FULL TITLE:	**Influence of a 3-month letermovir treatment on gut inflammation in ART-treated HIV-infected persons in an open labelled controlled randomized study**
Protocol No.	CTNPT 047
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Merck Canada (study drug supply)

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Version No. 5.0

Date: 11 October 2024

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 Sponsor-Investigator and his/her 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 Sponsor-Investigator.

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INVESTIGATOR AGREEMENT

Protocol Title:	**Influence of a 3-month letermovir treatment on gut inflammation in ART-treated HIV-infected persons in an open labelled controlled randomized study**
Protocol No.:	CTNPT 047
Version No.:	5.0
Date:	11 October 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 trial 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 a separate protocol deviation form and will be reported to applicable REB.

I agree to promptly report to the applicable ethics boards any deviations, 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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Investigational Product Supply	Merck Canada, Investigator Studies Program (MISP) Medical and Scientific Liaison

ABBREVIATIONS AND DEFINITONS

Acronym / Abbreviation	Definition
AIDS	Acquired Immune Deficiency Syndrome
AE	Adverse Event
ALT	Alanine transaminase
AST	Aspartate transaminase
ART	Antiretroviral Therapy
CBC	Complete Blood Count
CD	Cluster of Differentiation
CCR	C-C Chemokine Receptor
CHUM	Centre hospitalier de l'université de Montréal
CIHR	Canadian Institutes of Health Research
CMV	Cytomegalovirus
COCQ SIDA	Coalition des organismes communautaires québécois de lutte contre le syndrome immunodéficitaire acquis
CRP	C-Reactive Protein
CTN	CIHR Canadian HIV Trials Network
CVIS	Chronic Viral Illness Service
eCRF	Electronic Case Report Form
EIA	Enzyme Immune Assay
ELISA	Enzyme-linked Immunosorbent Assay
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
HBV	Hepatitis B Virus
HC	Health Canada
hCG	human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HDL	High Density Lipoprotein
HIV	Human Immunodeficiency Virus

ABBREVIATIONS AND DEFINITIONS (continued)

Acronym/Abbreviation	Definition
HLA-DR	Human leukocyte antigen-DR
hs-CRP	High-sensitivity C-reactive protein
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	Investigational Product
IP-10	Interferon-gamma induced protein-10
LBP	Lipopolysaccharide Binding Protein
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharide
MUHC	McGill University Health Centre
NK	Natural Killer (Cell)
PBMC	Peripheral Mononuclear Cell
PLWH	People Living with HIV
REB	Research Ethics Board
qPCR	Quantitative Polymerase Chain Reaction
ddPCR	Digital droplet Polymerase Chain Reaction
PD-1	Programmed Cell Death – 1
SAE	Serious Adverse Event
TILDA	Tat/Rev Induced Limiting Dilution Assay
TNF-α	Tumor Necrosis Factor-alpha

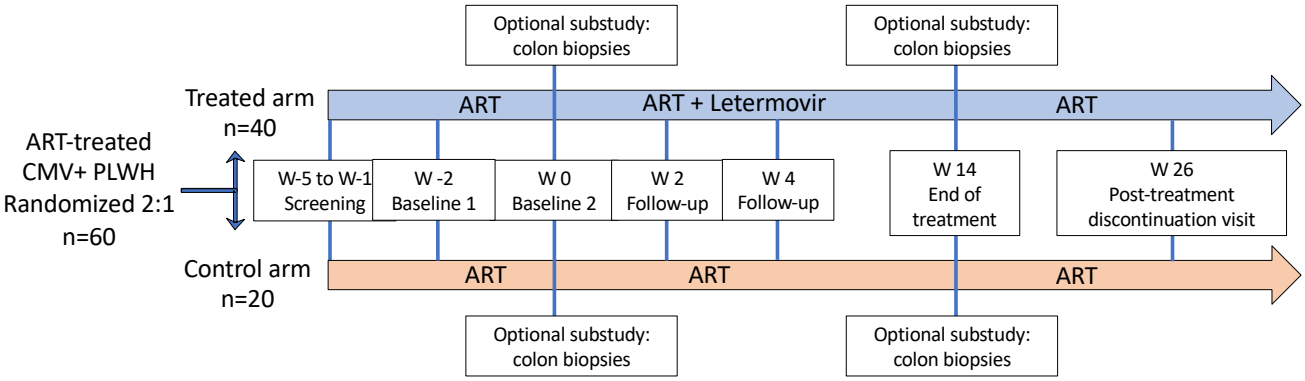
PROTOCOL SYNOPSIS

Full Title	Influence of a 3-month letermovir treatment on gut inflammation in ART-treated HIV-infected persons in an open labelled controlled randomized study
Short Title	Letermovir in ART-treated HIV-infected persons
Protocol and Version No.	CTNPT 047, Version 5.0
Clinical Phase	Phase 2
Study Duration	Enrollment period: July 2022 - June 2026
	Study period: July 2022 - December 2026
Sponsor-Investigator	Dr Jean-Pierre Routy
Number of Centres	3
Study Design	Multi-centre, open-label, randomized, controlled clinical trial
Primary Objective	To assess the influence of CMV replication inhibition with letermovir on gut translocation markers (LPS) in blood of PLWH.
Secondary Objectives	1. To assess the influence of CMV replication inhibition with letermovir on: • Gut permeability markers in blood of PLWH (REG3α and I-FABP). • Inflammation markers (IL-6, sCD14, hsCRP, d-dimers) in blood of PLWH. • Anti-CMV immune response (anti-CMV IgG titers and specific T-cell response) and CMV DNA detection in plasma and PBMCs • In a sub-study, in inflammation in colon biopsies obtained by coloscopy and in CMV DNA detection in gut mucosa 2. To assess the safety and tolerability of letermovir by evaluating adverse events, weight, waist circumference, hematology, serum chemistries, HIV virology and immunology over the course of the study.
Exploratory Objectives	To assess the influence of CMV replication inhibition with letermovir on: 1. HIV reservoir size in blood and mucosal samples. 2. Microbiota composition.
Sample Size	N = 60 (40 treated, 20 controls)
Randomization	2:1
Study Population	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. CMV seropositive (as per clinical lab test). 4. On ART for at least 3 years, and stable ART regimen (same prescription) for at least 3 months. 5.Undetectable viral load < 50 copies/ml for the past 3 years. Viral blips in the past 3 years, below 200 copies/ml, are allowed if preceded and followed by a HIV viremia below 50 copies/ml. 6. CD4 count >400 cells/µL of blood

Investigational Product Description	Anti-CMV specific drug: MK-8228 (letermovir) 480mg tablet MK-8228 (letermovir) 240mg tablet
Administration and Dosing	Participants in the treatment arm will take either 1 tablet of 480mg PO once daily or 2 tablets of 240mg PO once daily.
Duration of Treatment	14 weeks
Control	Standard of care (ART only)
Outcome Measures	Primary : - To assess the influence of letermovir on gut translocation marker LPS in blood of participants receiving or not receiving Letermovir treatment (ELISA in plasma).
	Secondary : 1. To assess the influence of letermovir on gut permeability markers I-FABP and REG3α, and microbial translocation marker BDG in blood of participants receiving or not receiving letermovir treatment (ELISA or Fungitell assay in plasma). 2. To assess the difference in inflammation markers IL-1β, IL-6, IL-8, TNF-α, sCD14, hsCRP, and D-Dimers in blood of participants receiving or not letermovir treatment (single and multiplex ELISA in plasma). 3. To assess the difference in immune activation (HLA-DR, CD38, PD-1) on CD4 and CD8 T-cells in blood (flow cytometry). 4. To assess anti-CMV immune response and CMV DNA detection before and after letermovir in PLWH, assessing anti-CMV IgG titers (ELISA) and CMV specific CD4 and CD8 T-cells responses (IFN-γ/TNF-α/IL-2/IL-10, IL-17 intracellular staining analysis by flow cytometry), and CMV DNA (by digital droplet (dd) PCR in plasma and PBMCs) 5. In a sub-study, to assess gut immune activation (HLA-DR, CD38, PD-1) and gut permeability (Claudin-3, Zonulin-1) in colon biopsies obtained by colonoscopy before and after letermovir treatment. 6. To assess the safety and tolerability of letermovir measured by evaluating adverse events, weight, waist circumference, number of white blood cells, neutrophils, monocytes and lymphocyte, blood hemoglobin concentration, levels of liver enzymes (ALP, ALT, AST), amylase, creatine kinase, D-dimer, HBA1c, Hs-CRP, Lipase, serum phosphatase, total cholesterol, triglycerides, as well as HIV viral load, CD4 count, CD8 count and CD4/CD8 ratio.
	Exploratory : 1. To assess changes in HIV reservoir size (HIV DNA qPCR) in blood and mucosal samples in the 2 study arms and to assess changes before and after letermovir. 2. To collect stool samples in letermovir treated and control groups in order to assess changes in microbiota composition, as a consequence of immune exhaustion

	variations. Microbiota composition will be analyzed in a future study on frozen stool samples.
Statistical Analyses	Analyzed parameters will be compared to mean of the 2 baselines for each participant and fold changes will be compared between the treated and the control group by non-parametric analysis such as Wilcoxon matched pairs test and Friedman test. Microbiota 16S rRNA analysis will be performed. As age and sex have been shown to influence response to different viruses, including HIV, we will assess the influence of those possible confounding factors in multivariable analyses (logistic regression).

STUDY FLOW CHART



1. INTRODUCTION, BACKGROUND, AND STUDY RATIONALE

Cytomegalovirus (CMV) seropositivity is frequent in the general population and even more so in persons living with HIV (PLWH) [1,2]. In the general population, high anti-CMV IgG-titer has been associated with earlier mortality, CD8 T-cell expansion, low CD4/CD8 ratio and increased CD57 senescence marker expression on T cells [3]. Such elevated IgG titer may be linked to transient CMV replication [4,5]. We have shown that gut permeability is correlated with anti-CMV IgG titers in PLWH on antiretroviral therapy (ART) [6]. Increased gut permeability is associated with microbial translocation and systemic inflammation, and correlates with frequency of non-infectious/non-AIDS comorbidities such as cardiovascular diseases, neurocognitive disorders and cancer [7–9]. Moreover, gut permeability has been linked with reduced response to commercialized vaccines [3]. We recently demonstrated that, in HIV elite controllers, a rare subgroup of PLWH who can control HIV replication in absence of ART, CMV coinfection and anti-CMV IgG levels were associated with CD4 count decay [10]. As HIV replication is well controlled with ART, our findings suggest that CMV co-infection is a driving factor behind increased gut permeability and inflammation in PLWH, contributing to immune exhaustion and senescence.

Hunt *et al*. have shown that administration of valganciclovir, an anti-CMV medication, in ART-treated PLWH for 4 weeks was associated with a decrease in immune activation markers compared to the control group [11]. However, long-term use of valganciclovir is not convenient due to its toxicity and its activity is not specific to CMV. Furthermore, its influence on gut permeability has not been assessed.

A novel anti-CMV agent with an excellent safety profile, letermovir, was approved in 2017 for anti-CMV prophylaxis in allogeneic hematopoietic cell transplant recipients. In these patients, a drastic reduction of both low-grade replication and CMV infections, combined with an improved immune reconstitution after the graft was reported [12,13]. *In vitro*, in intestinal tissues reconstituted from primary cells, letermovir anti-CMV treatment could prevent CMV-induced epithelial disruption [14].

1.1 Potential Risks and Benefits to Human Participants

1.1.1. Potential Risks Associated with letermovir

For this study, participants will be receiving two tablets of 240mg or one tablet of 480mg of MK-8228 (letermovir) daily, for 14 weeks in total. This corresponds to the dose recommended for anti-CMV prophylaxis in hematopoietic cell transplant recipients. In this latter population, which is the only one for which letermovir (PREVYMIS®) has been approved in Canada, most frequent side effects are nausea/vomiting and peripheral edema. Cardiac events constitute 13% of all adverse effects, including atrial fabrillation

in 3.5, as such an ECG will be performed during the screening visit and participants with any type of known arrythmia will be excluded. In case of cardiac symptoms during follow-up, another ECG will be performed and participants with arrythmia will be withdrawn from the study and referred to a cardiologist. In practice, those side effects are unfrequent, as illustrated in the pivotal clinical trial of letermovir in which frequency and severity of adverse events were not statistically increased in the letermovir group compared to placebo group (NEJM 2017, [15]).

Finally, based on the Canadian PREVYMIS® Monograph [21], drug-on-drug interactions are possible and the concomitant administration of following medications will constitute exclusion criteria: pimozide, ergotamine, and cyclosporine. For patients using lovastatin, rosuvastatin, simvastatin or bosentan, their dose of these statins will be reduced by 50% for the duration of the letermovir treatment. Due to the long-term effect of statins, such a short-period of dose reduction is not expected to increase cardiovascular risk to participants. Regarding HIV medication, exclusionary agents include darunavir, efavirenz, etravirine and nevirapine. Those drugs constitute parts of ART regimens and participants who receive them will need to be excluded. However, this should not significantly impact recruitment as these drugs are only rarely prescribed in HIV clinics in Quebec.
Exclusionary agents have been discussed and accepted by the MISP Safety Committee of Merck.

1.1.2. Potential risks to blood draws

The risks of drawing blood from a vein include: discomfort at the blood draw site (often momentary); possible bruising, redness, swelling, and/or bleeding at the site (will be managed by putting pressure on site of draw); feeling of lightheadedness when blood is drawn (will be managed by ensuring participants remains seated until dizziness abates); 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, and treatment in case of infection will be provided).

1.1.3. Potential risks and/or discomforts to colon mucosal biopsies

Participants from MUHC 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 this procedure, 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 (Appendix 6).

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. Light to moderate sedation using midazolam and fentanyl could be used during the procedure, in the absence of any contraindication. This sedation procedure is very safe, rare side effects include temporary memory loss, blood pressure drop or vomiting. If the participant experiences severe discomfort despite sedation during the colon mucosal biopsies, the procedure will be stopped immediately; he/she will be seen by a physician and the research nurse will monitor him/her until he/she is ready to go home.

1.1.4 Benefits to Participating in This Study

The effects of CMV latent infection has been widely reported and include persisting inflammation, gut leakage, decreased response to vaccines and to pathogens [3]. As such, a potential beneficial effect of letermovir on those parameters is expected, which is particularly meaningful in the light of the current COVID-19 pandemic. Altogether, findings from this study may clarify the influence of CMV on health, and help to improve treatments for PLWH. The study's results will be published and all participants will be given access to the study findings.

1.2 Study Rationale

Latent CMV infection and occasional replication has been linked with poorer outcome in PLWH. Our hypothesis is that inhibiting CMV replication with a safe anti-CMV agent as letermovir will prevent gut damage and favor gut epithelium repair, decreasing microbial translocation and lower non-AIDS comorbidity risks in ART-treated PLWH.

Based on our experience, and recent trials such at the single arm CTNPT27 Lilac study [16], we propose a randomized controlled trial with a control arm receiving standard of care (ART only) to ensure the specificity of any changes we could observe with letermovir (long term ART treatment could influence inflammation markers). Such a design is optimal for the development of future larger studies, in the case where results would show a clinical interest.

2. STUDY OBJECTIVES AND DESIGN

2.1 Overall Study Design

This is a multi-centre, open-label, randomized, controlled clinical trial to assess the influence of letermovir on gut translocation marker LPS in blood in ART-treated CMV-seropositive adult PLWH. The study will explore the influence of letermovir treatment on

gut damage, with no intention for label change. Participants (n=60) should have a CD4 count greater than 400 cells/µl and a negative viral load. Forty (40) participants will be randomized to receive letermovir (MK-8228 tablet: 1x480 mg or 2x240mg orally) in addition to their usual ART, and 20 participants will receive standard of care alone (ART only) for 14 weeks. Study visits will include screening, 2 baselines, followed by follow-up visits at 2 and 4 weeks and at 14 weeks after starting treatment and 12 weeks after end of letermovir treatment to assess a carry-over effect.
In an optional sub-study, colonoscopies and colon biopsies will be performed before and after letermovir treatment or standard of care alone to assess gut mucosa inflammation.

2.2 Primary Objective(s)

To assess the influence of CMV replication inhibition with letermovir on gut translocation markers (LPS) in blood of PLWH.

2.3 Secondary Objective(s)

1. To assess the influence of CMV replication inhibition with letermovir on:
   - Gut permeability markers in blood of PLWH (REG3α and I-FABP).
   - Inflammation markers (IL-6, sCD14, hsCRP, d-dimers) in blood of PLWH.
   - Anti-CMV immune response (anti-CMV IgG titers and specific T-cell response) and CMV DNA detection in plasma and PBMCs.
   - In a sub-study, inflammation in colon biopsies obtained by coloscopy and in CMV DNA detection in gut mucosa.
2. To assess the safety and tolerability of letermovir by evaluating adverse events, weight, waist circumference, hematology, serum chemistries, HIV virology and immunology over the course of the study.

2.4 Exploratory Objectives(s)

To assess the influence of CMV replication inhibition with letermovir on:

1. HIV reservoir size in blood and mucosal samples.
2. Microbiota composition.

2.5 Sub-studies

In an optional sub-study, colonoscopies and colon biopsies will be performed before and after letermovir treatment or standard of care alone to assess gut mucosa inflammation.

3. SELECTION AND ENROLLMENT OF PARTICIPANTS

3.1 Number of Participants

A total of 60 patients will be recruited, including 40 participants receiving letermovir and 20 control participants receiving standard of care alone.

Participants will be recruited in approximately 4 HIV clinics in Québec.

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. CMV seropositive (as per clinical lab test). This test will only be performed if no previous positive results are available.
4. On ART for at least 3 years, and stable ART regimen (same prescription) for at least 3 months.
5. Patients taking statins will be directed to reduce their statin dose by 50% for the duration of the study because of potential drug-drug interactions. This dose modification is not expected to increase cardiovascular risk.
6. Undetectable viral load < 50 copies/ml for the past 3 years. Viral blips in the past 3 years, below 200 copies/ml, are allowed if preceded and followed by a HIV viremia below 50 copies/ml.
7. CD4 count >400 cells/μL of blood
8. Able to communicate adequately in either French or English.
9. Able to understand and willing to provide written informed consent prior to screening.
10. Women of childbearing potential must have a negative serum pregnancy test.
11. 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:
    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, started at least 30 days before screening, 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. Another method approved by the Investigator with published data showing that the expected failure rate is <1% per year preferably with one barrier method.

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

12. 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.
13. Sexually active men with a female partner of childbearing potential must agree to one of the following methods of birth control:
    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.

3.3 Exclusion Criteria

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

1. Patients who are hypersensitive to letermovir or to any ingredient in the formulation of MK-8228 as described in the Letermovir (MK-8228) Investigator Brochure [20], including any non-medicinal ingredient, or component of the container.
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 including COVID-19 (as tested by PCR).
4. Co-infection with active Hepatitis B or C Virus.
5. Current use or have used in the past 3 months: immune-modulatory agents, antibiotics, Metformin or Morphine as these drugs modulate inflammation and/or gut microbiota composition.
6. Current use of any drug with known drug-on-drug interaction with letermovir, as described in the Letermovir (MK-8228) Investigator Brochure [20], including pimozide, ergot alkaloids, cyclosporine. Regarding HIV medication, exclusionary agents include darunavir, efavirenz, etravirine and nevirapine.
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 3 months, including any previous participation in this study.
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 at the screening visit, suggesting an alcohol abuse problem.
12. Patients with moderate hepatic impairment combined with moderate or severe renal impairment (CrCl less than 50 mL/min).

3.4 Strategies for Recruitment

Participants will be recruited in approximately 3 HIV clinics in Québec, with more than 6000 PLWH followed in total. Teleconferences and face-to-face meetings will be organized between the Qualified Investigators and study staff to help promote patient recruitment and follow-up during the study. The Sponsor-Investigator and all Qualified Investigators will promote the 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 induced by CMV. These talks will target primary care physicians that treat PLWH, other infectious diseases physicians, and PLWH community/advocacy groups in the cities where the study will be conducted.

3.5 Enrollment procedures

Study staff will provide information about the study and its procedures to prospective participants and ask about their general health. Study staff will review inclusion and exclusion criteria. Participants will be informed that they can withdraw from the study at any time. Participants will be informed that their participation in the study is confidential. 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. 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. No study procedure will be performed prior to acquiring informed consent from participants.

3.6 Sub-study Enrollment Procedures

Participants will be invited to enroll in an optional sub-study which includes coloscopies before and after treatment. Access to tissues as colon biopsies remains difficult; however, we have shown that colonoscopies are beneficial for participants as colon polyps are also removed during the procedure [17]. We expect a high degree of participation acceptance in the proposed sub-study, as PLWH in our clinic have a mean age of 54 years and would benefit from colon cancer screening. However, only 50% of gut sampling acceptance would be enough to ensure sufficient data collection. Based on previous clinical trials

including gut biopsies by colonoscopy [17–19], we expect to recruit a sufficient number of participants.

3.7 Co-enrollment Guidelines

Participants enrolled in this study must not be enrolled in other clinical trials during the duration of this study, nor should they enroll in any other clinical trial until 30 days after completion of this study to avoid potential overlapping bias. In addition, participants enrolled in this study (whether completed or not) shall not be re-enrolled at any point, regardless of assigned treatment arm. However, it is acceptable for participants to 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.
- Safety reasons (e.g. Serious adverse drug reactions, potential significant drug interaction).
- Pregnancy or desire to become pregnant during study period.
- Participant is 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 From (eCRF) and decide whether the participant is to be withdrawn.
- Loss to follow-up. The Investigator will attempt to reach the participant by phone, email or mail at least 3 times during a 3-week period 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 will be replaced to ensure a minimum of 60 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 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 End of Treatment Visit (Visit 6) are performed, including the assessment of AEs and medication compliance. If the participant discontinues the study within 15 days prior to either the Week 4, Week 14 or Week 26 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 will 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 procedure 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 will be entered into the eCRF as unscheduled visits. If a participant is withdrawn from the study due to study drug intolerability, an AE will be recorded **regardless of grade**.

5. RANDOMIZATION AND BLINDING PROCEDURES

5.1 Randomization

Participants will be randomized 2:1 (letermovir: standard of care). Randomization will be performed on a website, which will be set up by the CTN.

6. STUDY TREATMENTS

6.1 Investigational Product Description

Product: MK-8228
Generic Name: letermovir
Description: pink tablets of 480mg, or yellow tablets of 240mg
Mechanism of action: CMV terminase complex inhibitor

Non-medicinal ingredients:
Colloidal silicon dioxide, croscarmellose sodium, magnesium stearate, microcrystalline cellulose and povidone 25. Film-coating: hypromellose 2910, iron oxide yellow, and (only

for 480 mg tablets) iron oxide red, lactose monohydrate, titanium dioxide and triacetin. Carnauba wax is added as a polishing agent.

6.1.1 Expected Side Effects

The safety of letermovir (PREVYMIS®) was evaluated in a Phase 3 randomized, double-blind, placebo-controlled trial (P001) through Week 14 post-transplant and were followed for safety through Week 24 post-transplant [21]. The most commonly reported adverse reactions occurring in at least 1% of participants in the PREVYMIS® group through Week 24 post-transplant and at a frequency greater than placebo were nausea, diarrhea, and vomiting (see Table below).

Table 2 - P001 Adverse Reactions Reported in ≥1% HSCT Recipients in the PREVYMIS® Group and at a Frequency Greater than Placebo Through Week 24 Post-Transplant

Adverse Reaction	PREVYMIS® (N=373)	Placebo (N=192)
	n (%)	n (%)
Gastrointestinal		
nausea	27 (7.2)	7 (3.6)
diarrhea	9 (2.4)	2 (1.0)
vomiting	7 (1.9)	2 (1.0)

Serious adverse reactions through week 24 post-transplant were reported in 6 (1.1%) participants with 3 (0.8%) in the PREVYMIS® group and 3 (1.6%) in the placebo group. The reported serious adverse reactions, which had a temporal association but no other plausible causal relationship to study treatment, were pancytopenia, thrombocytopenia, and delayed engraftment in the PREVYMIS® group and Bowen's disease, mental status changes, and acute kidney injury in the placebo group.

Cardiac Adverse Events: Cardiac adverse events were more common in participants receiving PREVYMIS® (13%) compared to participants receiving placebo (6%). The most common cardiac adverse events were tachycardia (reported in 4% of PREVYMIS® participants and in 2% of placebo participants) and atrial fibrillation PREVYMIS® (reported in 3.5% of PREVYMIS® participants and in 1% of placebo participants). These adverse events were mostly considered mild or moderate in severity. Hypersensitivity was reported with PREVYMIS® in one participant. Overall, similar proportions of participants in each group discontinued study medication due to an adverse reaction (4.8% PREVYMIS® vs. 3.6% placebo). The most frequently reported adverse reactions that led to discontinuation of PREVYMIS® were nausea (1.6%), vomiting (0.8%), and abdominal pain (0.5%).

Abnormal Laboratory Findings: Hematologic, Clinical Chemistry and Other Quantitative Data overall, the percentage of participants with potentially clinically significant changes in laboratory values (e.g., hematology, chemistry, renal, and hepatic function) was similar in the PREVYMIS® and placebo groups. There were no differences in the incidence of, or time to, engraftment (defined as absolute neutrophil count ≥ 500/mm3 on 3 consecutive days after transplantation) between the PREVYMIS® and

placebo groups. Biomarkers of testicular toxicity were evaluated in male participants. The changes from baseline in male sex hormones (serum inhibin B, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone) were similar in the PREVYMIS® and placebo groups.

6.1.2 Formulation, Storage and Handling

MK-8228 (letermovir) is formulated as 240mg or 480mg tablets and will be provided in appropriated bottles by Merck. The bottles will be stored between 15°C to 30°C in a temperature controlled/monitored environment at the study site pharmacy until given to participants at Visit 3, 4 and 5.

6.2 Packaging and Labelling

MK-8228 (letermovir) will be provided by Merck in bottles of 17 tablets of 480mg or 240mg.

The MK-8228 (letermovir) 480 mg tablet is a pink oval, bi-convex tablet. Each tablet is debossed with “595” on one side and Merck logo on the other side.

The MK-8228 (letermovir) 240 mg tablet is a yellow oval tablet. Each tablet is debossed with “591” on one side and Merck logo on the other side.

6.3 Investigational Product Supply and Accountability

The study drug will be provided in-kind by Merck at the pharmacy of the MUHC. Each study site will then be supplied sufficient study product based on the number of participants expected to be enrolled. MK-8228 tablets will then be stored and dispensed to participants at each site by the respective hospital pharmacy.

6.4 Dosing and Administration

Participants in the treatment arm will be instructed to take either one tablet of 480mg or two tablets of 240mg of MK-8228 once daily. Tablets will be taken at the same time each day with a meal, preferentially breakfast.

6.5 Dose Reduction or Modification

Any adverse event that may be a symptom, sign or abnormal laboratory finding will be carefully monitored. If a symptom, sign or abnormal laboratory finding consistent with a side effect arise, the participant will be asked to stop taking MK-8228. However, the participant will be followed clinically until the side effect or abnormal lab result(s) resolves. In the case of persistent abnormal results after stopping treatment, the participant will be referred to the proper specialist.

6.6 Concomitant Medications/Natural Remedies/Foods

Concomitant medications and reported natural remedies 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. However, any changes in concomitant medications or supplement regimes (product or dosage) will be tracked and documented at each study visit.

Participants will be asked to refrain from consuming non-prescription drugs for 48h 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.

6.7 Concomitant Alcohol and "Street" Drug Use

No data is available on the concomitant use of alcohol or street drugs with letermovir. It will be recommended to participants to 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. 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 2-7). 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 letermovir effects but may negatively affect baseline values, cigarette smoking is an important parameter that will also need to be documented for each participant.
Regarding street drugs, participants will be informed to use fentanyl with caution as letermovir may increase the concentrations of this drug due to drug-drug interaction through CYP3A inhibition [21].

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 medical judgement.

In the 24 hours prior to a study visit participants will be instructed 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.

6.8 Prohibited Medications and Procedures

Medications with known drug-on-drug interactions with letermovir will be prohibited during the study as previously described in the exclusion criteria and Section 3.3: darunavir, efavirenz, etravirine and nevirapine, pimozide, ergot alkaloids, and cyclosporine. In addition, immune-modulatory agents, antibiotics, Metformin or Morphine will also be exclusion criteria as these drugs modulate inflammation and/or gut microbiota composition.

Based on PREVYMIS® Monograph [21]:
PREVYMIS® (letermovir) should be used with caution with drugs that are CYP3A substrates with narrow therapeutic ranges (e.g., alfentanil, fentanyl, and quinidine1) as co-administration may result in increases in the plasma concentrations of CYP3A substrates. Close monitoring and/or dose adjustment of co-administered CYP3A substrates is recommended.

Co-administration of PREVYMIS® with strong and moderate inducers of transporters (e.g., P-gp) and/or enzymes (e.g. UGTs) is not recommended due to the potential for a decrease in letermovir plasma concentrations.

Rifampin co-administration resulted in an initial increase in letermovir plasma concentrations (due to OATP1B1/3 inhibition) that is not clinically significant, followed by clinically relevant decreases in letermovir plasma concentrations with continued rifampin co-administration. This may result in loss of prophylactic efficacy and necessitate initiation of anti-CMV therapy for CMV reactivation and/or disease.

6.9 Participant Access to Study Medication at Study Closure

MK-8228 (letermovir) will not be provided at the end of the study, as clinical benefits will have not yet been demonstrated in this population.

7. RISK MANAGEMENT

7.1 Acceptable Methods of Birth Control

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:

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, started at least 30 days before screening, 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. Another method approved by the Investigator with published data showing that the expected failure rate is <1% per year preferably with one barrier method.

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

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.

Sexually active men with a female partner of childbearing potential must agree to one of the following methods of birth control during the study and for 2 weeks after study completion:

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.

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 with letermovir. 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 letermovir, the Investigator may use his or her medical judgment regarding whether to continue or to discontinue the study medication. If letermovir treatment is discontinued, the participant will be scheduled for follow-up visit(s) as required to treat the symptoms or adverse event related to letermovir 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 will be performed, if required. 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. In case of abnormal results, 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 (if no previous positive test result available)

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 becomes pregnant during the study will be immediately withdrawn from the study. A pregnancy will be documented as a serious protocol deviation in the eCRF 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: Laboratory evaluations

	Marker	Sample type	Objective	Method
Gut damage	I-FABP	Plasma	Secondary 1	ELISA
	REG3α	Plasma	Secondary 1	ELISA
	Claudin-3	Gut biopsies	Secondary 5	Microscopy
	Zonulin-1	Gut biopsies	Secondary 5	Microscopy
Microbial translocation	LPS	Plasma	Primary	ELISA
	BDG	Plasma	Secondary 1	Fungitell
Gut inflammation	sCD14	Plasma	Secondary 2	ELISA
Systemic inflammation	IL-1β	Plasma	Secondary 2	ELISA[1]
	IL-6	Plasma	Secondary 2	ELISA[1]
	IL-8	Plasma	Secondary 2	ELISA[1]
	TNF-α	Plasma	Secondary 2	ELISA[1]
	IL-10	Plasma	Secondary 2	ELISA[1]
	D-Dimers	Serum	Secondary 2	Clinical lab
	hsCRP	Serum	Secondary 2	Clinical lab
Immune activation/ exhaustion	HLA-DR	PBMCs + gut cells	Secondary 3, 5	Flow cytometry
	CD38	PBMCs + gut cells	Secondary 3, 5	Flow cytometry
	PD-1	PBMCs + gut cells	Secondary 3, 5	Flow cytometry
Anti-CMV immune response	TNF-α	PBMCs	Secondary 4	In vitro stimulation, flow cytometry[2]
	IFN-γ	PBMCs	Secondary 4	In vitro stimulation, flow cytometry[2]
	IL-2	PBMCs	Secondary 4	In vitro stimulation, flow cytometry[2]

	Marker	Sample type	Objective	Method
	IL-10	PBMCs	Secondary 4	In vitro stimulation, flow cytometry[2]
	IL-17	PBMCs	Secondary 4	In vitro stimulation, flow cytometry[2]
	IgG	Plasma	Secondary 4	ELISA
	CMV Protein IE, gE	Gut biopsies	Secondary 4	Microscopy
CMV viremia	CMV DNA detection	PBMCs + Plasma	Secondary 4	Digital droplet PCR[3]
HIV reservoir	HIV DNA	PBMCs + gut cells	Exploratory 1	nested qPCR
Microbiota composition		Stools	Exploratory 2	16S rRNA gene sequencing

1. Inflammatory markers will be assessed with multiplexes (MSD mesoscale) in a non-biased analysis.
2. Briefly, participant's PBMC will be incubated with pool-peptides covering the entire sequences of CMV pp65 protein for 6 hours in the presence of brefeldin A to prevent cytokine secretion. Those antigens were selected as dominant epitopes of both viruses, other epitopes could also be tested. By flow cytometry, intracellular production of cytokines IFN-γ, IL-2, TNF-α, IL-10, and IL-17 will be analyzed in CD4 and CD8 T-cells. Peptides pools of Influenza Matrix protein 1 (MP1) will be used as positive controls, in a similar method already published by our team [21].
3. Briefly, DNA will be extracted from PBMCs and plasma samples using appropriated kits. An in-house ddPCR assay will then allow the detection and quantification of CMV DNA with high sensitivity and specificity.

8.3.1 Research Blood Collection

Blood (~100 ml) will be collected into anti-coagulant tubes (EDTA for plasma and one SST tube for serum) at Visits 2, 3, 4, 5, 6 and 7 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 at each site. PBMCs will be flash frozen and stored in liquid nitrogen at each site. Plasma/serum and PBMC will be stored at each site until all study visits of the participants have been completed, and then shipped to the MUHC research lab of Dr. Routy under the supervision of Dr. Isnard and Dr Berini.

8.3.2 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)[1]. 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[49]. 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.

8.3.3 Microbiota composition

As an exploratory objective, 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 impact on other beneficial microbes (e.g. *Barnesiella* and *Turicibacter*).

8.4 Questionnaires

8.4.1 Full AUDIT Questionnaire

Participants will be administered a full AUDIT (Alcohol Use Disorders Identification Test, Appendix 1)[50] Questionnaire at screening by study staff. 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 (Appendix 2) will be administered by study staff at study visits 2-7. 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 letermovir on inflammation or HIV, but they won't be withdrawn from the study.

8.4.3 Letermovir Adherence Questionnaire and pill count

Participants in the treatment arm will be asked to complete a daily dosing diary (Appendix 3), as a memory aid, which will be given to the participants at week 0 and an adherence questionnaire (Appendix 4), administered by study staff at week 4 (visit 5) and the end of treatment visit (week 14, visit 6). The questionnaire will help determine when doses were missed (near the end or beginning of the study) and if participants were compliant with taking doses with a meal and at the same time of day. Adherence will be measured by calculating the total investigational product doses used/total doses expected (days in inter-visit period) at each visit. The amount used will be determined from the total IP doses dispensed – unused IP doses returned at each follow-up visit (Visit 4 and 5) and at the end of treatment visit (Visit 6). Pill counts of returned (unused) tablets will be performed by trained staff. Discrepancy between participant's questionnaire and pill count will be recorded. A sub-analysis will be performed after excluding those with reduced adherence. When discrepancies are observed between a pill count, participant questionnaire and participant diary, the pill count will take precedence.

Bristol score Questionnaire
Bristol score Questionnaire (Appendix 5) will be administered by study staff at study visits 1-7. This questionnaire will help determine if participants have experienced differences in bowel movements during the study.

8.5 Stored Research Specimens and Plans for Possible Future Testing

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

Once all study visits have been completed, every site will send all research specimens to the MUHC research lab of Dr. Routy.

Plasma/serum and PBMC samples will be stored at -80°C until ready to be analyzed at the MUHC research lab of Dr. Routy. PBMC may be sent to Dr Chomont's lab at the CHUM (Montréal) to perform TILDA, if sufficient samples are available.

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.

Fecal collection will be performed and stored 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 the start of the study (Baseline Visits 1 and 2), during the study (Visits 4 and 5), at the end of therapy (Visit 6) and at 12-weeks post-intervention (Visit 7) to assess microbial changes. Instructions on stool sample collection and sample collection bottle 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 sample will be stored in a -80°C freezer at the respective site. Once all stool samples have been collected, stool samples will be shipped to the MUHC for processing. DNA will be isolated and sent to Dr. Marette's lab at Laval University for rDNA sequencing.

9. STUDY PROCEDURES

9.1 Schedule of Events

Table 2: Schedule of Events

Visit Type	Screening	Study Visits					
		Baseline 1	Baseline 2	Treatment			Post-treatment
Visit Window **Procedures:**	**Week -5 to -1**	**Week -2 (±7 days)**	**Week 0 (Day 0)**	**Week 2 (±3 days)**	**Week 4 (±3 days)**	**Week 14 (±7 days)**	**Week 26 (±7 days)**
Visit No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Informed Consent	X						
Oral re-consent		X					
Eligibility Assessment	X	X	X				
Concomitant Medication	X	X	X	X	X	X	X
Medical History	X						
Complete Physical Exam, Vital Signs & Waist Circumference	X						
Targeted Physical Exam, Vital Signs & Waist Circumference		X	X	X	X	X	X
Adverse Event Assessment		X	X	X	X	X	X
Serum Pregnancy Test	X	X	X	X	X	X	X
Hematology*	X	X†	X	X	X	X	X
Serum Chemistry**	X	X†	X	X	X	X	X
Serology***	X		X				
Serology - HIV-1 Viral Load****	X	X†	X	X	X	X	X
Markers of gut barrier integrity, inflammation and microbial translocation++		X	X	X	X	X	X
Immune activation markers/cytokines (ELISA)****		X	X	X	X	X	X
Monocyte and T cell activation markers+		X	X	X	X	X	X
Anti-CMV IgG and IgM in Serum, anti-CMV CD4 and CD8 T-cells in PBMC, CMV DNA in plasma and PBMC		X	X	X	X	X	X
Size of HIV reservoir in Latently Infected CD4 T cells+++		X	X	X	X	X	X
Stool sample collection and microbiota composition++++		X	X	X	X	X	X
Alcohol use (AUDIT-Full), Appendix 1	X						
Alcohol use (AUDIT-C), Appendix 2		X	X	X	X	X	X
Bristol score questionnaire, Appendix 5	X	X	X	X	X	X	X
Investigational Product Dispensation#			X	X	X		
Dispense Dosing Diary#			X				
Collect Dosing Diary#				X	X	X	
Investigational Product Compliance#				X	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) if no positive test result available, Hepatitis B virus (HBV), 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: LPS, BDG, sCD14, I-FABP, Reg3a (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 14 samples to assess the HIV viral reservoir (Exploratory analysis).
++++qPCR of *A. muciniphila*, 16S and 18S rDNA sequencing for other members of the microbiota.
#Treatment arm only
##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)
‡ If required

9.2 Visit 1: Screening Visit (-5 to -1 weeks)

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, 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 3 years before screening.

•A physical exam will be performed; vital signs (seated blood pressure (BP), heart rate (HR), respiration rate, oral temperature) waist circumference weight and height will be measured.
•Blood (~20 ml) will be collected for:
- Hematology and serum chemistry tests including a pregnancy test for female participants of child-bearing capacity.
- Serology – HIV viral load, Cytomegalovirus (CMV) if no previous positive test result available, Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV).

•A Full AUDIT (Alcohol Use Disorders Identification Test) questionnaire will be administered by study staff to assess participant dependence on alcohol (scores of 8+ suggest alcohol abuse).
•A questionnaire will be administered by study staff to assess the quality and frequency of bowel movements, based on the Bristol score.
• Participant will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 2.
• Participants will be assigned a code at screening.

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 if eligibility requirements are met. If the participant meets eligibility criteria, randomization to the control or treated arm will be performed. The Investigator or study staff will perform a simple web-based randomization process. Eligible participants will be assigned to one of two treatment groups in a 2:1 ratio as per mentioned randomized protocol.

9.3 Visit 2: Baseline 1 (Week -2 ±7 days)

During this visit the following will take place:
- Confirmation of informed consent and eligibility criteria have been met.

- Review of any pre-existing medical conditions/signs/symptoms.
- Concomitant medications (including ART), alcohol use (modified AUDIT-C) and bowel movements will be surveyed.
- Targeted physical examination, if required, vital signs, and waist circumference.
- Blood (about 90 ml, including 70 ml for research) will be collected for:
    - Hematology and serum chemistry tests including a pregnancy test for female participants of child-bearing capacity.
    - Serology HBV, HCV, HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, BDG, REG3α, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
        - Anti-CMV IgG and CMV DNA detection by ddPCR
    - Peripheral blood mononuclear cell (PBMC) isolation and freezing in liquid nitrogen to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry, as well as CMV specific CD4 and CD8 T-cells.
    - 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 a stool collection kit and instructions for collecting a stool sample in the 24 hours prior to Visit 3.

9.4 Visit 3: Baseline 2 (Week 0, Day 0)

This visit must be scheduled between 7 and 21 days after Visit 2.
During this visit, the following will take place prior to product administration:

- Confirmation of informed consent and eligibility criteria have been met.
- Review of any pre-existing medical conditions/signs/symptoms.
- Concomitant medications (including ART), alcohol use (modified AUDIT-C) and bowel movements will be surveyed.
- Targeted physical examination, if required, vital signs and waist circumference.
- Blood (about 90 ml, including 70 ml for research) will be collected for:
    - Hematology and serum chemistry tests including a pregnancy test for female participants of child-bearing capacity.
    - Serology HBV, HCV, HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, BDG, REG3α, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
        - Anti-CMV IgG and CMV DNA detection by ddPCR
    - 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, as well as CMV specific CD4 and CD8 T-cells.

  - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR).
- AE assessment.
- 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.
- If a participant is randomized to the **treatment** arm, a 17-day (2 weeks + 3 days) supply of investigational product (IP)will be dispensed and a dosing diary given to complete daily during the 14 weeks of treatment.
- If a participant is randomized to the **control** arm, they will be required to continue their current care and medications for the duration of the study.

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 and CMV in the rectal mucosa.

9.5 Visit 4: Follow-up Visit 1 (Week 2 ± 3 days)

During this visit, the following will take place:

- Ongoing confirmation of informed consent
- Review of any pre-existing medical conditions/signs/symptoms.
- Concomitant medications (including ART), alcohol use (modified AUDIT-C) and bowel movements will be surveyed.
- Targeted physical examination, if required, vital signs and waist circumference.
- Blood (about 90 ml, including 70 ml for research) will be collected for:
  - Hematology and serum chemistry tests including a pregnancy test for female participants of child-bearing capacity.
  - Serology HBV, HCV- HIV viral load.
  - Plasma/serum isolation to check for given markers by ELISA:
    - Markers of gut barrier integrity (LPS, BDG, REG3α, I-FABP);
    - Immune activation (sCD14);
    - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
    - Anti-CMV IgG and CMV DNA detection by ddPCR
  - Peripheral blood mononuclear cell (PBMC) isolation and freezing in liquid nitrogen to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry, as well as CMV specific CD4 and CD8 T-cells.
  - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR)
- AE assessment.
- 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.

- Collection of unused IP and completed dosing diary pages, if applicable.
- For participant in the **treatment** arm, study product compliance will be assessed by using a pill count, and review of dosing diary.
- For participant in the **treatment** arm, a 17-day (2 weeks + 3 days) supply of IP will be dispensed. In addition, a second back-up bottle of IP may be dispensed to ensure no lapse in treatment due to scheduling. (partly due to restriction of supply packaging (17 tablets/bottle).
- Participant in the **control** arm will continue their current care and medications for the duration of the study.

9.6 Visit 5: Follow-up Visit 2 (Week 4 ± 3 days)

During this visit, the following will take place:

- Ongoing confirmation of informed consent.
- Review of any pre-existing medical conditions/signs/symptoms.
- Concomitant medications (including ART), alcohol use (modified AUDIT-C) and bowel movements will be surveyed.
- Targeted physical examination, if required, vital signs and waist circumference.
- Blood (about 90 ml, including 70 ml for research) will be collected for:
    - Hematology and serum chemistry tests including a pregnancy test for female participants of child-bearing capacity.
    - Serology HBV, HCV- HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, BDG, REG3α, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
        - Anti-CMV IgG and CMV DNA detection by ddPCR
    - Peripheral blood mononuclear cell (PBMC) isolation and freezing in liquid nitrogen to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry, as well as CMV specific CD4 and CD8 T-cells.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR)
- AE assessment.
- 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 6.
- Collection of unused IP tablets and completed dosing diary pages, if applicable.
- For participant in the **treatment** arm, investigational product compliance will be assessed by using an adherence questionnaire, pill count, and review of dosing diary.
- For participants in the **treatment** arm, an 11-week (10 weeks + 7 days) supply of IP will be dispensed (a small additional amount of IP tablets may be supplied (~8 tablets) to accommodate limitations of drug packaging (17 tablets/bottle)).

- Participant in the **control** arm will continue their current care and medications for the duration of the study.

9.7 Visit 6: End of Treatment Visit (Week 14 ± 7 days)

This visit will be of approximately one-hour duration. Participants must complete study medication (14 weeks) prior to visit. The participant will undergo the following procedures:

- Ongoing confirmation of informed consent.
- Concomitant medications (including ART), alcohol use (modified AUDIT-C) and bowel movements will be surveyed.
- Targeted physical examination, if required, vital signs and waist circumference.
- Blood (about 90 ml, including 70 ml for research) will be collected for:
    - Hematology and Chemistry tests including pregnancy test for female participants of child-bearing capacity.
    - Serology HBV, HCV- HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, BDG, REG3α, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
        - Anti-CMV IgG and CMV DNA detection by ddPCR
    - Peripheral blood mononuclear cell (PBMC) isolation and freezing in liquid nitrogen to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry, as well as CMV specific CD4 and CD8 T-cells.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR)
- 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 7.
- Collection of unused IP and completed dosing diary pages, if applicable.
- For participant in the **treatment** arm, investigational product compliance will be assessed by using an adherence questionnaire, pill count and review of dosing diary.
- Participant in the **control** arm will continue their current care and medications for the remainder of the study.

<u>Optional sub-study procedure: colon mucosal biopsy</u>

Participants that consent to the optional sub study (separate consent form, only in Montreal site) will have a colon mucosal biopsy to assess the amount of T cell activation and to determine the amount of HIV and CMV in the rectal mucosa.

9.8 Visit 7: Post-treatment discontinuation Visit (Week 26 ± 7 days*)*

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

- Ongoing confirmation of informed consent
- Concomitant medications (including ART), alcohol use (modified AUDIT-C) and bowel movements will be surveyed.
- Targeted physical examination, if required, vital signs and waist circumference.
- Blood (about 90 ml, including 70 ml for research) will be collected for:
    - Hematology and Chemistry tests including pregnancy test for female participants of child-bearing capacity.
    - Serology HBV, HCV- HIV viral load.
    - Plasma/serum isolation to check for given markers by ELISA:
        - Markers of gut barrier integrity (LPS, BDG, REG3α, I-FABP);
        - Immune activation (sCD14);
        - Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)/anti-inflammatory cytokine (IL-10).
        - Anti-CMV IgG and CMV DNA detection by ddPCR
    - Peripheral blood mononuclear cell (PBMC) isolation and freezing in liquid nitrogen to check monocytes and T cells for markers of activation (HLA-DR, CD38) and T cell exhaustion (PD-1) by flow cytometry, as well as CMV specific CD4 and CD8 T-cells.
    - Measuring the size of the HIV reservoir in PBMC-isolated latently infected CD4 T cells (assayed by nested qPCR)
- AE assessment.
- Stool sample collection for assessment of gut microbiota composition (stool sample may be brought from participant's home).

9.9 Missed Visits

If a participant misses the scheduled study visit, clinical research staff will try to contact him/her and schedule a visit as soon as possible. Participants who stop taking the study product for any reason will be encouraged to complete any remaining visits through to the final study visit (Visit 7).

9.10 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, will 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, if applicable;
3. Investigational products are returned and inventoried, if applicable;

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.

9.11 Re-contact of Participants after Trial Termination

Participants may be contacted after early termination from the trial due to any safety concerns, AE or serious AE (SAE) identified with the study product after the trial termination. All participants may be contacted after trial termination due to safety concerns, AEs or SAEs identified with the study product. Participants would be contacted by clinical staff and informed of any safety concerns. The Investigator or designated clinic staff would then schedule a follow-up visit with the participant if required to assess the potential safety concern. 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 will also be contacted after the trial if extra testing needs to be 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 for 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, administered 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 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. AEs 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 study treatment.

Stable chronic conditions which are present prior to clinical trial entry and do not increase in severity or frequency 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

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 his/her 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 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 drug 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

Study sites are required to report all SAEs occurring during the course of the study to Dr JP Routy within 24 hours of the site becoming aware of the event.

SAEs will be reported to:

Dr Jean-Pierre Routy

Attention:	Dr Jean-Pierre Routy
Phone:	+ 1 514-843-1558
Fax:	+ 1 514-843-1418
E-mail:	Jean-pierre.routy@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 (or sub-investigator) and Medical Monitor agrees that the event is satisfactorily resolved, becomes chronic, or no further follow-up is required.

The Sponsor-Investigator will ensure that all participating study sites are notified of any 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 SAEs as per the local requirements.

The CTN will be responsible for reporting in an expedited manner, any serious unexpected adverse drug reactions to Health Canada on behalf of the Sponsor-Investigator within the required timelines:

a. Where it is neither fatal nor life-threatening, within 15 days after becoming aware of the information;

b. Where it is fatal or life-threatening, within 7 days after becoming aware of the information. Within 8 days after having initially informed Health Canada of the fatal or life-threatening ADR, submit as complete a report as possible. Follow-up reports of fatal or life-threatening reactions **must** include an assessment of the importance and implication of the findings, including relevant previous experience with the same or similar drugs.

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, questions about changes in medication, and any new symptoms reported.

All AEs (including SAEs) will be followed until resolution or until the Qualified Investigator (or sub-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 Follow-up

Any female participant who becomes pregnant during the study will be instructed to stop taking the study product as soon as they are aware of the pregnancy, to eliminate further exposure to the embryo/fetus, and to inform the Qualified Investigator.

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 will be recorded as a major protocol deviation.

If a participant becomes pregnant during the study, the Qualified 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 will 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 Qualified Investigator’s attention after the participant completed the study and that was considered by the Qualified Investigator to be possibly related to the investigational product must be promptly reported.

11. STATISTICAL CONSIDERATIONS

11.1 General Study Design

The effect of a 14-week course of letermovir therapy on the amount of microbial translocation, immune activation and inflammation will be assessed in this open labelled, randomized interventional study. The comparison will be made by repeated measures of the outcomes (plasma LPS and cytokines levels) at baselines, during and after letermovir use. Intra-patient variability will also be assessed by comparing markers of microbial translocation and inflammation in blood and stool assessed two weeks apart in the same patient prior to letermovir treatment. Baseline level will constitute an adjustment variable given the small number of participants, which might result in an imbalance in the baseline levels between groups.

11.2 Sample Size Considerations/Justification

Comparison of CMV seropositive and CMV seronegative ART-treated PLWH showed an average of 1.7-fold difference of plasma levels of gut I-FABP, LPS or IL-6. As such, a minimum sample size of 30 participants (15 per arm) would allow the detection of changes before/after letermovir therapy, as compared to the control (no letermovir) group. However, we chose a sample size of 60 (40 in the treated arm, 20 in the control arm) to ensure sufficient power to detect significant variation greater than the standard deviation of those markers after letermovir treatment. Statistical analysis for sample size determination was performed by Dr C. Richard (Université de Montréal). Results of this randomized pilot study are descriptive.

11.3 Endpoints and Outcome Measures

For each outcome, two separate regression analyses will be performed:

- A groups comparison of outcome levels at each time point (Visits 4, 5, 6, 7), with baseline level of the outcome as adjustment variable to assess if the change relative to baseline is significantly different between groups.
- A “within group” comparison, to assess if the change relative to baseline is significant within each group.

11.3.1 Primary Endpoint:

1. We will assess the changes in plasma microbial translocation marker LPS (ELISA) after treatment with letermovir compared to the control group.

11.3.2 Secondary Endpoints:

1. We will assess the changes in plasma gut permeability markers I-FABP and REG3α, in microbial translocation marker BDG (ELISA or Fungitell assay in plasma) after treatment with letermovir compared to the control group.

2. We will assess the changes in plasma inflammation markers IL-1β, IL-6, IL-8, TNF-α, sCD14, hsCRP, and d-dimers after treatment with letermovir compared to the control group.

3. To assess the changes in immune activation (HLA-DR, CD38, PD-1) on CD4 and CD8 T-cells in blood (flow cytometry) after treatment with letermovir compared to the control group.

4. To assess the changes in anti-CMV immune response (anti-CMV IgG titers by ELISA and CMV specific CD4 and CD8 T-cells responses with IFN-γ/TNF-α/IL-2/IL-10, IL-17 intracellular staining analysis by flow cytometry) and CMV DNA detection (digital droplet (dd) PCR in plasma and PBMCs) after treatment with letermovir compared to the control group.

5. In a sub-study, to assess the changes in immune activation (HLA-DR, CD38, PD-1) and gut permeability (Claudin-3, Zonulin-1) in colon biopsies obtained by colonoscopy after treatment with letermovir compared to the control group.

6. To assess the safety and tolerability of letermovir measured by evaluating adverse events, weight, waist circumference, number of white blood cells, neutrophils, monocytes and lymphocyte, blood hemoglobin concentration, levels of liver enzymes (ALP, ALT, AST), amylase, creatine kinase, D-dimer, HBA1c, Hs-CRP, Lipase, serum phosphatase, total cholesterol, triglycerides, as well as HIV viral load, CD4 count, CD8 count and CD4/CD8 ratio.

11.3.3 Exploratory Endpoints:

1. To assess the changes in HIV reservoir size (HIV DNA qPCR) in blood and mucosal samples after treatment with letermovir.
2. To assess the changes in microbiota composition after treatment with letermovir.

11.3.4 Sub-study Endpoint and Outcome Measures

1. We will evaluate composition and gut mucosa architecture in the colon sub study at Baseline (Visit 3, Week 0) versus end of letermovir treatment (Visit 6, Week 14). 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).

2. We will evaluate the association between baseline gut microbiota composition (16S rDNA sequencing), and markers of gut integrity (I-FABP, tissue staining) and inflammation (T cell activation, inflammatory cytokines).

3. We will detect CMV proteins by microscopy and DNA by ddPCR in the gut mucosa.

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), with CD4 count >400 cells/μL of blood 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 visits (approximately two weeks apart) agrees, a mean of the data will be used as the true baseline for each parameter. If the two readings do not agree, then 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.

The participant demographic information 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 may also be reported here

11.5 Other Considerations

- The time between Screening (Visit 1) and Baseline (Visits 2 and 3) will be variable for each participant but this will have no impact on the study results.

- The second Baseline (Visit 3) will always be considered Week 0 and that is the start of the treatment.

Each subsequent visit (Week 2, Week 4, Week 14 and Week 26) can vary based on clinic availability and participant availability. Week 2 and 4 visits will be scheduled within a window of ± 3 days. Participants (treatment arm) are required to complete 14 weeks of treatment and the end-of-treatment visit (week 14, visit 6) will be scheduled within a window of ±7 days.
Participants will be asked to minimize the time between end of treatment and study Visit 6.

11.6 Interim Analysis

Interim analysis will be performed after 30 participants have completed the Week 14 visit assessments. The main focus of the interim analyses will be futility on the primary outcome of changes in plasma microbial translocation marker LPS. Analysis of key secondary outcomes by treatment arm will also be performed in order to provide context for the Data Safety Monitoring Committee to make their recommendation about continuance of the study. Adverse events by treatment arm will also be reported.

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. All Investigators (Qualified investigators and sub-investigators) will be thoroughly familiar with the appropriate use of the study treatment as described in the protocol and PREVYMIS® Product Monograph [21].

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's 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.

No genetic testing will be performed on the host DNA in any of the samples (blood, stool or biopsies).

12.2 Informed Consent

All participants will be given detailed oral and written information about the study. Consent forms 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 centre's REB prior to any procedures being done specifically for the trial. Each participant will have sufficient opportunity to discuss the study, have all of 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 form 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 of the signed document will be provided to the participant.

The informed consent process will 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 Case Report Forms, laboratory specimens, evaluation forms, reports, etc. will be kept strictly confidential. All records will be kept in a secure, locked location and only accessible to research staff. Electronic files will be password-protected and hosted on secured hospital networks. Participants will be

identified only by means of a coded number specific to each participant (refer to Section 5). All computerized databases will identify participants by numeric codes only, and will be password protected.

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

12.4 Ethics Committee, or Research Ethics Board

The Ethical Committee or 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/IRB 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 Conduct 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 will also be submitted to the ethics committee/REB.

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 or hazards 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 departures 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, study-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 must 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. Remote or 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 will 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.

13.3.2 Data Corrections

Corrections of data 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 will 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 within Canada 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. L. Royston) 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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Appendix 1: 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:



12 oz. of beer (about 5% alcohol) = **8-9 oz. of malt liquor** (about 7% alcohol) = **5 oz. of wine** (about 12% alcohol) = **1.5 oz. of hard liquor** (about 40% alcohol)

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 2: 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?	0, 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	

Appendix 3: Dosing diary sample

Complete dosing diary will be provided to each participant.



DOSING DIARY – 1 Pill / CTN PT047
Dr. Jean-Pierre Routy

Participant ID Number: ____ ____
Participant Code: ____ ____

Week 1 - Daily Dose: 480mg		
Date	Dose 1 (Every 24h)	ARV taken
Day 1 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 2 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 3 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 4 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 5 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 6 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 7 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		

Week 2 - Daily Dose: 480mg		
Date	Dose 1 (Every 24h)	ARV taken
Day 1 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 2 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 3 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 4 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 5 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 6 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		
Day 7 __/___/____ DD / MMM / YYYY	☐ 1 Pill __:__ ☐ Missed dose	☐ Yes ☐ No __:__ ☐ Missed dose
Comments (Please report any side effects):		

Version: 480mg - 27Jul2022

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Appendix 4 : Adherence Questionnaire

Letermovir study

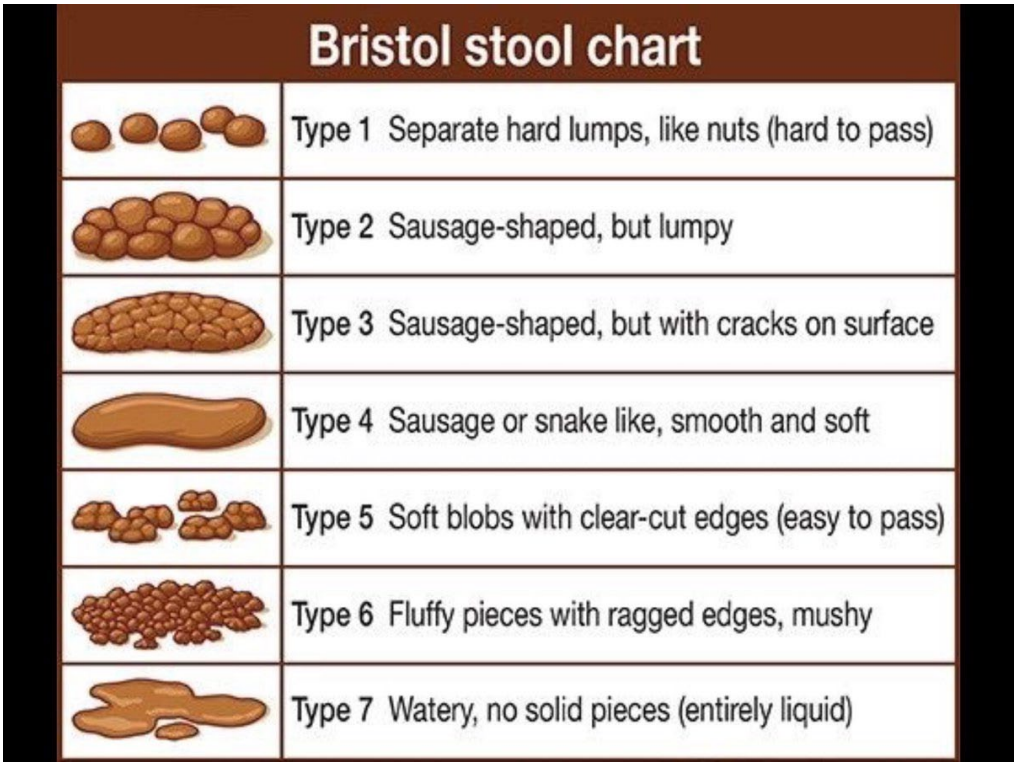
Adherence Questionnaire

This questionnaire asks about study medications that you took recently. Many people find it hard to always remember their pills. For example,

- Some people get busy and forget to carry their pills with them.
- Some people find it hard to take their pills according to special instructions, such as to increase the amount of medication you are taking if you have a herpes flare.
- Some people decide to skip pills to avoid side effects or to just not be taking pills that day.

1.	How many doses of letermovir did you miss in the past month?	○ None ○ One ○ Two ○ Three ○ Four ○ Five or more
2.	Letermovir should be taken once daily. How closely have you followed this specific schedule during the study period?	○ Never ○ Some of the time ○ About half of the time ○ Most of the time ○ All of the time
3.	When was the last time you missed any of your letermovir pill?	○ Within the past week ○ 1-2 weeks ago ○ 2-4 weeks ago ○ 1 month ago or more ○ Never

Appendix 5: Bristol score Questionnaire



Appendix 6: Colonoscopy preparation information sheet

Centre universitaire de santé McGill | McGill University Health Centre

INSTRUCTIONS FOR ANY PICOSULFATE BASED SOLUTION

ADULT DIVISION OF GASTROENTEROLOGY

DO NOT TAKE A PICOSULFATE BASED SOLUTION

If you have the following medical conditions: **Renal or hepatic failure , Ascites**
IMPORTANT- verify with your doctor or pharmacist that this bowel preparation is adequate for you

IMPORTANT • YOU MUST HAVE SOMEONE TO ACCOMPANY YOU HOME AFTER THE TEST AND THE PERSON NEEDS TO PICK YOU UP ON THE UNIT OR THE TEST WILL BE CANCELLED.
• YOU MUST NOT DRIVE FOR THE NEXT 24 HOURS AFTER YOUR EXAM.

IMPORTANT – Please READ the sheet on medication information such as blood thinners and speak to your physician regarding if they need to be stopped. Please bring a copy of your medication list.

HOW TO PREPARE FOR A COLONOSCOPY?

- Purchase **ANY PICOSULFATE BASED SOLUTION (Pico-Salax®, Purg-Odan™)** at your pharmacy *(available without prescription)*
- Purchase **1 BOTTLE OF MAGNESIUM CITRATE**

BOWEL PREPARATION

Day before your appointment :

- **NO SOLID FOOD all day**
- Drink **ONLY CLEAR LIQUIDS** (see list below)

At 6:00 PM (18h00)

- Empty contents of **first** sachet into mug and add 150 ml (5 oz) of cold water. Stir frequently
- Drink the **ENTIRE** prepared picosulfate based solution
- Drink 1.5 to 2 liters of clear liquids over the next 4 hours
- It may take 1 to 3 hours for your bowel movements to become progressively loose and watery (clear)

Other Instructions:

Day of your appointment:

- **NO SOLID FOOD**
- **4 hours before your appointment time**, you must **drink the ENTIRE second** sachet prepared picosulfate based solution (follow instructions as above) **AND** drink **ONLY HALF a bottle of Magnesium Citrate**
  You should continue to drink clear liquids but must **STOP DRINKING ALL LIQUIDS** (including water) **2 hours** before your appointment

WHAT TO DRINK AND EAT TO PREPARE FOR MY COLONOSCOPY?

☑ ALLOWED: Clear Liquid Diet	☒ STOP
NO RED, PURPLE, BLUE or GREEN colored liquids **NO liquids containing PULP** • Clear soup, broth or bouillon • Sports drinks (Gatorade) or Soft drinks (7-Up, Ginger Ale, etc.) • Clear fruit juices • Kool-Aid, Jello • Tea, coffee (no milk nor cream) • Water	**1 week** before colonoscopy: • Iron supplements • Nuts, seeds
Please present yourself to : ☐ **Montreal General Hospital** **1650 Cedar Avenue, room C7-200** **Montreal, QC, H3G 1A4** If you do not have a MGH (red card) please present yourself to room **L6-130** at least 30 minutes prior to your appointment time.	**Please present yourself to**: ☐ **Royal Victoria Hospital, (Glen Site)** **1001 Decarie Blvd, room C03-7158** **Montreal,QC,H4A-3J1** If you do not have a RVH (blue card) please present yourself to room **CRC.0046** at least 30 minutes prior to your appointment time.
☐If you need more information or to cancel your appointment call 514-934-8308 or send fax to 514-934-8531	☐ If you need more information or to cancel your appointment call 514-843-1616 or send fax to 514-843-1421

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