to the sensation felt with the urge to have a bowel movement. The participant may also feel a small amount of abdominal cramping. The most serious risk of the procedure is perforation, i.e. poking a hole in the lining of the intestine; this risk is extremely uncommon, occurring once out of every 10,000 procedures. Some bleeding will likely occur at the place where the biopsies are done. If the participant experiences severe discomfort during the colon mucosal biopsies, the procedure will be stopped immediately; he/ she will be seen by a physician (gastro-enterologist or investigator) and the research nurse will monitor him/ her until he/ she is ready to go home.

1.3.4 Benefits to Participating in This Study

We do not know whether taking part in this study will be of direct benefit to the participant. Based on previous studies, FMT may decrease chronic inflammation, modulate gut microbe composition, ameliorate gut barrier integrity, or aid in CD4 T cell recovery during the study. However, it is unknown if this will result in a marked improvement to the participant's health. Findings from this study may help us to improve treatments for PLWH. The study's results will be published and all participants will be given access to the study findings.

1.4 Study Rationale

PLWH on ART experience increased inflammation putting them at risk for inflammation-related diseases including cardiovascular diseases, fatty liver, neurocognition dysfunction and cancer[1]. Persistent inflammation has been associated with dysbiosis, an unbalanced composition of the gut microbiota. Therefore, we wish to evaluate if modification of the gut microbiota can positively affect HIV-infected patients on ART in terms of reduced inflammation, improved gut microbiota and potentially decreased HIV latent reservoir.

2. STUDY OBJECTIVES AND DESIGN

2.1 Overall Study Design

This single-blind, randomized, placebo-controlled, interventional study will enroll 20 PLWH participants. These participants will have received ART for at least 3 years, on the same stable ART regimen for at least 3 months, have had undetectable viral load for at least 3 years, and have a CD4 count between greater than 200 cells/μL of blood and a CD4:CD8 ratio below 1 to select people with higher risks of inflammatory non-AIDS comorbidities and dysbiosis. Participants will be randomized 1:1 to receive FMT or placebo capsules. Study participants will undergo a bowel cleanse and will then receive two doses of FMT or placebo taken orally 22 days apart. The comparison between the two groups will be made by evaluating the outcome measures at each visit: two baselines to assess intraparticipant variability, intervention (Day 0 and Day 22); and post-intervention (3 and 9 weeks after FMT/placebo treatment).

Participants at the Montreal site will have the option to participate in a sub-study that will evaluate gut mucosa architecture, inflammation and reservoir size using colon biopsies performed before (Baseline 2) and at the end of the study, 9 weeks after the 2nd dose of FMT or placebo (Week 12).

2.2 Study hypothesis

FMT will counteract gut dysbiosis, increasing the abundance of *A. muciniphila* and other beneficial microorganisms, and reduce epithelial damage, microbial translocation, and inflammation in ART treated participants.

2.3 Primary Objective

To evaluate changes in plasma levels of the marker of gut permeability REG3α before and after FMT compared to placebo.

2.4 Secondary Objectives

To assess, before and after FMT, compared to the placebo arm:

1. Safety and tolerability of FMT measured by evaluating adverse events, hematology, serum chemistries and stool classification (Bristol chart) over the course of the study. These evaluations will include HIV viral load, glucose levels, a lipid profile and plasma levels of hsCRP and D-dimer.
2. Changes in plasma levels of gut damage markers including I-FABP and LPS.
3. Changes in plasma levels of pro-inflammatory markers ((1-3)-β-D-Glucan, IL-1β, IL-6, IL-8. IP-10, IL-17A and F, IL-22) and anti-inflammatory markers (IL-10, IL-37).
4. Changes in T and myeloid cell activation in blood, as assessed by flow cytometry expression of HLA-DR and CD38, and CD83 and CD86 respectively

2.5 Exploratory Objective

To assess, before and after FMT, compared to the placebo arm:

1. Changes in microbiota composition and diversity in stools, as assessed using 16s rDNA sequencing.
2. Changes in HIV reservoir size in blood and gut mucosa by PCR

2.6 Sub-study Objective

To assess, before and after FMT, compared to the placebo arm:

1. The gut mucosa architecture in a subset of participants agreeing to have colon biopsies before and 12 weeks after the first FMT/placebo.
2. Changes in inflammatory cytokines by qPCR as well as T and myeloid cell activation in colon biopsies, as assessed by flow cytometry expression of HLA-DR and CD38, and CD83 and CD86 respectively.
3. Changes in HIV reservoir size in colon CD4 T-cells as assessed by qPCR.

3. SELECTION AND ENROLLMENT OF PARTICIPANTS

3.1 Number of Participants

A total of 20 PLWH participants will be enrolled at the Chronic Viral Illness Service (CVIS) at the McGill University Health Centre, Glen Site, Montreal, QC.

3.2 Inclusion Criteria

Participants will be eligible for the study if they meet the following criteria:

1. Male or female adults ≥18 years of age.
2. Documented HIV-1 infection by Western Blot, Enzyme Immuno Assay (EIA) or viral load assay.
3. On ART for at least 3 years, and stable ART regimen (same prescription) for at least 3 months.
4. Undetectable viral load < 50 copies/ml for the past 3 years. Viral blips below 200 copies/ml, are allowed if preceded and followed by a HIV viremia below 50 copies/ml.
5. CD4 count greater than 200 cells/μL and a CD4/CD8 ratio below 1 to select people with higher risks of inflammatory non-AIDS comorbidities and dysbiosis.
6. Able to communicate adequately in either French or English.
7. Able and willing to provide written informed consent prior to screening.
8. Women of childbearing potential must have a negative serum pregnancy test at Screening
9. Women of childbearing potential must agree to use one of the following approved methods of birth control while in the study and until 2 weeks after completion of the study (See Section 7.1):
    a. Complete abstinence from penile-vaginal intercourse from the screening period until 2 weeks after study completion.
    b. Double barrier method (acceptable barrier methods include diaphragm, coil, contraceptive foam, sponge with spermicide, or condom).
    c. Oral, injectable or implant contraceptives plus one barrier method.
    d. Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year (not all IUDs meet this criterion) plus one barrier method.
    e. Male partner sterilization confirmed prior to the female participant's entry into the study; this male is the sole partner for that participant.
    f. Approved hormonal contraception, started at least 30 days before screening, preferably with one barrier method.
    g. Another method approved by the Investigator with published data showing that the expected failure rate is <1% per year preferably with one barrier method.
10. Women of non-child-bearing potential as defined as either post-menopausal (12 months of spontaneous amenorrhea and ≥ 45 years of age) or physically incapable

of becoming pregnant with documented tubal ligation, hysterectomy or bilateral oophorectomy.

11. Sexually active men with a female partner of childbearing potential must agree to one of the following methods of birth control (See Section 7.1):
    a. The use of at least one barrier method of contraception (e.g. condom) with a female partner using a second approved method of contraception (IUD, hormonal contraceptive pill, diaphragm, spermicide, etc.) during the study and until two weeks after study completion.
    b. Have had a successful vasectomy.
    c. Be confirmed sterile.

Any contraception method must be used consistently, in accordance with this study protocol, and for the duration of the study until two weeks after study completion.

3.3 Exclusion Criteria

Participants are not eligible to participate in the study if they meet any of the following conditions:

1. Known allergy/hypersensitivity Polyethylene glycol.
2. Current AIDS-related event or serious health condition including systemic infections in the last 3 months.
3. Severe systemic diseases (e.g. uncontrolled hypertension, chronic renal failure), or active uncontrolled infections.
4. Co-infection with active Hepatitis B or C Virus.
5. Current use or have used in the past 3 months: immune-modulatory agents, prophylactic antibiotics[45]/antibiotics, or Morphine as these drugs modulate gut microbiota.
6. Diagnosis of diabetes mellitus (HbA1c≥6.5%) as defined by the Canadian Clinical Practice Guidelines for the Prevention and Management of Diabetes[46].
7. Recent changes in dietary habits, intermittent fasting, chronic constipation or laxative use as these can affect gut microbiota.
8. Psychiatric or cognitive disturbance or any illness that could preclude compliance with the study.
9. Current participation in an experimental therapy study or receipt of experimental therapy within the last 6 months.
10. Women who are planning to become or who are pregnant, or breast-feeding.
11. A score of higher than 8 on a Full AUDIT questionnaire (See Appendix 2) at the screening visit, suggesting an alcohol abuse problem.

3.4 Strategies for Recruitment

Participants will be recruited at the McGill University Health Centre in Montréal, QC, under the supervision of Dr. Jean-Pierre Routy. The Qualified Investigator works in medical clinics that provide care to more than 2000 HIV-infected persons. Teleconferences and face-to-face meetings will be organized between the Qualified Investigator and study staff to help promote participant recruitment and follow-up during the study. The Sponsor-Investigator and Qualified Investigator will promote the Gutsy Study when attending or

presenting at local meetings and HIV-related conferences such as the CTN semi-annual meetings. In these presentations the problems associated with inflammation during HIV-infection will be discussed as well as innovative therapies to alleviate inflammation, such as FMT. These talks will target primary care physicians that treat PLWH, other infectious diseases physicians, and PLWH community/advocacy groups in the Montréal area where the study will be conducted.

3.5 Enrollment Procedures

Study staff will provide information about the study and procedures to prospective participants and ask about their general health. Study staff will review inclusion and exclusion criteria. Participants will be informed that their participation in the study is voluntary and confidential and that they can withdraw from the study at any time. If interested, the participant will be scheduled for a screening visit. At the screening visit, study staff will make sure the participant understands the study, including the risks, benefits and procedures, and will review the informed consent form. If willing to participate, the participant will be asked to sign the informed consent document for the main study. The participant will then proceed to the screening and enrollment activities.

3.6 Sub-study Enrollment Procedures

There will be an optional colon biopsy sub-study. Participants recruited will be given the option to participate in this sub-study, but only after giving informed consent to participate in the main study and being shown to be eligible for the main study (after screening). Participants cannot only participate in the sub-study. The sub-study will have a separate informed consent document.

3.7 Co-enrollment Guidelines

Participants enrolled in this study must not be enrolled in other experimental therapy studies for the duration of this study. However, participants can enroll in other qualitative studies such as survey research that do not involve an investigational product.

4. WITHDRAWAL OF PARTICIPANTS

4.1 Withdrawal criteria

Participants are free to withdraw from the study at any time and for whatever reason, specified or unspecified, and without prejudice to his or her medical care by a physician. A participant may be withdrawn from the study due to the reasons listed below including, but not limited to:

- Participant’s request.
- Investigator’s request.
- Sponsor-Investigator’s request.

- Safety reasons (e.g. Serious adverse drug reactions, potential significant drug interaction).
- Pregnancy or desire to become pregnant during the study period.
- Participant non-compliance with study procedures.
- Participant's use of, or need for, concomitant therapy liable to interfere with the interpretation of study endpoints. The Investigator will report all such information on the electronic Case Report Form (eCRF) and decide whether the participant is to be withdrawn.
- Loss to follow-up. The Investigator will attempt to reach the participant before considering the participant lost-to-follow-up. These actions will be reported on the appropriate page of the eCRF and in the participant's file.

Participants that are withdrawn before the second round of FMT or placebo capsules (Visit 4) will be replaced to ensure a minimum of 20 participants complete the study.

4.2 Procedures for Discontinuation

If a participant withdraws or is removed from the study for any reason prior to the completion of the study, the reason for, date of the discontinuation, and the date of the last dose of the study medication must be recorded in the participant's file and the appropriate section of the eCRF.

At the time of discontinuation, every effort should be made to ensure that:

1. Procedures and evaluations scheduled for the Final Visit (Visit 6) are performed, including the assessment of AEs. If the participant discontinues the study within 15 days prior to either Day 22, Week 6 or Week 12 visits, the laboratory testing may be performed;
2. Study products are returned and inventoried; and
3. Study staff will make an appointment for safety follow-up visit(s) as required.

All premature discontinuations and their causes must be documented by the Investigator or designated study staff on the appropriate eCRF pages (e.g., Final Participant Status, AE). Participants not completing the entire study should be fully evaluated (i.e., Final Visit procedures performed) whenever possible.

Any participant who discontinues participation due to an unresolved clinically significant AE will be followed until satisfactory clinical resolution is achieved and the AE documented in the eCRF regardless of disease severity. For all AEs that require the participant to be discontinued from the study, relevant clinical assessments and laboratory tests will be repeated at least on a monthly basis until final resolution or stabilization of the event(s). These visits should be entered into the eCRF as unscheduled visits. If a participant is withdrawn from the study due to study drug intolerability, an AE should be recorded **regardless of grade**.

5. RANDOMIZATION AND BLINDING PROCEDURES

5.1 Randomization

Participants will be randomized to one of two groups in a 1:1 ratio. The study arms are:

Group 1: FMT capsules
Group 2: Placebo capsules

The randomization will also allocate the number of placebo capsules, ranging from 30 to 40, per dose.

Prior to study commencement, a statistician will develop a randomization list and prepare sequentially numbered sealed envelopes containing the randomization group assignment. In this single blind study, participants will not know which arm they have been assigned to.

After screening, upon validation of eligibility, study staff will open the next sequentially numbered envelope to determine which group the study participant has been assigned to. Randomization after screening will allow sufficient time to prepare capsules and have them shipped to the study site before the first treatment visit (Visit 3-Day 0). The randomization group will be recorded on the randomization electronic case report form (eCRF).

6. STUDY TREATMENTS AND PROCEDURES

FMT capsules are the only study intervention.

6.1 Investigational Product Description

The study product consists of fecal microbiota capsules prepared by Dr. Silverman's team in London, Ontario, Canada. Dr. Silverman has received approval from Health Canada to evaluate FMT in patients with metastatic malignant melanoma or with fatty liver within a clinical trial setting (CTA control nos. 235387and 195078 respectively).

Approximately 30 to 40 capsules will be prepared from 80-100g of healthy human feces from a single healthy donor and administered as a single dose. Capsules will be prepared by a modified method as described by Kao et al, 2017[47,48] (see Investigator brochure).

Donor inclusion criteria:
An individual between the age of 18-50 years, who has a normal body mass index (BMI of 18.5-30) and who does not have the following criteria will be eligible and further screened for all known potential transmissible agents will be selected from a pool of donors available in the Infectious Diseases clinic at St. Joseph's Hospital supervised by Dr. Silverman and screened for all known transmissible agents at the Microbiology and

Immunology laboratory at St. Joseph's Hospital, London public health unit, Toronto public health unit and Mount Sinai hospital, Toronto. This selection process requires screening of 100 participants in order to select 5 to 10 for stool donation.

Donor exclusion criteria:

1) Any underlying metabolic disease including; hypertension, hyperlipidemia, diabetes, insulin insensitivity, atherosclerosis
2) Positive stool test or Nasal Swab for any Multi-Drug Resistant Organisms (extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, vancomycin-resistant enterococci (VRE), carbapenem-resistant Enterobacteriaceae (CRE), SARS-CoV-2 and methicillin-resistant Staphylococcus aureus (MRSA))
3) Any of the following symptoms 14 days prior to any stool collection: fever, cough, loss of taste, anosmia, fatigue/malaise, myalgia, sore throat, nausea, abdominal pain, diarrhea, dyspnea, chest pain, rash conjunctivitis, headache
4) Any symptoms suggestive of SARS-CoV-2 or a history of SARS-CoV-2 infection within the last 3 months
5) New rash or spots indicative of monkeypox (Mpox)
6) Men who have sex with men or women who have sex with a man who has sex with other men as this is a requirement by Health Canada
7) Mpox virus or may have been recently infected or exposed to Mpox virus within the last 3 months
8) A history of any gastrointestinal, liver or biliary disorders or cancers. Including but not limited to; gastroesophageal reflux, peptic ulcer disease, celiac disease, inflammatory bowel disease (Crohn's disease or ulcerative colitis), microscopic colitis, motility disorders (including gastroparesis and irritable bowel syndrome) diverticular disease.
9) Previous surgery to the intestine, liver or gallbladder (except remote appendectomy).
10) History of any malignancy(cancer)
11) Used any antibiotics or probiotics within the last 3 months
12) Been hospitalized within the last 3 months
13) Recently traveled to a developing country (within 3 months) https://worldpopulationreview.com/country-rankings/developing-countries
14) A new sexual partner (within the last 3 months)
15) A history of street drug use
16) A family history of diabetes, early onset coronary disease or gastrointestinal or liver disease, colon cancer, familial malignancy
17) A history of psychiatric illness (major affective disorder, psychotic illness, ongoing use of any psychiatric medications)
18) Any positive laboratory results for a transmissible pathogen

19) Alcohol intake greater than 1 alcohol containing drink/day for women or 2/day for men

Multiple stool donors (2 or 3) will be used for the participants who will receive FMT in our study.

6.2 Expected Side Effects

In Dr. Silverman's team experience, the most common complaints associated with FMT include loose stools, abdominal pain, and dizziness, largely consistent with the previously published data[40]. There is a possibility of as-yet-unknown risks associated with FMT given the complex interplay between transplanted microbiota and the recipient bowel[42-44]. Possible allergy and/or anaphylaxis to antigens in donor stool; theoretical risk of developing disease possibly related to donor gut microbiota including but not limited to: obesity, metabolic syndrome, cardiovascular disease, autoimmune conditions, allergic/ atopic disorders, neurologic disorders, psychiatric conditions, and malignancy (refer to FMT capsules Investigator brochure).

6.3 Formulation

Formulation: 80-100g of healthy donor stool processed and encapsulated in gelatin capsules (modified Kao et al, 2017).

Physical Chemical and Pharmaceutical Properties and Formulation of FMT capsules:
Donor Fecal samples will be inspected visually for color and consistency (See details in Bristol Stool chart in Appendix 1), also to assure no gross particulate matter present prior to administration. Type 3-4 are considered normal and acceptable for the study. Donor samples will be then processed and capsules prepared according to the modified protocol by Kao et al, 2017[48]. Each capsule will be filled with approximatively 450µL of fecal slushy and encapsulated in size 0 and size 00 capsules (see Investigator brochure).

Placebo capsules for FMT: microcrystalline cellulose, for equivalence in weight and color, will be encapsulated in gelatin capsules. Each capsule will be filled with approximatively 5.5g of microcrystalline cellulose, and encapsulated in size 0 and size 00 capsules.

6.4 Packaging, Labelling and Storage

FMT capsules or placebo capsules will be prepared by trained staff from the Infectious Diseases Care Program at St. Joseph's Health Care, London, ON, Canada. Capsules will be shipped to the study site in Montréal, QC, Canada together with a temperature monitor. Capsules will be stored at -80°C by the site laboratory or the pharmacy. Both the manufacturing and the study sites have temperature controlled, monitored freezer for storage of FMT or placebo capsules.

Product will be labeled for investigational use in accordance with regulatory requirements at the Infectious Diseases Care Program at St. Joseph's Health Care, London, ON, Canada.

6.5 Study Product Supply and Accountability

FMT or placebo capsules will be stored and dispensed to participants at each site by the respective hospital pharmacy or laboratories.

6.6 Dosing and Administration

Two doses of FMT or placebo will be administered orally with water at Day 0 and Day 22. Thirty (30) to 40 capsules will be consumed under the direct observation by trained staff at the study site. The variable number of capsules depends on the fiber content of the donor stools.

A random number of placebo capsules, ranging from 30 to 40, will be dispensed in the placebo arm.

Participants will be instructed to continue taking their ART during the entire study.

6.7 Dose Reduction or Modification

In the unlikely event that a patient vomits during capsule intake or shortly after, the reasons will be evaluated by the investigator. A decision will be made to do repeat dosing or exclude the participant from the study.

Any adverse event that may be a symptom, sign or abnormal laboratory finding will be carefully monitored. If the symptom, sign or abnormal laboratory finding persists after the 1st treatment, the 2nd treatment will not be proposed to the participant. However, the participant will be followed clinically until the side effect or abnormal laboratory result(s) resolves. In the case of persistent abnormal results after stopping treatment, the participant will be referred to the proper local specialist.

6.8 Concomitant Medications and supplements

Concomitant medications and supplements will be collected at study visits and recorded in the eCRF.

All study participants must continue to take their ART medications during the full study period.

There are no restrictions on the use of concomitant medications/ natural remedies/ foods in the study except those listed in the exclusion criteria.

Participants will be asked to refrain from consuming non-prescription drugs for 48 hours prior to the study visit unless approved by the Investigator. Any deviation from this protocol will be documented in the eCRF and the Investigator will determine if the protocol deviation warrants exclusion from the trial.

6.9 Concomitant Alcohol and "Street" Drug Use

For maximum benefit of FMT, it is recommended that participants limit their alcohol use during the study period (maximum one alcoholic drink per day, preferably less than daily) as alcohol has been shown to increase inflammation and negatively modulate gut microbiota in HIV-infected persons[49]. Alcohol consumption will be assessed during screening using a full AUDIT Questionnaire and will be surveyed at subsequent study visits using a modified AUDIT-C Questionnaire (administered by Study Staff at Visits 2-6). The Investigator may exclude an individual from participating in the trial after screening if their Full AUDIT Questionnaire score signals the participant has an alcohol abuse problem (scores of higher than 8).

Since cigarette smoking may not affect FMT effects but may negatively affect baseline values of inflammatory markers, cigarette smoking is an important parameter that will need to be documented for each participant.

Street drug, cigarette smoking, non-prescription medication and marijuana/cannabis products use will be surveyed by research staff and recorded at each visit. Study continuation will be based on the Investigator's judgement.

In the 24 hours prior to a study visit participants will be asked to refrain from using marijuana/ cannabis products and limit alcohol to no more than one alcoholic beverage with dinner the night before the study visit.

6.10 Prohibited Medications and Procedures

No medications are prohibited during the study.
Participants will be asked to share with the study team any new medication or procedure they are undertaking. Such information will be documented in the eCRF.

6.11 Participant Access to Study Medication at Study Closure

FMT capsules will not be provided at the end of the study, as clinical benefits will have not yet been demonstrated.

7. RISK MANAGEMENT

7.1 Acceptable Methods of Birth Control

While abstinence from sexual activity is the only certain method to prevent pregnancy, female participants of childbearing potential who are or who anticipate the possibility of becoming sexually active with a male partner during the must practice an acceptable method of contraception while in the study and until 2 weeks after completion of the study such as:

1. Complete abstinence from penile-vaginal intercourse from the screening period until two weeks after the study completion.
2. Double barrier methods (acceptable barrier methods include diaphragm, coil, contraceptive foam, sponge with spermicide, condom).
3. Oral, injectable or implant contraceptives plus one barrier method.
4. Any intrauterine device (IUD) with published data showing that the expected failure rate is <1% per year (not all IUDs meet this criterion) plus one barrier method.
5. Male partner sterilization confirmed prior to the female participant's entry into the study; this male is the sole partner for that female participant.
6. Approved hormonal contraception (pills, injection or implant), started at least 30 days before screening, preferably with one barrier method.
7. Another method approved by the Investigator with published data showing that the expected failure rate is <1% per year preferably with one barrier method.

Male participants who are or anticipate the possibility of becoming sexually active with a female partner of childbearing potential during the study and for 2 weeks after study completion must practice an acceptable method of contraception such as:

1. Use a barrier method of contraception (condom) with a female partner who practices an acceptable method of contraception (see above).
2. Previous vasectomy.
3. Be proven to be sterile.

Contraceptive measures will be reviewed with participants at all study visits over the course of the study.

7.2 Risk Management

Risk minimization, management, and assessment procedures have been implemented in the study to minimize and assess potential risks to participants who participate in this clinical study. Components include specific study entry and exclusion criteria to ensure that participants who have underlying characteristics that potentially increase their risk for an adverse outcome are excluded; monitoring for adverse events for the duration of the study; overview surveillance by an Independent Data Safety Monitoring Committee (DSMC); risk identification and mitigation management over the course of the study (and the sub-study).

When side effects are perceived to be related to study treatment, the Investigator can use his or her clinical judgement regarding whether to continue or to discontinue the study medication. If study treatment is discontinued, the participant will be scheduled for follow-up visit(s) as required to treat the symptoms or adverse event related to FMT or placebo treatment.

8. CLINICAL AND LABORATORY EVALUATIONS

8.1 Clinical Evaluations

At screening (Visit 1) a medical history and medication history will be recorded (to be conducted by study staff through chart review and/or patient interview). A complete physical examination will be conducted. At subsequent visits participants will be asked about any changes in health, or newly diagnosed medical conditions or changes to current medications or new medications started since their last study visit will be recorded and a targeted physical examination, if required, will be performed. Blood will also be collected for laboratory testing as outlined below.

8.2 Laboratory Evaluations and Specimen Collection

Blood samples will be obtained for clinical laboratory evaluations at each visit. Initial testing during screening will include hematology, serum chemistry, and serology evaluations listed in Table 1. CBC, CD4 and CD8 T cell counts will be taken at each visit along with HIV copy number. Participants will be provided with the results of the clinical laboratory tests. Participants with any clinically significant abnormalities at screening that in the opinion of the Investigator would preclude their enrolment, will be excluded from the study and referred to their physician for medical follow-up as necessary.

Table 1: Clinical Laboratory Tests

Clinical Blood tests (~20 mL)		
Hematology (1 tube, 5ml)	**Serum Chemistry (1 tube, 8ml)**	**Serology (1 tube, 5ml)**
• CBC • CD4 T cell counts • CD8 T cell counts • Blood Pregnancy test (hCG levels measured: only in women of child-bearing potential)	• Alkaline phosphatase • ALT (alanine aminotransferase) • Amylase • AST (aspartate aminotransferase) • Bilirubin (total) • Total Cholesterol • Creatine kinase • Creatinine • D-dimer • Fasting Blood Glucose • HbA1c • HDL/LDL • hs-CRP • Lipase • Serum phosphate • Triglycerides •	• HIV viral load • HBV • HCV • CMV

ALT – alanine aminotransferase; AST – aspartate aminotransferase; CBC-Complete blood count; hCG – human chorionic gonadotropin; HDL/LDL-High Density Lipoprotein/Low Density Lipoprotein; HbA1c-glycolated hemoglobin; hs-CRP – high sensitivity C-Reactive Protein, HIV-Human immunodeficiency Virus; HBV-Hepatitis B Virus; hCG – Human chorionic gonadotropin; HCV-Hepatitis C Virus; CMV-Cytomegalovirus.

8.2.1 Research Blood Collection

Female participants of childbearing potential must have a negative serum pregnancy test (measuring human chorionic gonadotropin-hCG levels) prior to study entry. A serum pregnancy test will be performed for all fertile female participants at each subsequent study visit. Any female who are pregnant during screening will be excluded from the study.

Any female who becomes pregnant during the study will be withdrawn from the study. A pregnancy will be documented as a protocol deviation in the eCRF (See Section 9.7) and followed to term.

8.3 Research Sample Collection and Laboratory Assays

Samples for the research tests will be collected as per Table 2.

Table 2: Research Laboratory Assays including Sub-Study Colon Biopsies

Blood for Research	Stool Sample Analysis	Sub-Study Colon Biopsies
Approximately 80 ml of blood will be taken at visits 2, 3, 4, 5, and 6 • PBMCs isolated and frozen (for cell marker analysis by flow cytometry and isolation of CD4+ T cells for subsequent HIV reservoir quantification (see below) • Plasma/serum isolated and frozen (for research tests including ELISAs and Mulitplex tests for gut damage markers, cytokines/inflammation markers). Extra will be used for measuring exploratory markers of gut damage (REG3α), microbial translocation (16S rDNA) or inflammation (T cell activation markers).	Stool samples will be collected at visits 2, 3, 4, 5 and 6. • Stools will be frozen until ready to be processed for microbiota analysis	Colon biopsies will be taken in sub-study participants at visits 3 and 6 • Collected colon biopsies (up to 32 per sub-study participant) will be either paraffin embedded or frozen for gut architecture analysis, inflammation assessment and HIV reservoir localization analysis

PBMCs-Peripheral Blood Mononuclear Cells

8.3.1 Research Blood Collection

Blood (~80 ml) will be collected into anti-coagulant tubes (EDTA for plasma and one SST tube for serum) at Visits 2, 3, 4, 5 and 6 and will be processed to separate out plasma/ serum and peripheral blood mononuclear cells (PBMCs) by centrifugation. Plasma/ serum will be flash frozen and stored at -80°C. PBMCs will be flash frozen and stored in liquid nitrogen. Plasma/serum and PBMC will be stored at the MUHC research lab of Dr. Routy under the supervision of Dr. Isnard. Plasma/ serum and PBMCs will be processed as outlined in Section 8.2.

8.3.2 Fecal Collection

Fecal collection will be performed according to the International Human Microbiota Standard (IHMS) guidelines standard operating procedure (SOP) V2 (http://www.microbiome-standards.org/). Participant stool samples will be collected before FMT/ placebo administration (Baseline Visit 2 and Visit 3): after the first FMT/ placebo administration and before the 2nd FMT/ Placebo (Visit 4), at 6-weeks post-intervention (Visit 5) and at 12-weeks post intervention (Visit 6) to assess microbial changes. Instructions on stool sample collection and sample collection bottles will be given to the study participant one visit prior to the visit of collection. Study participants will collect stool samples in the clinic or at their home within 24 hours of their study visit. The stool samples will be stored in a -80°C freezer at the site. DNA will be isolated processed for rDNA sequencing. Stool samples will be processed as per Section 8.3.2.

8.3.3 Optional colon mucosal biopsies

Optional colon mucosal biopsies will be done before FMT/ placebo administration (Visit 3; Baseline 2, Day 0) and at the end of study (Visit 6, week 12). The day before the procedure, the consenting participant will be required to take measures to clear his/ her bowels for the examination; an information sheet detailing the steps will be provided. If the appointment is in the morning, the participant will be asked to take a liquid laxative once during the day and once in the evening the day before the exam. If the appointment is in the afternoon, the liquid laxative must be taken in the evening before bed the day before the exam and again first thing the morning of the exam. Additionally, the participant will be asked to take two laxative pills the day before the exam. The participant will also be asked to adhere to a clear-liquids diet for 24 hours prior to the procedure. He/ she may request mild sedation although this is usually not required. If sedated, driving is prohibited until the next morning.

Procedures
All procedures will be performed in a procedure suite in the Inpatient Unit of the Gastroenterology Department of the Royal Victoria Hospital (Glen Site).

The procedure will last about 30 minutes. Before Dr. Bessissow or Dr. Lakatos, the gastroenterologists, can perform the colon mucosal biopsies, the participant will be asked to remove all clothing and will be given a hospital gown to wear. The participant will then be positioned on the left side of a bed with knees bent. A flexible tube will be inserted through the rectum and slowly advanced into the colon. Several samples will be taken from inside the colon. The participant should not feel any pain but may experience some discomfort. A standard endoscopic exam will be performed first to detect any recto-sigmoid mucosa abnormalities. Disposable, flexible biopsy forceps (a tube having a sample collection tool in the form of a pair of pivotally-mounted opposed cups on its end) will be inserted into the rectum. The tube, which will be new and sterile, has a diameter of a few millimetres. The tube will then be utilized to collect samples about the size of a pinhead. Up to 32 samples will be obtained before the tube is removed. A small amount of blood may be visible with the first bowel movement following the exam. It is recommended that the participant refrain from engaging in receptive anal activity for one day following the procedure.

Colon biopsies will be collected and cells isolated, or frozen or fixed in formaldehyde on site. Some biopsies will then be paraffin embedded and stained under the supervision of Dr. Isnard at the research lab in MUHC.

The optional colon biopsies will be processed as per Sections 8.3.3 and 8.3.7.

8.3.4 Assessment of gut damage, microbial translocation and inflammation

To evaluate gut epithelial damage, we, and others, have validated markers that will be measured in the plasma by ELISA before, after the 1st and the 2nd FMT/Placebo, at week 6 and 12[11,50-52]. LPS, a common marker of bacterial translocation[53], soluble Suppression

of Tumorigenicity (sST2)[20] and Intestinal-Fatty Acid Binding Protein (I-FABP) will be measured to assess gut barrier integrity. Immune activation markers (sCD14) and pro- (IL-1β, IL-6. IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines will be quantified. Activation of monocytes and CD4+ and CD8+ T cells will be assessed *ex vivo* by flow cytometry with HLA-DR and CD38 staining. CD4+ and CD8+ T cells will also be assessed for PD-1 expression as a marker of T cell exhaustion and plasma will be assayed for beta D-glucan as a marker of fungal infection. REG3α and 16S rDNA as well as other markers of microbial translocation and gut damage may be tested in plasma as well. Any unforeseen tests could be performed for the same purpose: for instance, in the event a new marker of gut damage is discovered, it could be tested on participant's samples.

8.3.5 Microbiota composition

qPCR for *A. municiphila* will be performed on fecal DNA samples. Gut microbiota composition will be further studied by 16S and 18S rDNA sequencing to determine the modification of the gut microbiota.

8.3.6 HIV Reservoir quantification

If differences in microbial translocation and/ or inflammation are observed, HIV reservoirs will be quantified in blood and mucosa. CD4+ T cells will be sorted from peripheral blood mononuclear cells (PBMCs) (and mucosal biopsies in the optional sub-study) by flow cytometry to assess HIV reservoirs by nested qPCR.

If sampling permits, isolated CD4+ T cells from PBMCs will also be assayed for HIV reservoir using Tat/rev Induced Limiting Dilution Assay (TILDA) at Baseline Week 0 and End-treatment Week 12 timepoints.

In mucosal biopsies, HIV DNA and RNA will also be quantified by DNA/RNAscope[22,54] (optional sub-study). If sampling permits, CD4+ T cells will be isolated from these biopsy samples to measure the HIV reservoir.

Any unforeseen tests could be performed for the same purpose: in the event a new technic is discovered to quantify HIV reservoirs, it could be applied to participant's samples.

8.3.7 Gut mucosa architecture (optional colon biopsy/ sub-study)

Biopsy inclusion in paraffin will be performed by the MUHC Histopathology core facility. Gut architecture will be monitored by immunochemistry and immunostaining of the epithelial tight junctions (Claudin-3/Occludin)[53]. If a diminution in inflammation is noted, myeloperoxidase staining will be performed to allow for the quantification of inflammatory myeloid cells in the gut.

For other analyses gut cells will be separated from tissues by enzyme digestion using a collagenase-based method as reported previously[55]. Fresh tissue biopsies will be

incubated with type II collagenase for 30 minutes at 37°C in a shaking incubator. Tissue pieces will be separated from debris using Ficoll-Hypaque and density gradient centrifugation. The resulting lymphocyte suspension will be stained with monoclonal Antibodies (mAbs) against CD3, CD4, CD8, and myeloid markers. The total frequency of activated CD4 and CD8 T cells will be determined by flow cytometry as described above.

8.4 Questionnaires

8.4.1 Full AUDIT Questionnaire

Participants will be administered a full AUDIT (Alcohol Use Disorders Identification Test)[56] Questionnaire at screening by study staff (See Appendix 2). This will help determine if there are alcohol abuse problems that could negatively influence the participant's ability to adhere to the study protocol procedures. Participants with a score of greater than 8 at screening, signaling an alcohol abuse problem, will be excluded from the study.

8.4.2 Modified AUDIT-C Questionnaire

A Modified AUDIT-C Questionnaire will be administered by study staff at study visits 2-6 (See Appendix 3). This questionnaire will help determine if participants have stayed in the light-to-moderate drinking range (score up to 4) or if there are signs of alcohol abuse during the study period. Participants with scores above 4 may have their data presented separately as excessive alcohol may confound the positive effects of FMT on inflammation or HIV[5,49].

8.5 Stored Research Specimens and Plans for Possible Future Testing

Only the study team will have access to participants' study samples.

Plasma/ serum and PBMC samples will be stored at -80°C until ready to be analyzed at the MUHC research lab of Dr. Routy.

Stool samples will be stored at -80°C until ready to be processed at the MUHC and extracted DNA will be sequenced for microbiota sequencing.

Colon biopsy samples will be stored and analyzed at the MUHC research lab of Dr. Routy.

Samples collected and stored for this study will be used only for the purpose of this study's aims. Samples will be stored as described above for a maximum duration of 10 years after the end of the study and will be subsequently destroyed.

9. STUDY PROCEDURES

9.1 Schedule of Events

Table 3: Schedule of Events

Visit Type	Screening	Study visits					
		Baseline 1	Bowel cleanse	Baseline 2, Treatment 1	Treatment 2	Follow-up	
Visit Window **Procedures:**	**Visit 1 Day -56 to -7 days**	**Visit 2 Day -14 (±7 days)**	**Day -1**	**Visit 3 Day 0**	**Visit 4 Day 22 (±7 days)**	**Visit 5 Week 6 *(±7 days)***	**Visit 6 Week 12 (±7 days)**
Visit No.	1	2		3	4	5	6
Informed Consent	X						
Eligibility Assessment	X	X		X			
Concomitant Medication	X	X		X	X	X	X
Medical History	X						
Complete Physical Exam and Vital Signs	X						
Targeted Physical Exam‡ and Vital Signs		X		X	X	X	X
Adverse Event Assessment				X	X	X	X
Serum Pregnancy Test	X	X		X	X	X	X
Hematology*	X	X†		X	X	X	X
Serum Chemistry**	X	X†		X	X	X	X
Serology***	X			X			
Serology - HIV-1 Viral Load***	X	X†		X	X	X	X
Randomization	X††						
Immune activation markers/cytokines (ELISA)****		X		X	X	X	X

Visit Type	Screening	Study visits					
		Baseline 1	Bowel cleanse	Baseline 2, Treatment 1	Treatment 2	Follow-up	
Visit Window **Procedures:**	**Visit 1 Day -56 to -7 days**	**Visit 2 Day -14 (±7 days)**	**Day -1**	**Visit 3 Day 0**	**Visit 4 Day 22 (±7 days)**	**Visit 5 Week 6 *(±7 days)***	**Visit 6 Week 12 (±7 days)**
Monocyte and T cell activation markers[+]		X		X	X	X	X
Markers of gut barrier integrity, inflammation and microbial translocation[++]		X		X	X	X	X
Size of HIV reservoir in Latently Infected CD4 T cells[+++]		X		X	X	X	X
Stool sample collection and microbiota composition[++++]		X		X	X	X	X
Bristol Chart		X		X	X	X	X
Alcohol use (AUDIT-Full), Appendix 2	X						
Alcohol use (AUDIT-C), Appendix 3		X		X	X	X	X
Bowel cleanse			X[†††]				
Study Product Dispensation (FMT or placebo)				X	X		
Colon mucosal biopsies[#]				X			X

*CBC, CD4 and CD8 T cell counts.
**Alkaline phosphatase, ALT, Amylase, AST, Bilirubin (total), Creatine kinase, Creatinine, D-dimer, fasting blood glucose, HbA1c, high sensitivity C-reactive protein (hsCRP), Lipase, lipid profile (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), Triglycerides), serum phosphate.
***Serology measurements include: Cytomegalovirus (CMV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) and HIV viral load. Since HIV viral load will be measured at each visit, it was put as a separate line item.
****Immune activation markers/cytokines include: soluble CD14, pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokine IL-10. Measured in plasma by ELISA.

+Monocyte and T cell activation markers include: HLA-DR and CD38. T cell exhaustion marker: PD-1. Measured by staining and flow cytometry.
++Markers of gut barrier integrity, microbial translocation and inflammation: lipopolysaccharide, soluble ST2, I-FABP (measured in plasma by ELISA).
+++PBMCs will be isolated and then latent CD4 T cells will be isolated by flow cytometry. HIV viral reservoir in the latent CD4 T cell population will be measured by nested qPCR. More specific TILDA analysis will be performed on Baseline Week 0 and End-treatment Week 12 samples to assess the HIV viral reservoir (Exploratory analysis).
++++qPCR of A. muciniphila, 16S and 18S rDNA sequencing for other members of the microbiota.
Optional sub-study procedure.
† Not required when the same tests have been performed at the screening visit within the past 14 days, with the exception of CBC, CD4, CD8 (and serum pregnancy test)
†† *upon validation of eligibility.*
††† Bowel cleanse the day before Visit 3 (Baseline 2).
‡ If required.

9.2 Screening and Baseline Procedures

9.2.1 Visit 1: Screening Visit: -56 to -7 days (up to 8 weeks prior to Baseline 2 (Visit 3))

Screening will be performed before the baseline assessment as a preliminary assessment of the participant's eligibility. This will take approximately an hour and a half to complete the assessment.
During this visit, the following will be performed:

- Obtain participant's written informed consent prior to initiating any screening or study procedures. Provide participant with a copy of the signed and dated consent form. Document participant's informed consent in the participant's medical record.
- The first assessment of the eligibility criteria.
- Collection of demographic information and participant's medical history:
    - Age, education, ethnicity, sexual preference.
    - Review of any pre-existing medical conditions/signs/symptoms.
    - List of current medications and supplements, dosage and date of treatment initiation.
    - Duration of HIV infection.
    - Current ART regimen (duration in months) and review of ART history.
    - CD4 T cell count within the past three months if available.
    - A Nadir CD4+ T cell count.
    - Viral load within at least 2 years before screening.
- Complete physical exam including vital signs (seated blood pressure (BP), heart rate (HR), respiration rate, oral temperature), and weight, height and waist circumference will be measured.
- Blood (~20 ml) will be collected for:
    - Hematology and serum chemistry tests (see Table 1) including a pregnancy test for female participants of child-bearing capacity.
    - Serology – HIV viral load, Cytomegalovirus (CMV), Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV).

- A Full AUDIT (Alcohol Use Disorders Identification Test, see Appendix 2) questionnaire will be administered by study staff to assess participant dependence on alcohol (scores of 8+ suggest alcohol abuse).
- Participants will be instructed to use contraception throughout the duration of the study as described in Section 7.1.
- Participant will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 2.

The Investigator will inform the participant that the information gathered at this Screening Visit will be reviewed and the participant will be contacted to attend a Baseline Visit (Visit 2) if eligibility requirements are met.

Eligible participants will be given the following instructions to prepare for each visit:
- Refrain from consuming non-prescription drugs 48 hours prior to the study visits;
- Refrain from marijuana use 24 hours prior to the study visits;
- Refrain from consuming alcohol 24 hours prior to the study visits, other than one drink at the previous night's meal.

Upon validation of eligibility, participants will be randomized to the treatment or the placebo arms using the method described section 5.1.

9.2.2 Baseline Visits

There will be two baseline visits which will help assess intra-patient variability with respect to the parameters described below.

Visit 2: Baseline 1 (day -14 ±7 days)
This visit will last approximately 30 minutes. During this visit the following will take place:
- Confirmation eligibility criteria have been met.
- Review of any pre-existing medical conditions/signs/symptoms.
- Concomitant medications (including ART) and supplements, alcohol use (modified AUDIT-C, see Appendix 3) and stool classification (Bristol chart, Appendix 1) will be surveyed.
- Targeted physical examination, if required, and vital signs.
- Blood (about 100 ml, including 80 ml for research) will be collected for:
    - Hematology and serum chemistry tests (see Table 1) including a pregnancy test for female participants of child-bearing capacity†
    - Serology - HIV viral load†.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, sST2, I-FABP, REG3α);
        - Immune activation (sCD14);

† *If Baseline 1 (Visit 2) visits occurs within 14 days of Screening (Visit 1), hematology, serum chemistry tests and HIV viral load (serology) will not be repeated, with the exception of CBC, CD4 and CD8 counts (and serum pregnancy test).*

        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
    - Peripheral blood mononuclear cell (PBMC) isolation to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR).
- Stool sample collection for assessment of gut microbiota composition (stool sample brought from home).
- Participant will be provided with instruction to buy a liquid laxative and instructions for conducting a bowel cleanse the day before Visit 3.
- Participant will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 3.

Visit 3: Baseline 2 (Day 0)
This visit must be scheduled between 7 and 21 days after Visit 2 and within 56 days of the screening visit. This visit will last approximately 1 hour.
During this visit, the following will take place prior to product administration:
- Confirmation of eligibility.
- Concomitant medications (including ART) and supplements, alcohol use (modified AUDIT-C, see Appendix 3) and stool classification (Bristol chart, Appendix 1) will be surveyed.
- Review of any pre-existing medical conditions/signs/symptoms.
- Targeted physical examination, if required, and vital signs.
- Blood (about 100 ml, including 80 ml for research) will be collected for:
    - Hematology and serum chemistry tests (see Table 1) including a pregnancy test for female participants of child-bearing capacity.
    - Serology: HIV viral load, CMV, HBV, and HCV.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, sST2, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10);
        - New exploratory markers of gut damage (REG3α), microbial translocation (16S rDNA) or inflammation (T cell activation markers).
    - Peripheral blood mononuclear cell (PBMC) isolation to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR). Tat/rev induced limiting dilution assay (TILDA) will also be used as a more specific measure of the HIV reservoir in latently infected CD4 T cells (exploratory analysis).
- Stool sample collection for assessment of gut microbiota composition (stool sample brought from home).
- Participant will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 4.

Product Administration
- 30 to 40 capsules of FMT or placebo will be dispensed to participants with water, under direct observation by trained staff.
- Adverse event (AE) assessment

Optional Sub-study Procedure: Colon Mucosal Biopsy
Participants that consent to the optional sub-study (Montreal site) will have a colon mucosal biopsy to assess the amount of T cell activation and to determine the amount of HIV in the rectal mucosa. (Refer to sections 8.3.3 and 8.3.7).

9.3 Visit 4: Treatment Visit (day 22 ± 7 days)

At this study visit, which will last approximately 1 hour, the study participant will undergo the following procedures:
- Concomitant medications (including ART) and supplements, alcohol use (modified AUDIT-C, see Appendix 3) and stool classification (Bristol chart, Appendix 1) will be surveyed.
- AE assessment.
- Targeted physical examination, if required, and vital signs.
- Blood (about 100 ml, including 80 ml for research) will be collected for:
    - Hematology and serum chemistry tests (see Table 1) including a pregnancy test for female participants of child-bearing capacity.
    - Serology: HIV viral load, CMV, HBV, and HCV.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, sST2, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10);
        - New exploratory markers of gut damage (REG3α), microbial translocation (16S rDNA) or inflammation (T cell activation markers).
    - Peripheral blood mononuclear cell (PBMC) isolation to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR). Tat/rev induced limiting dilution assay (TILDA) will also be used as a more specific measure of the HIV reservoir in latently infected CD4 T cells (exploratory analysis).
- Stool sample collection for assessment of gut microbiota composition (stool sample brought from home).
- Participant will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 5.

Product Administration
- 30 to 40 capsules of FMT or placebo will be dispensed to participants with water, under the direct observation by trained staff.

9.4 Visit 5: Follow up (Week 6 ± 7 days)

This visit will be of approximately 1 hour duration. The participant will undergo the following procedures:

- Concomitant medications (including ART) and supplements, alcohol use (modified AUDIT-C, see Appendix 3) and stool classification (Bristol chart, Appendix 1) will be surveyed.
- Targeted physical examination, if required, and vital signs.
- Blood (about 100 ml, including 80 ml for research) will be collected for:
    - Hematology and Chemistry tests (See Table 1) including pregnancy test for female participants of child-bearing capacity.
    - Serology - HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, sST2, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/ anti-inflammatory cytokine (IL-10);
        - New exploratory markers of gut damage (REG3α), microbial translocation (16S rDNA) or inflammation (T cell activation markers).
    - Peripheral blood mononuclear cell (PBMC) isolation to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells by nested qPCR and TILDA (exploratory analysis).
- AE assessment.
- Stool sample collection for assessment of gut microbiota composition (stool sample may be brought from participant's home).
- Participant will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 6.

9.5 Visit 6: Final Study Visit (Week 12 ±7days)

This final study visit will be approximately 30 minutes in duration. The participant will undergo the following procedures:

- Concomitant medications (including ART) and supplements, alcohol use (modified AUDIT-C, see Appendix 3) and stool classification (Bristol chart, Appendix 1) will be surveyed.
- Targeted physical examination, if required, and vital signs.
- Blood (about 100 ml, including 80 ml for research) will be collected for:
    - Hematology and chemistry tests (See Table 1) including a pregnancy test for female participants of child-bearing capacity.
    - Serology - HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, sST2, I-FABP);
        - Immune activation (sCD14);

        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10);
    - New exploratory markers of gut damage (REG3α), microbial translocation (16S rDNA) or inflammation (T cell activation markers). Peripheral blood mononuclear cell (PBMC) isolation to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells by nested qPCR.
- AE assessment.
- Stool sample collection for assessment of gut microbiota composition.

Optional sub-study procedure: colon mucosal biopsy
Participants that consent to the optional sub study (separate consent form, Montreal site, will have a colon mucosal biopsy to assess the amount of T cell activation and to determine the amount of HIV in the rectal mucosa. Refer to sections 8.3.3 and 8.3.7.

9.6 Missed Visits

If participants miss the scheduled study visit, clinical research staff will try to contact them and schedule a visit as soon as possible.

9.7 Early Termination Visit

If a participant withdraws or is removed from the study for any reason prior to the completion of the study, the reason for, and date of, the discontinuation, and date of the last dose of study medication, must be recorded in the appropriate section of the eCRF.

At the time of discontinuation, every effort should be made to ensure that:
1. Procedures and evaluations scheduled for the Final Visit (Visit 6) are performed, including assessment of AEs;
2. Study treatment compliance is assessed;
3. Study products are returned and inventoried;
4. Study staff will make an appointment for safety follow-up visit(s) if required.

Clinical research staff trying to contact the participant will be careful to maintain participant's privacy and will not tell anyone about his/her participation in this HIV study.

9.8 Unscheduled Visits

Unscheduled visits may be performed at any time at the discretion of the Investigator for the assessment of laboratory parameters or clinical safety, or for the follow-up of AEs.

9.9 Re-contact of Participants after Trial Termination

Participants may be contacted after the trial if there are any safety concerns regarding the study product. The Investigator or clinical staff will contact participants and inform them of any safety concerns and schedule a follow-up visit with the participant to assess the potential safety concern if required. This visit would be documented in the eCRF if scheduled before the database lock. The participant would be referred to a specialist if required.

Participants could also be contacted after the trial if there was to be extra testing performed on their collected specimens outside the scope of what was initially written in their informed consent form. In this instance, the Investigator or designated clinical research staff would contact the participant and schedule them an appointment to explained the protocol amendments and ask them to sign a new informed consent form.

10. EVALUATION, RECORDING, AND REPORTING OF ADVERSE EVENTS

10.1 Definitions

10.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or clinical investigation participant, following the administration of a study medication/intervention, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) study medication/intervention, whether or not the AE is related to the medicinal (investigational) study medication/intervention.

During each follow-up visit with the participant, information on AEs will be gathered and documented accordingly. Adverse Events will be graded as mild, moderate, severe or life-threatening and assessed by causality as probably related, possibly related, unlikely to be related or not related to FMT.

Stable chronic conditions which are present prior to clinical trial entry and do not worsen are not considered AEs and will be accounted for in the participant's medical history.

10.1.2 Serious Adverse Events (SAEs)

An SAE is defined as an AE meeting one of the following criteria at any dose:

- Results in death during the period of protocol-defined surveillance;
- Is a life-threatening event (defined as a participant at immediate risk of death at the time of the event);

- Results in in-patient hospitalization or prolongation of existing hospitalization during the period of protocol-defined surveillance;
- Results in persistent or significant disability or incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions);
- Is a congenital anomaly or birth defect.

Any other important medical event that may not result in one of the above outcomes, may be considered a SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

Participants will be monitored during the study period for SAEs. If an SAE is ongoing at the time a participant discontinues/completes the study, the SAE will be followed until the Investigator agrees that the event is satisfactorily resolved, becomes chronic, or that no further follow-up is required.

10.2 AE Descriptions and Recording

All Adverse Events reported in the study will be documented in the eCRF, regardless of intensity (severity) or relationship to study product.

10.2.1 Intensity

The intensity (severity) of each AE (including SAEs) will be graded according to the toxicity table and toxicity guidelines provided by the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables).

10.2.2 Relationship to Study Treatment

For all collected AEs (including SAEs), the clinician who examines and evaluates the participant will determine the AE's causality based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below:

Definitely Related: There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to Investigational Product (FMT or placebo) administration and cannot be explained by concurrent disease or other products or chemicals. The response to withdrawal of the product (de-challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory re-challenge procedure if necessary.

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

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

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to Investigational Product administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other products or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

Not related: The AE is completely independent of Investigational Product administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

10.3 Reporting and Evaluation of SAEs and Other Clinically Significant AEs

10.3.1 SAEs

All SAEs which occur during the course of the study must be reported to Carolina Berini **within 24 hours** of the site becoming aware of the event.

SAEs will be reported to:

Attention:	Carolina Berini
Phone:	+ 514 934 1934 ext. 34240
Fax:	+1 514 933 1562
E-mail:	carolina.berini@muhc.mcgill.ca

All additional follow-up evaluations must also be reported as soon as possible in the same manner. All SAEs will be followed until the Qualified Investigator/Medical Monitor agrees that the event is satisfactorily resolved, becomes chronic, or no further follow-up is required.

The Sponsor-Investigator will ensure that both the manufacturing and study sites are notified of any AEs and SAEs that are definitely, probably or possibly related to the intervention. Each Qualified Investigator is responsible for ensuring that their local Research Ethics Board (REB) is notified of any AEs and SAEs as per the local requirements.

The Canadian HIV Trials Network will be responsible for reporting expedited, reportable SAEs to Health Canada on behalf of the Sponsor-Investigator, in accordance with Health Canada guidance and applicable regulations.

10.4 Follow-up for Adverse Events

Any AE that occurs between the second baseline visit (Visit 3, Week 0) and the time that she/he departs the study at the end of the final study visit (or at the time of early discontinuation of the participant from the study for any reason) will be captured and recorded. At each contact with the participant, the Investigator (or designate) must seek information on AEs by specific questioning and, as appropriate, by examination.

AEs that had previously been reported by the study participant will also be reassessed for duration, intensity and possible reoccurrence. Assessment of safety will include clinical observation and questions about changes in medication and any new symptoms reported.

All AEs (including SAEs) will be followed until resolution or until the Qualified Investigator and the Medical Monitor are in agreement that the AE has resolved, stabilized or become chronic and no further follow-up is required.

10.5 Pregnancy Reporting

Any female participant who becomes pregnant during the study will be instructed to inform the Investigator as soon as they are aware of the pregnancy and not to receive further treatment (if found before Day 22) to eliminate further exposure to the embryo/ foetus.

If pregnancy occurs during the period of protocol-defined surveillance, it is not to be considered an AE or SAE requiring monitoring and follow-up; however, any complication related to pregnancy would be considered an AE. Pregnancy should be recorded as a protocol deviation.

If a participant becomes pregnant during the study, the Investigator must inform the Sponsor-Investigator and collect follow-up data regarding the pregnancy, birth, and status of the child. The Sponsor-Investigator will provide a special eCRF for data collection in the case of pregnancy.

The Investigator must report the pregnancy information for female participants or female partners of male participants using a pregnancy eCRF. The pregnancy should be followed up by the Investigator to determine the outcome (including premature termination) and

the status of the mother and of the child, which must also be reported using a pregnancy outcome eCRF. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or as an SAE. Spontaneous abortions must be reported as an SAE.

Any SAE that occurred in association with a pregnancy that was brought to the Investigator's attention after the participant completed the study and that was considered by the Investigator to be possibly related to the study product must be promptly reported. Refer to Sections 10.1 and 10.3.

11. STATISTICAL CONSIDERATIONS

11.1 General Study Design

The effect of 2 rounds of FMT on the amount of immune activation, on the composition of the gut microbiota, and decreasing the size of the viral reservoir will be assessed in this Single blind, randomized, double arm, interventional study. The comparison will be made by repeated measures of the outcomes (Plasma I-FABP, LPS and cytokines levels, percentage of activated CD8 T cells, the composition of the gut microbiota, and size of the viral reservoir) at baselines, during the 1st and 2nd FMT and 6 and 12 week of follow-up. FMT effect will be compared to the same repeated outcome in the placebo arm. Intra-patient variability will also be assessed by comparing markers of inflammation and microbial transfer in blood and stool assessed two weeks apart in the same patient prior to study treatment.

11.2 Sample Size Considerations/Justification

To our knowledge, the effect of FMT therapy on the inflammation, size of the HIV viral reservoir, and gut microbiota composition/architecture in the context of HIV infection has is not confirmed yet. Therefore, a convenient sample of 20 participants was chosen without formal power calculations. The data that we will obtain in this study should suffice for such calculations for future studies. The absolute numbers of activated CD4 and CD8 T cell subsets will be computed using: (i) the white blood cell (WBC) count; (ii) the percentage of lymphocytes; (iii) the percentage of CD4 or CD8 T cells; and (iv) the percentage of the CD4 or CD8 T cell memory subset.
The following formula will be used:

Absolute count of activated cells/µl =
WBC count x % lymphocytes x % CD4 (or CD8) T cells x %CD4 (or CD8) T cell memory subset

Analyzed parameters will be compared to baseline for each participant and fold changes will be compared between the treated and the placebo group by t-test or non-parametric analysis such as Wilcoxon rank sum test as appropriate. 95% confidence interval of the estimated difference will be computed. Microbiota 16S rRNA diversity will be interpreted

using high dimensional class comparisons via linear discriminant analysis of effect size (LEfSe) and volcano plot, as well as DESeq2 analyses.

11.3 Endpoints/Outcome Measures

11.3.1 Primary Endpoint and Outcome Measures:

1. Changes in plasma REG3α levels will be measured as markers of gut permeability (ELISA).

11.3.2 Secondary Endpoint and Outcome Measures:

1. Safety and tolerability of FMT measured by evaluating adverse events, hematology, and serum chemistries over the course of the study. These evaluations will include: HIV viral load, glucose levels, a lipid profile and plasma levels of hsCRP and D-dimer.[28]

2. Changes in plasma levels of gut damage markers including I-FABP and LPS (ELISA).

3. Changes in plasma levels of pro-inflammatory markers ((1-3)-β-D-Glucan, IL-1β, IL-6, IL-8. IP-10, IL-17A and F, IL-22) and anti-inflammatory markers (IL-10) will be measured by Multiplex or ELISA.

4. Changes in T and myeloid cell activation in blood. CD4+ and CD8+ T cell and Monocyte activation will be measured by the expression of CD38 and HLA-DR and T cell exhaustion by PD-1 (Flow cytometry (Baselines compared to each other; Mean Baseline vs. post treatment and follow-up visits, fold changes compared between treated arm and placebo arm)).

11.3.3 Exploratory Endpoint and Outcome Measures:

1. Changes in microbiota composition and diversity in stools, as assessed using 16rDNA sequencing.

2. Changes in HIV reservoir size in blood by qPCR.

11.3.4 Sub-study Endpoint and Outcome Measures

1. Changes in inflammatory cytokines by qPCR as well as T and myeloid cell activation in colon biopsies, as assessed by flow cytometry expression of HLA-DR and CD38, and CD83 and CD86 respectively.

2. Changes in HIV reservoir size in colon CD4 T-cells as assessed by qPCR.

11.4 Summary of Demographic and Baseline Data

For this study we will be screening so that all participants will be HIV-infected, will be on ART treatment for at least three years with an undetectable viral load (<50 copies/ml), and will be on a stable ART regimen for at least 3 months. To ensure the baseline data is accurate, there will be two baseline visits for each participant. If the data collected from the two baseline visits (approximately 14 days apart) are consistent, a mean of the data will be used as the true baseline for each parameter. If the two readings are not consistent, it will be up to the Investigator to determine if the mean of the two measurements represents an accurate baseline measure, or if one reading should be omitted because it represents a lab error. *Baseline 2 (day 0, visit 3) may be used as the default if uncertainty about laboratory errors appears, as it represents the gut mucosa biopsy.*

The participant demographic information (all 20 participants will be included) will be presented in Table form (in an anonymous fashion). Depending on the journal requirements, this Table will either be in the results section or as an appendix. Included in the table will be information on average age, ethnicity, gender, sexual preference, time of infection, length of time on ART, and also information on how many completed the study and at which point the study was terminated (i.e. if there were SAEs or AEs leading to termination or not). CD4 and CD8 T cell ratio, and baseline levels of *A. municiphila* may also be reported here.

12. STUDY ETHICAL CONSIDERATIONS

12.1 Ethical Conduct of the Study

This study will be conducted in accordance with the ICH-GCP Guidelines, applicable Health Canada regulations and the principles of the Declaration of Helsinki. The Investigator will be thoroughly familiar with the appropriate use of the study treatment as described in the protocol and FMT Investigator Brochure.

The study protocol, informed consent document and additional study-related documentation will be submitted for approval to each sites Research Ethics Board. A Clinical Trial Application will be submitted to Health Canada and the study will not be initiated until a No Objection Letter is received. All participants will be given adequate explanation of the aims, methods, anticipated benefits and potential risks of the study. Participants will be required to sign an informed consent document prior to the initiation of the screening process.

12.2 Informed Consent

All participants will be given detailed oral and written information about the study. Consent documents describing in detail the study medication/intervention(s), study procedures and risks will be given to each participant and written documentation of informed consent is required prior to starting study medication/intervention. Participants must sign an informed consent document that has been approved by a participating center's REB prior to any procedures being done specifically for the trial. Each participant should have sufficient opportunity to discuss the study, have their questions addressed, and consider the information in the consent process prior to agreeing to participate. Participants may withdraw consent at any time during the course of the study without prejudice. The informed consent document will be signed and dated by the participant and the Investigator or delegate. The original signed informed consent form will be retained in the participant's study files and a copy will be provided to the participant.

The informed consent process must be conducted, and form signed before the participant undergoes any screening procedures that are performed solely for the purpose of determining eligibility for the study.

12.3 Confidentiality

All participant-related information including eCRF, laboratory specimens, and other study documents will be kept strictly confidential. Only study staff will have access to records with participant names. Otherwise, participants will be identified only by means of a coded number specific to each participant. All records will be kept in a secure, locked location and only accessible to research staff. All computerized databases will identify participants by numeric codes only and will be password protected.

Upon request, and in the presence of the Investigator or his/her representative, participant records will be made available to the study Sponsor-Investigator, monitoring groups representative of the study Sponsor-Investigator, representatives of funding groups, the research ethics board (REB) and applicable regulatory agencies for the purpose of verification of clinical trial procedures and/or data, as is permissible by local regulations.

12.4 Research Ethics Board

The REB will review all appropriate study documentation to safeguard the rights, safety, and well-being of the participants. The study will be conducted only at sites where ethics approval has been obtained. A copy of the protocol (including protocol amendments), all versions of informed consent forms, other information to be completed by participants such as survey instruments or questionnaires, and any proposed advertising/recruitment materials must be reviewed and approved by the REB of each participating centre prior to implementation of the trial. The Qualified Investigator will be responsible for obtaining REB approval of the annual Continuing Review throughout the duration of the study. The Qualified Investigator will notify the REB of serious adverse events as applicable. The Qualified Investigator will seek prior ethics approval for any protocol deviations except

when the change is intended to eliminate an immediate hazard to participants. In this case, the protocol deviation will be promptly reported.

13. GENERAL TRIAL CONSIDERATIONS

13.1 Adherence to Protocol

13.1.1 Protocol Amendments

All protocol amendments will be reviewed and approved and if applicable submitted to the applicable regulatory agencies for prior approval or notification. The Qualified Investigator must sign and date the amendment prior to implementation. All protocol amendments must also be submitted to the ethics committee.

13.1.2 Protocol Deviations

No deviations from this protocol will be permitted without the prior written approval of the Sponsor-Investigator, except when the modification is needed to eliminate an immediate hazard(s) to participants. Any deviations that may affect a participant's treatment or informed consent, especially those increasing potential risks, must receive prior approval from the REB unless performed to remove an immediate safety risk to the participants. In this case it will be reported to the REB and the Sponsor-Investigator immediately thereafter. Any deviations from the protocol must be documented.

13.2 Monitoring & Auditing

13.2.1 Data Safety Monitoring Committee

The study will be monitored by the CTN's Data Safety Monitoring Committee (DSMC). The DSMC's primary responsibility will be to monitor participant safety and enrolment. The DSMC will meet every 6 months to review the rate of recruitment, loss to follow-up, adherence to protocol, drug-related AE's and all SAE's. The DSMC will convene ad hoc when necessary via teleconference to discuss any safety issues that arise. In conjunction with the Sponsor-Investigator and the Medical Monitor, the DSMC can recommend changes to the protocol and procedures and stopping the trial due to safety concerns or lack of enrolment.

The Sponsor-Investigator must provide the DSMC with semiannual reports outlining the above stated data.

13.2.2 Study Monitoring

The study sites agree to allow monitors from the Sponsor-Investigator and/or their representatives, direct access to the study records, the medical records or participants enrolled in the clinical study, and to drug accountability records. Adequate monitoring

space and time will be provided for the Clinical Research Associates. Monitoring for this study will consist of data management review focused on erroneous data, illogical entries, and missing data, and on central monitoring of clinical and operational data reviewed for outliers and trends. On-site monitoring will be targeted based on the results of data management and of central monitoring reviews.

13.2.3 Early Termination of the Trial

As part of their duties to ensure that research participants are protected, the REB or other government organizations may discontinue the study at any time. Regulatory authorities and the Sponsor-Investigator retain the authority to suspend additional enrollment for the entire study as applicable.

The trial may be terminated early on the advice of the independent DSMC based on their review of AEs and SAEs. The trial may also be terminated early due to low enrollment.

13.3 Record Keeping

13.3.1 Data Collection

The Qualified Investigator must maintain detailed records on all study participants. Data for this study will be recorded in the participant's chart and applicable data entered into the study specific eCRFs. Applicable data from the participant's chart should be recorded in the eCRFs completely and promptly, taking time to correct any mistakes.

13.3.2 Data Corrections

Corrections of date entered on source documents/data collection worksheets must be entered into the study eCRF. Data corrections in the eCRF will be automatically tracked.

13.3.3 Source Documents

The Qualified Investigator must maintain adequate and accurate source documents upon which the eCRF for each participant is based. They are to be separate and distinct from the eCRF except for cases in which the Sponsor-Investigator has pre-determined that direct data entry into specified pages of the participant's eCRF is appropriate. These records should include detailed notes on:

- Oral and written communication with participant regarding the study treatment (risks/benefits);
- Participation in trial and signed and dated informed consent forms;
- Inclusion and exclusion criteria details;
- Visit dates;
- Adverse events and concomitant medication;
- Results of relevant examinations;
- Laboratory printouts;

- Participant's exposure to any concomitant therapy (start/stop dates, dosing details);
- Reason for premature discontinuation (if applicable);
- Enrollment number;
- Methods of contraception and fertility status (if applicable);
- Compliance/non-compliance and information about protocol deviation(s) (if applicable).

13.3.4 Data Management

Instructions concerning the recording of study data on the eCRFs will be provided by CTN Data Management group in the form of a Database User Manual. Each study site is responsible for submitting the data in a timely fashion. All data will be maintained on secure computer servers by the CTN.

It is the responsibility of the CTN Data Management group to assure the quality of computerized data for this study. Detailed aspects of data handling will be described in the Data Management Plan.

13.3.5 Record Retention

The Qualified Investigator will maintain all study records according to the ICH-GCP and applicable regulatory requirement(s). Records will be retained for 15 years, in accordance with applicable regulatory requirement(s). If the Qualified Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility and the Sponsor-Investigator must be notified.

14. DISCLOSURE AND PUBLICATION POLICY

The final study results are expected to be published in peer-reviewed journals. The Sponsor-Investigator (Dr. J.-P. Routy) and Collaborator (Dr. S. Isnard) will determine authorship for each manuscript based on the contributions to the study design, study execution, and manuscript completion. No author will be included without prior authorization by the Sponsor-Investigator; authorship will include study investigators who made active contributions to the manuscript as outlined in the ICJME criteria for authorship which states that authorship credit should be based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Authors should meet all 3 criteria.

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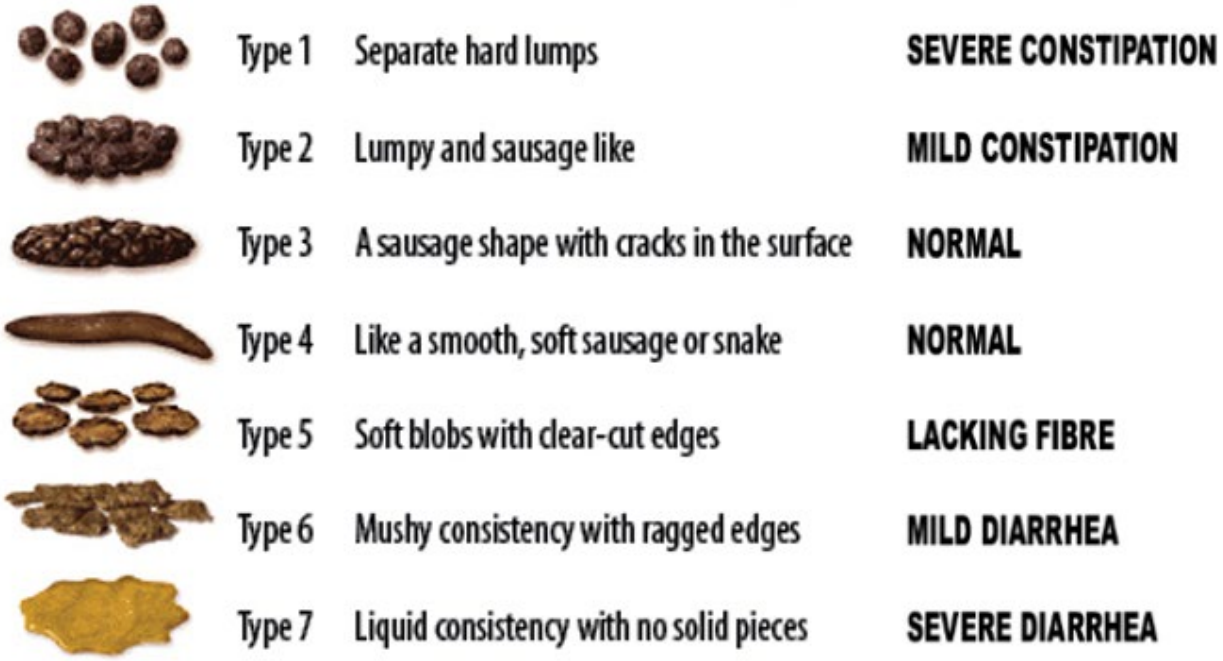
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15. APPENDICES

Appendix 1: Bristol stool scale.



Appendix 2: Full AUDIT Questionnaire

AUDIT

PATIENT: Because alcohol use can affect your health and can interfere with certain medications and treatments, it is important that we ask some questions about your use of alcohol. Your answers will remain confidential, so please be honest.

For each question in the chart below, place an X in one box that best describes your answer.

NOTE: In the U.S., a single drink serving contains about 14 grams of ethanol or "pure" alcohol. Although the drinks below are different sizes, each one contains the same amount of pure alcohol and counts as a single drink:



Questions	0	1	2	3	4	
1. How often do you have a drink containing alcohol?	Never	Monthly or less	2 to 4 times a month	2 to 3 times a week	4 or more times a week	
2. How many drinks containing alcohol do you have on a typical day when you are drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more	
3. How often do you have 5 or more drinks on one occasion?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
4. How often during the last year have you found that you were not able to stop drinking once you had started?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
5. How often during the last year have you failed to do what was normally expected of you because of drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
6. How often during the last year have you needed a first drink in the morning to get yourself going after a heavy drinking session?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
7. How often during the last year have you had a feeling of guilt or remorse after drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
8. How often during the last year have you been unable to remember what happened the night before because of your drinking?	Never	Less than monthly	Monthly	Weekly	Daily or almost daily	
9. Have you or someone else been injured because of your drinking?	No		Yes, but not in the last year		Yes, during the last year	
10. Has a relative, friend, doctor, or other health care worker been concerned about your drinking or suggested you cut down?	No		Yes, but not in the last year		Yes, during the last year	
					Total	

Note: This questionnaire (the AUDIT) is reprinted with permission from the World Health Organization. To reflect drink serving sizes in the United States (14g of pure alcohol), the number of drinks in question 3 was changed from 6 to 5. A free AUDIT manual with guidelines for use in primary care settings is available online at *www.who.org*.

Excerpted from NIH Publication No. 07-3769 **National Institute on Alcohol and Alcoholism** *www.niaaa.nih.gov/guide*

Appendix 3: Modified AUDIT-C Questionnaire

Questions	0	1	2	3	4	Score
How often during the study period did you have a drink containing alcohol?	Never	Monthly or less	2 to 4 times per month	2 to 3 times per week	4 or more times per week	
How many drinks containing alcohol did you have on a day when you were drinking?	1 or 2	3 or 4	5 or 6	7 to 9	10 or more	
How many occasions did you have 5 or more alcoholic drinks during the study period?	Never	Less than monthly	Monthly	Weekly	Daily	