

BMJ Open Influence of letermovir treatment on gut inflammation in people living with HIV on antiretroviral therapy: protocol of the open-label controlled randomised CIAO study

Léna Royston,^{1,2,3,4} Stéphane Isnard,^{1,2,3} Carolina A. Berini,^{1,2,5} Simeng Bu,^{1,2} Peter L. Lakatos,⁶ Talat Bessissow,⁶ Nicolas Chomont,⁷ Marina Klein,^{1,2,3} Bertrand Lebouché,^{1,2,8,9} Alexandra de Pokomandy,^{1,2,9} Nadine Kronfli,^{1,2} Cecilia T. Costiniuk,^{1,2,3} Rejean Thomas,¹⁰ Cécile Tremblay,⁷ Guy Boivin,¹¹ Jean-Pierre Routy^{1,2,12}

To cite: Royston L, Isnard S, Berini CA, *et al.* Influence of letermovir treatment on gut inflammation in people living with HIV on antiretroviral therapy: protocol of the open-label controlled randomised CIAO study. *BMJ Open* 2023;**13**:e067640. doi:10.1136/bmjopen-2022-067640

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2022-067640>).

Received 23 August 2022
Accepted 08 December 2022



© Author(s) (or their employer(s)) 2023. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

For numbered affiliations see end of article.

Correspondence to

Dr Jean-Pierre Routy;
jean-pierre.routy@mcgill.ca and
Dr Léna Royston;
lena.royston@mail.mcgill.ca

ABSTRACT

Introduction Chronic cytomegalovirus (CMV) infection is very frequent in people living with HIV (PLWH). High anti-CMV IgG titres, which may be linked to transient CMV replication, have been associated with earlier mortality, CD8 T-cell expansion, lower CD4/CD8 ratio and increased T-cell senescence. We previously showed that anti-CMV IgG titres correlated with gut permeability in PLWH on antiretroviral therapy (ART), which was associated with microbial translocation, systemic inflammation and non-infectious/non-AIDS comorbidities. Letermovir, a novel anti-CMV drug with a good safety profile, was recently approved for anti-CMV prophylaxis in allogeneic haematopoietic stem cell transplant recipients. A drastic and selective reduction of both low-grade replication and clinically significant CMV infections, combined with an improved immune reconstitution have been reported. *In vitro*, letermovir prevented CMV-induced epithelial disruption in intestinal tissues. Based on these findings, we aim to assess whether letermovir could inhibit CMV subclinical replication in CMV-seropositive PLWH receiving ART and, in turn, decrease CMV-associated gut damage and inflammation.

Method and analysis We will conduct a multi-centre, open-label, randomised, controlled clinical trial, including a total of 60 CMV-seropositive ART-treated PLWH for at least 3 years, with a viral load <50 copies/mL and CD4⁺ count >400 cells/μL. Forty participants will be randomised to receive letermovir for 14 weeks and 20 participants will receive standard of care (ART) alone. Plasma, peripheral blood mononuclear cells (PBMCs), and stool samples will be collected. Colon biopsies will be collected in an optional substudy. We will assess the effect of letermovir on gut damage, microbial translocation, inflammation and HIV reservoir size.

Ethics and dissemination The study was approved by Health Canada and the Research Ethics Boards of the McGill University Health Centre (MUHC-REB, protocol number: MP37-2022-8295). Results will be made available through publications in open access peer-reviewed journals and through the CIHR/CTN website.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ The CIAO study seeks to analyse the effect of cytomegalovirus replication inhibition with letermovir on microbial translocation and inflammation in people living with HIV (PLWH) treated with antiretroviral therapy (ART).
- ⇒ This is a controlled randomised multicentric study including 60 PLWH on ART, 40 participants receiving letermovir for 14 weeks and 20 controls receiving only standard of care.
- ⇒ Extensive measurements related to inflammation, microbial translocation, HIV reservoir size and microbiota composition will be performed.
- ⇒ Potential limitations include a restricted number of participants and the open-label design of the study.

Trial registration number NCT05362916.

INTRODUCTION

Cytomegalovirus infection in people living with HIV receiving antiretroviral therapy

Before widespread antiretroviral therapy (ART) implementation, people living with HIV (PLWH) were subject to frequent and prolonged herpesvirus reactivations with dramatically high morbidity and mortality rates, mainly due to cytomegalovirus (CMV) infection. ART revolutionised the prognosis of PLWH. However, despite suppressed HIV viral load and restored CD4 T-cell counts, PLWH still exhibit increased risk of non-AIDS comorbidities such as cardiovascular disease, neurocognitive disorders and cancer.¹ Evidence associating immune activation, immunosenescence and accelerated ageing in PLWH are increasingly reported,

and coinfections with other viruses including CMV have been shown to contribute to exacerbation of HIV-induced immune activation and the development of non-AIDS comorbidities.²

CMV chronic infection is frequent in the general population and even more common in PLWH.^{3,4} In Canada, the prevalence of CMV infection is the second lowest worldwide but there is still 50% of seropositivity in the general population and 84% in PLWH.³ Following primary infection, CMV establishes true episomal latency mainly in haematopoietic stem cells, and its persistence is characterised by intermittent episodes of productive infection and shedding in absence of cytopathogenic effect.⁵ The gastrointestinal tract represents the largest site of CMV persistence, with ongoing low-grade replication inducing local and systemic inflammation.⁶

In the general population, CMV chronic infection, which is reflected by high anti-CMV IgG titre, has been associated with earlier mortality, CD8 T-cell expansion, low CD4/CD8 ratio and increased CD57 senescence marker expression on T-cells.⁷ In elite HIV controllers, a rare subgroup of PLWH who can control HIV replication in absence of ART, we reported the association of anti-CMV IgG levels with a faster CD4 T-cells count decay, independently of age.⁸ Such elevated anti-CMV IgG titre may be linked to transient CMV replication events.^{9,10}

Role of CMV in gut damage and immune activation during HIV infection

Damage to the gastrointestinal epithelial gut barrier and subsequent translocation of microbes and their byproducts in the circulation constitute hallmarks of HIV infection and contributes to systemic inflammation, immune activation and non-AIDS comorbidities development.^{11,12} Increased gut permeability has been shown to correlate with development of non-infectious/non-AIDS comorbidities such as cardiovascular diseases, neurocognitive disorders and cancer.^{12–14} Moreover, gut permeability has been linked to reduced response to commercialised vaccines.⁷ The precise mechanisms responsible for gut damage and epithelial permeability in PLWH are not fully understood and those damages do not recover completely with ART. Bacterial translocation, with markers including lipopolysaccharide (LPS) and soluble CD14 (sCD14), is known to constitute a major cause of immune activation.^{11,15} Moreover, we have recently shown that circulating beta-D-glucan (BDG), a marker of fungal translocation, also contributes to immune activation, independently of LPS.^{12,16,17}

Increasing evidence suggests that CMV infection is an important contributor to gut permeability in PLWH. In a recent cross-sectional study, we have reported that CMV seropositivity was associated with higher plasma levels of gut damage markers (intestinal fatty-acid binding protein, I-FABP) and microbial translocation (LPS, BDG) in both PLWH and HIV-uninfected participants.¹⁸ Interestingly, gut leakage is associated with an increase of pro-inflammatory cytokines (CXCL13, interleukin 6 (IL-6),

IL-8) only in PLWH.¹⁸ A correlation between gut damage/microbial translocation markers and anti-CMV IgG levels was found, independently to anti-EBV IgG levels or total IgG, IgM, IgA. In addition, and as previously reported by other groups, CMV seropositivity was associated with elevated CD8 T-cell counts and lower CD4/CD8 ratio. In a study analysing gut biopsies of 19 ART-treated PLWH, CMV detection was associated with disrupted epithelial barrier, with decreased zonula occludens-1 immunostaining, a marker of tight junctions.⁶ Altogether, these results strongly suggest that CMV coinfection is a driving factor behind increased gut permeability and inflammation in PLWH, contributing to immune exhaustion and senescence.

Interventions to inhibit CMV replication to reduce inflammation in PLWH

In 2011, Hunt *et al* showed that a 4-week administration of valganciclovir, an anti-CMV medication, in ART-treated PLWH was associated with a decrease in immune activation markers compared with a control group.¹⁹ However, long-term use of valganciclovir is not recommended due to its haematological toxicity. Moreover, valganciclovir activity is not specific to CMV and can inhibit replication of other herpesviruses. Finally, its influence on gut permeability has not been assessed.

Letermovir, a CMV DNA terminase complex inhibitor, has been approved in 2017 for primary anti-CMV prophylaxis in allogeneic haematopoietic stem cell transplant (HSCT) recipients.^{20,21} This drug specifically inhibits CMV replication and has a better safety profile compared with other anti-CMV drugs like ganciclovir or foscarnet, with very few low-grade drug-related adverse events.^{20,22} In HSCT recipients, a drastic reduction of both low-grade replication and CMV infections, combined with an improved immune reconstitution after HSCT was reported.^{20,23} *In vitro*, in intestinal tissues reconstituted from primary cells, letermovir prevents CMV-induced epithelial disruption.⁶ Outside of the transplant recipient patients and in PLWH, the effect of letermovir-induced CMV inhibition remains unknown.

Objective

The aim of this study is to assess the influence of CMV replication inhibition with letermovir on microbial translocation and inflammation in PLWH receiving ART.

Primary outcomes

The primary outcome will be to evaluate the effect of a 14-week administration of letermovir on microbial translocation markers (LPS) in the blood of PLWH, assessed by means of ELISAs, compared with PLWH receiving standard of care alone.

Secondary outcomes

The secondary objectives will be to assess the changes of the following, before and after a 14-week administration

of letermovir and 8 weeks after letermovir discontinuation, compared with PLWH receiving standard of care alone:

1. Gut permeability markers in plasma (BDG, LPS binding protein, REG3 α and I-FABP).
2. Inflammation markers (IL-6, sCD14, high sensitivity C reactive protein (hsCRP), d-dimer) in plasma.
3. Anti-CMV immune response (anti-CMV IgG titres and specific T-cell response) and CMV DNA detection in plasma and PBMCs.

Exploratory outcomes

The exploratory outcomes of this study will be the following:

1. Difference in HIV reservoir size in blood samples before and after a 14-week administration of letermovir.
2. Difference in microbiota composition (16S rDNA sequencing) before and after a 14-week administration of letermovir.

Substudy outcomes

In a subset of participants who will consent to have colon biopsies collected before and at the end of the 14-week administration of letermovir, substudy outcomes will be the following:

1. Changes in gut mucosa architecture.
2. Changes in local gut inflammation and CMV DNA detection in colon biopsies.
3. Difference in HIV DNA reservoir size in colon mucosal samples.

METHODS AND ANALYSIS

Study design, settings, sample size and recruitment strategy

Trial CTN PT047, referred as the CIAO ('CMV Inhibition with letermovir in ART-treated people living with HIV: an

Open-label, randomized, controlled trial') study is a multi-centre, open-label, randomised, controlled clinical trial to assess the influence of letermovir on gut translocation (LPS and BDG markers) in blood in ART-treated CMV-seropositive adult PLWH; protocol version # 2.1; 27 July 2022. The study sponsor is Dr. Jean-Pierre Routy and the study support are the CIHR Canadian HIV Trials Network (CTN) and Merck Sharp & Dohme Corp (study drug supply in-kind). The study protocol fulfils the requirements of the 2013 Standard Protocol Items: Recommendations for Interventional Trials guidelines.^{24 25}

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/ μ L and an undetectable viral load. Forty participants will be randomised to receive letermovir (PREVYMIS 480mg or 2 \times 240mg orally) daily 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, two baseline visits to assess intraparticipant variability in all parameters, followed by follow-up visits at 2, 4 and 14 weeks after starting treatment, as well as 12 weeks after ending the letermovir treatment to assess a possible carry-over effect.

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

Assessment of outcomes will be made through various measures at baseline and throughout the study period (figure 1 and table 1).

A total of 60 ART-treated participants living with HIV will be enrolled in four clinical centres: (1) at the Chronic Viral Illness Service at the McGill University Health Centre (MUHC), Montreal, QC; (2) Centre de recherche

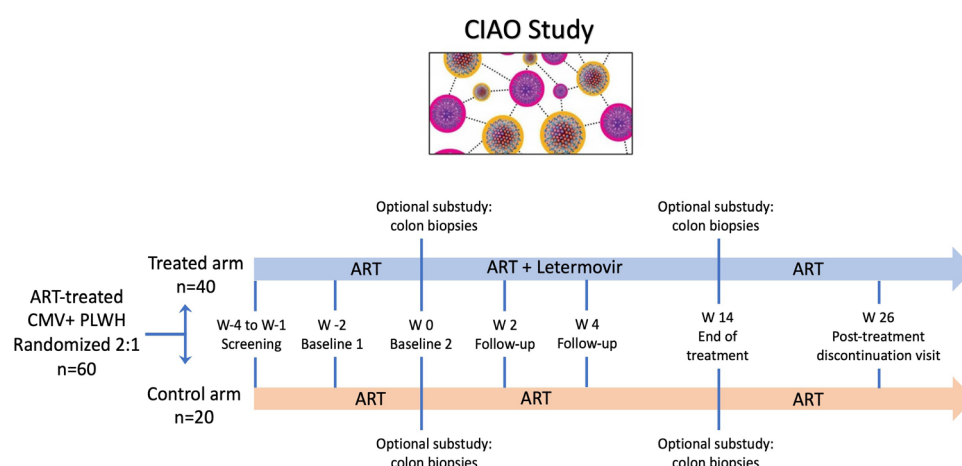


Figure 1 Study flow chart. Visit 1, the screening visit, will take place 1–4 weeks prior to the second baseline visit (week 0, visit 3) and informed consent document will be explained to the participant and will be signed. Two baseline visits will be conducted, the second one being at week 0 and all visits after that will be relative to this baseline week 0 visit. Data collected at these two baseline visits will be directly compared with determine intrapatient variability. For the treated arm, treatment will be a 1 \times 480mg or 2 \times 240mg of PREVYMIS taken with a meal, at the same time each day for 14 weeks. The control arm will receive standard of care alone (only ART). See schedule of events (table 1) for more details on tests and visits. The substudy is only available to participants at the Montreal site. ART, antiretroviral therapy; CMV, cytomegalovirus; PLWH, people living with HIV.

Table 1 Schedule of events

Visit type	Screening	Study visits					
		Baseline 1	Baseline 2	Treatment			Post-treatment
Visit window procedures	Week -4 to -1 (±7 days)	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 examination and vital signs	X						
Targeted physical examination and vital signs		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
Haematology*	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), online supplemental appendix 1	X						
Alcohol use (AUDIT-C), online supplemental appendix 2		X	X	X	X	X	X
Bristol score questionnaire, online supplemental appendix 5	X	X	X	X	X	X	X
Study product dispensation††			X	X	X		
Dispense dosing diary††			X				
Collect dosing diary††				X	X	X	
Study product adherence††				X	X	X	
Colon mucosal biopsies			X			X	

Continued

Table 1 Continued

Visit type	Study visits						
	Screening	Baseline 1	Baseline 2	Treatment			Post-treatment
Visit window procedures	Week -4 to -1 (±7 days)	Week -2 (±7 days)	Week 0 (day 0)	Week 2 (±3 days)	Week 4 (±3 days)	Week 14 (±7 days)	Week 26 (±7 days)
<p>*CBC, CD4 and CD8 T cell counts, erythrocyte sedimentation rate.</p> <p>†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).</p> <p>‡Alkaline phosphatase, ALT, amylase, AST, bilirubin (total), creatine kinase, creatinine, d-dimer, fasting blood glucose, HbA1c, high sensitivity C reactive protein, lipase, lipid profile (total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides), serum phosphate, urea.</p> <p>§Markers of gut barrier integrity, microbial translocation and inflammation: lipopolysaccharide (LPS), beta-D-glucan, LPS binding protein, soluble CD14 (sCD14), intestinal-fatty acid binding protein, Reg3a (measured in plasma by ELISA).</p> <p>¶Monocyte and T-cell activation markers include: HLA-DR and CD38. T-cell exhaustion marker: PD-1. Measured by staining and flow cytometry.</p> <p>¶¶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 tat/rev limiting dilution assay analysis will be performed on baseline week 0 and end-treatment week 14 samples to assess the HIV viral reservoir (exploratory analysis).</p> <p>**qPCR of <i>Akkermansia muciniphila</i>, 16S and 18S rDNA sequencing for other members of the microbiota.</p> <p>††Treatment arm only.</p> <p>Optional substudy procedure.</p> <p>Serology measurements include: cytomegalovirus (CMV), 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.</p> <p>Immune activation markers/cytokines include: soluble CD14, pro-inflammatory cytokines (interleukin (IL) 1β, IL-6, IL-8, TNF-α, soluble TNF receptor-1) and anti-inflammatory cytokine IL-10. Measured in plasma by ELISA.</p>							

du CHU de Québec, Université Laval; (3) Clinique d'infectiologie virale chronique, Centre Hospitalier de l'Université de Montréal, Montréal and (4) Clinique médicale l'Actuel, Montréal, all in Quebec, Canada.

Comparison of CMV seropositive and 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 PREVYMIS therapy, as compared with the control (no PREVYMIS) group with a power estimated at 0.8. However, we chose a larger 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 (SD) of those markers after PREVYMIS treatment. Statistical analysis for sample size determination was performed by Dr C Richard (Université de Montréal, Qc). Results of this randomised study will be descriptive.

Participants will be recruited at the four above-mentioned centres in Canada. Altogether, these participating medical centres provide care to more than 6000 PLWH. Teleconferences and face-to-face meetings will be organised between the Qualified Investigators and study staff to help promote participant recruitment and follow-up during the study.

At screening, a medical history and medication history will be recorded by study staff through chart review and/or patient interview. Date of diagnosis, date of ART initiation, nadir CD4 count, mode of HIV acquisition and previous AIDS defining illnesses will be

extracted from medical charts and records. Previous use of ART drugs and other medication will also be documented at each visit, including detailed information on ART adherence, given the known association between low ART adherence and inflammation. If the participant meets eligibility criteria, randomisation to the control or treated arm will be performed. The Investigator or study staff will perform a simple web-based randomisation process, using a randomisation tool designed by the CTN/CIHR. Eligible participants will be assigned to one of two treatment groups in a 2:1 ratio (letermovir:standard of care). Investigators 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 a study specific Electronic Case Report Form. All study records will be maintained according to the ICH-GCP and applicable regulatory requirements. Records will be retained for 15 years, in accordance with applicable regulatory requirements. 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.

Recruitment started in September 2022 and is expected to end by December 2023.

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 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 an HIV viraemia below 50 copies/mL; (6) CD4 count > 400 cells/ μ L of blood; (7) participants in the treated arm taking statins will be directed to reduce their statin dose by 50% for the duration of the study due to potential drug–drug interactions with letermovir. This dose modification is not expected to increase cardiovascular risk, since the duration of time for this dose reduction is relatively short it is unlikely to impact long-term cardiovascular risk. (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 an approved methods of birth control while in the study and until 2 weeks after completion of the study (any contraception method must be used consistently, in accordance with the approved product label and for the duration of the study until 2 weeks after study completion); (12) women of non-child-bearing potential as defined as either post-menopausal (12 months of spontaneous amenorrhoea 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 an approved methods of birth control.

Exclusion criteria

Participants are not eligible to participate in the study if they meet any of the following conditions: (1) hypersensitivity to letermovir or to any ingredient in the formulation of PREVYMIS, 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 (eg, uncontrolled hypertension, chronic renal failure (CrCl less than 50 mL/min), relapsing-remitting diseases associated with microbial translocation such as Crohn disease,²⁶ coeliac disease,²⁷ systemic lupus erythematosus,²⁸ or multiple sclerosis,²⁹ or active uncontrolled infections including COVID-19 (as tested by PCR); (4) coinfection with active hepatitis B or C virus; (5) current use or have used in the past 3 months: immunomodulatory agents, prophylactic antibiotics, proton pump inhibitors, metformin or morphine as these drugs are known to 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 PREVYMIS Product Monograph, 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 homeostasis; (8) psychiatric or cognitive disturbance or any illness that could preclude adherence with the study; (9) current participation in an experimental therapy study or receipt of experimental therapy within the last 6 months; (10) women who are planning to become or who are pregnant, or breast feeding; (11) a score of higher than 8 on a Full AUDIT questionnaire at the screening visit, suggesting an alcohol abuse problem and (12) moderate hepatic impairment combined with moderate or severe renal impairment (CrCl less than 50 mL/min).

Study intervention

Participants in the letermovir arm will be instructed to take PREVYMIS 1 \times 480 mg or 2 \times 240 mg orally in addition to their usual ART, and 20 participants will receive standard of care alone (ART only) for 14 weeks. Tablets will be taken at the same time each day with a meal, preferentially breakfast. PREVYMIS can be taken with ART as no interactions are expected.

No data are available on the concomitant use of alcohol or street drugs with PREVYMIS. Use of street drugs, cigarette smoking, non-prescription medications and marijuana/cannabis products use will be recorded in questionnaires by a research staff at each visit. Study continuation will be based on the Investigator's judgement. In the 24 hours prior to a study visit participants will be 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 as they could influence inflammation markers in blood and gut microbiota in stools.³⁰

Adverse events and toxicity management

The safety of PREVYMIS was evaluated in phase 3 randomised, double-blind, placebo-controlled trial (P001) through week 14 post-transplant and were followed for safety through week 24 post-transplant. 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, diarrhoea and vomiting.

During each follow-up visit with the participant, information on adverse events (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 PREVYMIS. Stable chronic conditions which are present prior to clinical trial entry and do not worsen will not be considered AEs and will be accounted for in the participant's medical history.

Risk minimisation, management and assessment procedures have been implemented in the study to minimise

and assess potential risks to participants who participate in this clinical study with PREVYMIS. 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; risk identification and mitigation management over the course of the study (and the sub-study). When side effects are considered to be related to PREVYMIS, the Investigator can use his clinical judgement regarding whether to continue or to discontinue the study medication. If PREVYMIS treatment is discontinued, the participant will be scheduled for follow-up visit(s) as required to treat the symptoms or adverse event related to PREVYMIS intake.

Clinical and laboratory assessments

Assessment of gut damage, microbial translocation and inflammation

To evaluate gut epithelial damage, we will measure markers in the plasma, mainly by ELISA before, during and after the study period.¹¹ To assess gut barrier integrity, LPS, a common marker of bacterial translocation³¹ and LPS binding protein, BDG, a marker of fungal translocation,¹² I-FABP and regenerating family member three alpha (REG3a) will be measured.³² Immune activation markers (sCD14), pro-(IL-1 β , IL-6, IL-8, TNF- α , soluble TNF receptor-1) and anti-inflammatory (IL-10) cytokines will be also quantified,³³ as well as hsCRP. We will assess ex vivo activation of monocyte, CD4⁺ and CD8⁺ T-cells by flow cytometry with CD83, CD86 and HLA-DR and CD38 staining, respectively. PD-1 expression on CD4⁺ and CD8⁺ T-cells will also be assessed as a marker of T-cell exhaustion.

Assessment of anti-CMV immune responses

To assess anti-CMV immune responses, anti-CMV IgG titres (by ELISA) and CMV specific CD4 and CD8 T-cells responses (*in vitro* stimulation with pp65 antigen/IE-1 peptides and intracellular staining analysis by flow cytometry of IFN- γ /TNF- α /IL-2/IL-10, IL-17) will be performed at the beginning and throughout the study period. Responding cells will be further characterised using KLRG1, KIR, and CD57 surface markers.

Detection of CMV DNA

CMV DNA will be detected in plasma, isolated PBMCs and mucosal colon samples (for participants of the optional colon biopsy/substudy). Briefly, DNA will be extracted from plasma, PBMCs and tissues samples using appropriated kits. An in-house PCR assay using the digital droplet technology will then allow the detection and quantification of CMV DNA with high sensitivity and specificity.

Assessment of microbiota composition (exploratory objectives)

As an exploratory objective, qPCR for the quantification of a gut protective bacterium, *Akkermansia muciniphila* will be performed on faecal DNA samples.³⁴

Gut microbiota composition will be further studied by 16S rRNA and ITS DNA sequencing to monitor changes in the microbiota composition.³⁵

Measures of HIV reservoir (exploratory objective)

As an exploratory objective, we will assess changes in the markers of HIV persistence. HIV DNA (LTR-gag) and HIV RNA (LTR-gag) will be measured in isolated CD4⁺T cells from the blood and in rectal biopsies using established assays.^{36,37} In addition, we will use the tat/rev limiting dilution assay to assess changes in the size of the inducible HIV reservoir in circulating CD4⁺T cells. As CMV is known to transactivate HIV, cell-associated HIV RNA will be monitored.

Assessment of gut mucosa architecture (optional colon biopsy/substudy)

Biopsies will be included in paraffin at the MUHC Histopathology core facility. Gut architecture will be monitored by immunochemistry and immunostaining of the epithelial tight junctions (Claudin-3/Occludin), intestinal villi length and apoptosis degree.³¹ 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.³⁸ Briefly, fresh tissue biopsies will be incubated with type II Liberase (Roche, Basel, Switzerland) at 37°C in a shaking incubator. The resulting lymphocyte suspension will be stained with monoclonal antibodies against CD3⁺, CD4⁺, CD8⁺ and myeloid markers. The frequency of activated CD4⁺ and CD8⁺ T-cells will be determined by flow cytometry as described above.

Assessment of letermovir blood concentrations

In the case we do not find any effect of the study drug in participants, letermovir blood concentrations and pharmacokinetic assessments will be performed to evaluate adherence (method as reported previously²³). In addition, CMV letermovir resistance will be assessed (PCR, UL56 sequencing).

Statistical analysis

Data collected from plasma, stool and colon biopsies will be compared between cases and controls at baselines, week 2, week 4, week 14 and week 26. Data will be analysed by parametric or non-parametric statistical tests such as the Wilcoxon matched pairs test and the Friedman test, as appropriate. To examine the change in plasma LPS relative to baseline, linear mixed-effects regression will be used. Time will be considered as a categorical variable in the model to allow flexible modelling of the time trend. All measurements will be included as outcome variable in the model. Demographics including age, sex, sexual practice and HIV history data will be included in multivariable analyses as they have been shown to influence immune activation in ART-treated PLWH.³⁹

Patient and public involvement

- ▶ The initial design of the study has been presented to community groups.
- ▶ Description of the study in lay language has been published on the website of the Canadian AIDS Treatment Information Exchange (<https://www.catie.ca/about-catie-what-we-do/about-catie>).
- ▶ Adherence questionnaires completed by participants throughout the study will allow for an assessment of their respective experiences.
- ▶ Results generated by the study are expected to be published in both formal scientific and lay language; however, individual study findings will not be shared with each study participant.

ETHICS AND DISSEMINATION PLAN

All participants will be given detailed oral and written information about the study. Consent documents (online supplemental material) describing in detail the study medication and interventions, 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 and the investigator or delegate must sign an informed consent document that has been approved by a participating centre's research ethics board (REB) prior to any procedures being done specifically for the trial. The study was approved by Health Canada and the Research Ethics Boards of the McGill University Health Centre (MUHC-REB, protocol number: MP37-2022-8295), ethics approval from the other sites is pending. All potential protocol amendments will be submitted to Health Canada and the respective research ethics board of the participating centres. Protocol deviations must first receive ethics approval and be reported to the data safety and monitoring committee of the CTN by the Investigator. The sole exception is when the suggested change intends to eliminate an immediate hazard to study participants. Results will be made available through publications in open access peer-reviewed journals and through the CIHR/CTN website.

Dissemination plan

The results of the trial will be disseminated through the traditional routes of scientific peer-reviewed publications, through international and national specialist conferences and through the press release by CTN. An open access journal will be chosen to ensure access to study results to all. Locally, results from the study will be shared with the McGill community. Study results will be submitted for publication in the Montréal LGBTQ+Community journal *Fugues*. Moreover, both the Sponsor-Investigator and Qualified Investigator will promote the CIAO study when attending or presenting at local, national, and international meetings.

Author affiliations

- ¹Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre, Montreal, QC, Canada
- ²Chronic Viral Illness Service, McGill University Health Centre, Montreal, QC, Canada
- ³Canadian HIV Trials Network, Canadian Institutes of Health Research, Vancouver, BC, Canada
- ⁴Division of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland
- ⁵CONICET, Universidad de Buenos Aires, Instituto de Investigaciones Biomédicas en Retrovirus y SIDA (INBIRS), Buenos Aires, Argentina
- ⁶Division of Gastroenterology, McGill University Health Centre, Montreal, QC, Canada
- ⁷Département de Microbiologie, Maladies Infectieuses et Immunologie, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Montreal, QC, Canada
- ⁸Centre for Outcomes Research and Evaluation, Research Institute of the McGill University Health Centre, Montreal, QC, Canada
- ⁹Department of Family Medicine, McGill University Health Centre, Montreal, QC, Canada
- ¹⁰Clinique Médicale L'Actuel, Montreal, QC, Canada
- ¹¹Division of Pediatrics, Faculty of Medicine, Laval University, Quebec city, QC, Canada
- ¹²Division of Hematology, McGill University Health Centre, Montreal, QC, Canada

Acknowledgements The authors would like to thank Angie Massicotte, Josée Girouard for their help with coordination, and Dr Tina Scardochio for coordination with Merck Sharp & Dohme Corp. The authors are grateful to members of the CIHR/CTN and Merck Investigator Studies Programme Review Committee (MISP-RC) whose insights paramount in the development of the protocol as well as Dana Nohynek for her help with regulatory affairs.

Contributors J-PR and LR designed the study, with insights from SI, CB, NC, GB and CT. LR and CB wrote the manuscript. J-PR, GB, CT, RT, CTTC, NK, AdP, BL, MK, TB and PLL will participate in participant recruitment and follow-up, and in data collection. LR, SI, CB and SB will participate in data collection and analysis. All authors critically reviewed the manuscript and approved the final version.

Funding This study was funded by the Canadian Institutes of Health Research (CIHR, grant 469701 to J-PR), the CIHR/Canadian HIV trials Network (CTN) PT047 grant to LR and J-PR), and by the Canadian HIV Cure Enterprise (CanCURE) (Team Grant HB2-164064 to J-PR). This study also received support from Fonds de la Recherche Québec-Santé (FRQ-S): Réseau SIDA/Maladies Infectieuses. Authors who are CTN and/or CanCURE members were involved in the study design and in the writing of the manuscript, and will also be involved in data collection, analysis, and interpretation. The study will be monitored by the CTN's Data Safety Monitoring Committee (DSMC). The study drug (PREVYMIS) has been provided in-kind by Merck investigator initiated studies programme (MISP) review committee (grant 60376 to J-PR).

Competing interests LR is a post-doctoral fellow supported by the Swiss National Science Foundation (SNSF) and the CTN/CIHR. SI is a post-doctoral fellow from the Fonds de recherche du Québec en santé, and from the CIHR/CTN. J-PR is the holder of the Louis Lowenstein Chair in Hematology and Oncology, McGill University. J-PR has performed contract research and/or served on Advisory Boards for Gilead Sciences Canada, Merck Canada, Abbvie, Viiv Healthcare, Bristol Myers Squibb, Janssen. NC has received research funding from EMD Serono and has served on the Advisory Board of Gilead Sciences Canada. AdP is a senior Clinical Research Scholar supported by Fonds de la recherche Québec -Santé (FRQ-S). BL is a senior Clinical Research Scholar supported by FRQ-S and received consultancy fees and/or honoraria from Gilead, Merck, and Viiv, and obtained research funds from Gilead, Merck, and Viiv, and support to attend educational conferences from Viiv Healthcare and Gilead. CTC is supported by a Fonds de recherche du Québec-Santé (FRQS) Junior 2 career award and has received consultancy fees and/or honoraria from Gilead and Viiv, and obtained research funds from Gilead, Merck, and Viiv, and support to attend educational conferences from Viiv Healthcare and Gilead. NK reports research funding from Gilead Sciences, advisory fees from Gilead Sciences, Viiv Healthcare, Merck and Abbvie, and speaker fees from Gilead Sciences, Abbvie and Merck, all outside of the submitted work. NK is supported by a career award from the Fonds de Recherche Québec – Santé (FRQ-S; Junior 1). CTC is the Pfizer/Université de Montréal Chair on HIV translational research and has received consultancy fees and honoraria from Gilead, Merck, GSK, Astra-Zeneca, Medicago, Sanofi. This study is supported in part (study drug in-kind) by a research grant from Investigator-Initiated Studies Program of Merck Sharp & Dohme Corp. The opinions expressed in this paper are those of the authors and do not necessarily represent those of Merck Sharp & Dohme Corp.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

ORCID iDs

Alexandra de Pokomandy <http://orcid.org/0000-0001-6809-1357>

Cecilia T. Costiniuk <http://orcid.org/0000-0002-4547-714X>

Jean-Pierre Routy <http://orcid.org/0000-0001-9897-7589>

REFERENCES

- Hsu DC, Sereti I. Serious non-AIDS events: therapeutic targets of immune activation and chronic inflammation in HIV infection. *Drugs* 2016;76:533–49.
- Routy J-P, Royston L, Isnard S. Aging with grace for people living with HIV: strategies to overcome leaky gut and cytomegalovirus coinfection. *J Acquir Immune Defic Syndr* 2022;89:S29–33.
- Zuhair M, Smit GSA, Wallis G, et al. Estimation of the worldwide seroprevalence of cytomegalovirus: a systematic review and meta-analysis. *Rev Med Virol* 2019;29:e2034..
- Lichtner M, Cicconi P, Vita S, et al. Cytomegalovirus coinfection is associated with an increased risk of severe non-AIDS-defining events in a large cohort of HIV-infected patients. *J Infect Dis* 2015;211:178–86.
- Sinclair J, Sissons P. Latency and reactivation of human cytomegalovirus. *J Gen Virol* 2006;87:1763–79.
- Maidji E, Somsouk M, Rivera JM, et al. Replication of CMV in the gut of HIV-infected individuals and epithelial barrier dysfunction. *PLoS Pathog* 2017;13:e1006202.
- Royston L, Isnard S, Lin J, et al. Cytomegalovirus as an Uninvited guest in the response to vaccines in people living with HIV. *Viruses* 2021;13. doi:10.3390/v13071266. [Epub ahead of print: 29 Jun 2021].
- Isnard S, Ramendra R, Lin J. Anti-CMV IgG is linked to CD4 T-cell count decay in HIV elite controllers. *Clin Infect Dis* 2020.
- Morris SR, Chen B, Mudd JC, et al. Inflammascent CX3CR1+CD57+CD8+ T cells are generated and expanded by IL-15. *JCI Insight* 2020;5. doi:10.1172/jci.insight.132963. [Epub ahead of print: 04 Jun 2020].
- Ballegaard V, Brændstrup P, Pedersen KK, et al. Cytomegalovirus-Specific T-cells are associated with immune senescence, but not with systemic inflammation, in people living with HIV. *Sci Rep* 2018;8:3778.
- Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2006;12:1365–71.
- Isnard S, Lin J, Bu S, et al. Gut leakage of Fungal-Related products: turning up the heat for HIV infection. *Front Immunol* 2021;12:656414.
- Mudd JC, Brenchley JM. Gut mucosal barrier dysfunction, microbial dysbiosis, and their role in HIV-1 disease progression. *J Infect Dis* 2016;214 Suppl 2:S58–66.
- Tincati C, Douek DC, Marchetti G. Gut barrier structure, mucosal immunity and intestinal microbiota in the pathogenesis and treatment of HIV infection. *AIDS Res Ther* 2016;13.
- Sandler NG, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. *Nat Rev Microbiol* 2012;10:655–66.
- Mehraj V, Ramendra R, Isnard S, et al. Circulating (1→3)-β-D-glucan Is Associated With Immune Activation During Human Immunodeficiency Virus Infection. *Clin Infect Dis* 2020;70:232–41.
- Isnard S, Fombuena B, Sadouni M, et al. Circulating β-D-glucan as a marker of subclinical coronary plaque in antiretroviral therapy-treated people with human immunodeficiency virus. *Open Forum Infect Dis* 2021;8:ofab109.
- Ramendra R, Isnard S, Lin J. Cmv seropositivity is associated with increased microbial translocation in people living with HIV and uninfected controls. *Clin Infect Dis* 2019.
- Hunt PW, Martin JN, Sinclair E, et al. Valganciclovir reduces T cell activation in HIV-infected individuals with incomplete CD4+ T cell recovery on antiretroviral therapy. *J Infect Dis* 2011;203:1474–83.
- Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med* 2017;377:2433–44.
- Food and Drug Administration.. *Letermovir data*. Washington, DC: Food and Drug Administration, 2017. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209939Orig1s000,209940Orig1s000lbl.pdf
- Royston L, Masouridi-Levrat S, Gotta V, et al. Therapeutic drug monitoring of orally administered Letermovir prophylaxis in allogeneic hematopoietic stem cell transplant recipients. *Antimicrob Agents Chemother* 2022;66:e0065722.
- Royston L, Royston E, Masouridi-Levrat S, et al. Letermovir primary prophylaxis in high-risk hematopoietic cell transplant recipients: a matched cohort study. *Vaccines* 2021;9:372.
- Chan A-W, Tetzlaff JM, Götzsche PC, et al. Spirit 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586.
- Chan A-W, Tetzlaff JM, Altman DG, et al. Spirit 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200–7.
- Sepehri S, Kotlowski R, Bernstein CN, et al. Microbial diversity of inflamed and noninflamed gut biopsy tissues in inflammatory bowel disease. *Inflamm Bowel Dis* 2007;13:675–83.
- Sapone A, Lammers KM, Casolaro V, et al. Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med* 2011;9:23.
- Hevia A, Milani C, López P, et al. Intestinal dysbiosis associated with systemic lupus erythematosus. *mBio* 2014;5:e01548–01514.
- Buscarinu MC, Romano S, Mechelli R, et al. Intestinal permeability in relapsing-remitting multiple sclerosis. *Neurotherapeutics* 2018;15:68–74.
- Yan J, Ouyang J, Isnard S, et al. Alcohol use and abuse Conspires with HIV infection to aggravate intestinal dysbiosis and increase microbial translocation in people living with HIV: a review. *Front Immunol* 2021;12:741658.
- Ortiz AM, Flynn JK, DiNapoli SR, et al. Experimental microbial dysbiosis does not promote disease progression in SIV-infected macaques. *Nat Med* 2018;24:1313–6.
- Isnard S, Ramendra R, Dupuy FP, et al. Plasma levels of C-type lectin REG3α and gut damage in people with human immunodeficiency virus. *J Infect Dis* 2020;221:110–21.
- Mehraj V, Ramendra R, Isnard S, et al. Cxcl13 as a biomarker of immune activation during early and chronic HIV infection. *Front Immunol* 2019;10:289.
- Ouyang J, Lin J, Isnard S, et al. The Bacterium *Akkermansia muciniphila*: A Sentinel for Gut Permeability and Its Relevance to HIV-Related Inflammation. *Front Immunol* 2020;11:645.
- Isnard S, Lin J, Fombuena B, et al. Repurposing metformin in nondiabetic people with HIV: influence on weight and gut microbiota. *Open Forum Infect Dis* 2020;7:ofaa338.
- Vandergeeten C, Fromentin R, Merlini E, et al. Cross-Clade ultrasensitive PCR-based assays to measure HIV persistence in large-cohort studies. *J Virol* 2014;88:12385–96.
- Leyre L, Kroon E, Vandergaeten C, et al. Abundant HIV-infected cells in blood and tissues are rapidly cleared upon art initiation during acute HIV infection. *Sci Transl Med* 2020;12. doi:10.1126/scitranslmed.aav3491. [Epub ahead of print: 04 Mar 2020].
- Planas D, Pagliuzza A, Ponte R, et al. LILAC pilot study: effects of metformin on mTOR activation and HIV reservoir persistence during antiretroviral therapy. *EBioMedicine* 2021;65:103270.
- Vujkovic-Cvijin I, Sortino O, Verheij E, et al. HIV-Associated gut dysbiosis is independent of sexual practice and correlates with noncommunicable diseases. *Nat Commun* 2020;11:2448.

Appendix 1.

Main Informed Consent Form

Version 2.1
July 27, 2022

INTRODUCTION

You are being invited to take part in this research study because you are living with the Human Immunodeficiency Virus (HIV) and you are taking anti-HIV medication.

Before you decide to take part in this study, it is important that you read and understand the following explanation about the study and its risks and benefits. This form may contain words you do not fully understand. Please ask the study doctor or study team to explain anything you do not understand and make sure that all your questions have been answered. You may also want to discuss this study with your family doctor, a family member or close friend before you make your decision about taking part. Being in this study does not replace your regular medical care.

If you decide to take part in this study, you will be asked to sign this form and a copy will be given to you.

BACKGROUND

Antiretroviral therapy (ART) is able to manage your HIV infection and help improve your health and quality of life. However, even when ART is taken correctly people living with HIV are at higher risk of developing certain inflammatory-related health conditions including diabetes, fatty liver, cardiovascular disease, brain-health related diseases and cancer. Essentially HIV keeps immune cells in an active or inflammatory state, partly due to the damage HIV does to the gut.

The lining of the gut acts as a barrier and prevents undesirable gut contents (e.g. bad bacteria) from traveling through the gut lining and into the blood. These gut compounds entering the bloodstream trigger inflammation and over time can increase the risk of inflammatory-related health conditions. ART medication can help repair some of the damage caused by HIV, however ART does not reduce the number of bacterial compounds crossing the gut lining to levels seen in healthy HIV-negative people. There is a need to find therapies to decrease this chronic inflammation in people living with HIV.

In addition to HIV itself, co-infection with other viruses such as the **cytomegalovirus (CMV)** has been shown to enhance inflammation in people living with HIV. CMV is a very frequent virus, which is usually well tolerated during acute infection. However, similarly to HIV, this virus has the ability to persist lifelong, mostly in deep tissues such as the gut and lymph nodes. Although the infection remains mostly silent if the immune system is strong, CMV chronic infection perturbs the immune system, induces inflammation and increases leaky gut. This virus has also been shown to decrease the immune response against other pathogens, and also to impair the response to vaccines.

We want to explore how CMV increases inflammation in tissues of people living with HIV taking ART. In this study, we will try to inhibit CMV with a new and specific drug called letermovir, in order to decrease leaky gut markers and inflammation. Conversely to previous

anti-CMV drugs, this new medication is very well tolerated, and is also very efficient in preventing CMV.

PURPOSES OF THE RESEARCH STUDY

The main purpose of this study is to evaluate if taking tablets of letermovir (Prevymis®) in addition to your ART medications will reduce inflammation and leaky gut. There will be one group of participants who will receive the drug, and another group receiving only their usual ART, as a control. By comparing the two groups, we will evaluate if letermovir is able to reduce inflammation, leaky gut and also to decrease the markers of CMV silent infection.

Optional sub-study (Montreal site only)

Participants at the Montreal site that agree to take part in this study will be invited to take part in an optional sub-study that will involve colon biopsies before and at the end of the study. The purpose of the sub-study is to directly assess changes in gut mucosa. The mucosa is the lining of the gut which helps stop pathogens from entering the body. The sub-study will be explained in a separate informed consent form.

BRIEF STUDY DESCRIPTION

A total of 60 participants will be enrolled in this study from up to four sites in Canada.

You will be asked to come to the clinic for a screening visit. You will be assessed to see if you meet the eligibility criteria to take part in the study. Once you are confirmed to be eligible to take part in the study, your participation will last about 26 weeks, not including the screening visit. At the beginning of the study, you will be informed if you have been randomized in the group of participants receiving letermovir in addition to their ART or receiving ART only. Over the course of the study, you will be asked to come to the clinic for 7 visits and you will be asked to give blood and stool samples. You will be asked to take either the study treatment, Prevymis®, or only your usual ART, for 14 weeks. In total, your participation will take about 5-6 hours over the duration of the study.

STUDY INCLUSION CRITERIA

To be eligible for the study you must be 18 years of age or older, be CMV positive, be HIV positive, be on ART for at least 3 years, and on the same ART medications for at least 3 months. If you are taking statin medication, the dose of your statin will be reduced by 50% for the duration of the study. You must have an undetectable viral load (the quantity of HIV virus in your blood must be less than 50 copies/ml, except for one blip not higher than 200 copies/ml per year in the last 3 years) for at least 3 years and have a CD4 count of more than 400.

STUDY EXCLUSION CRITERIA

You will not be able to take part in the study if you have an active co-infection with Hepatitis B or C virus, or if you have any underlying conditions, such as diabetes, cardiovascular disease or renal dysfunction. Your study doctor or nurse will review your medical history with you and make sure you do not have a health condition that will exclude you from the study.

Some drugs and herbal supplements such as immunomodulatory drugs: methotrexate, antibiotics, metformin, morphine and derivatives, aluminum containing phosphate binders, chemotherapeutics, niacin, anticoagulant (including Warfarin, Coumadin® and Jantoven®), pimozide, ergot alkaloids, and cyclosporine are banned during the study because of known interactions with letermovir. Regarding HIV medication, darunavir, efavirenz, etravirine and nevirapine will also be banned due to interactions. During the screening visit, please tell the

study nurse or doctor about any prescription drugs, natural health products (including supplements or herbal products), pre- or probiotics or other non-prescription drugs (including cannabis and street drugs) that you are taking or plan on taking. Alcohol is not banned, but participants will be screened using an alcohol questionnaire (AUDIT, see details below) and excluded if alcohol abuse is suspected. Those with pre-existing conditions that could prevent study compliance such as street drug abuse or psychiatric illness will be excluded at the discretion of the study doctor.

You will not be able to take part in this study if you are currently participating in another clinical trial or have done so in the past 3 months.

Females who are pregnant, planning on becoming pregnant, or who are breast-feeding will be excluded from the study.

STUDY MEDICATION

Eligible participants in the medication group will be asked to take either 1 tablet of 480mg or 2 tablets of 240mg of Prevymis® (letermovir) orally once a day, at about the same time each day, for 14 weeks.

Prevymis® is an anti-CMV drug that inhibits the correct formation of the viral particles. This drug has been approved since 2017 for the prevention of CMV infection in persons receiving a bone marrow transplant, who suffer from frequent infections.

Prevymis® is approved for this latter use by Health Canada, but in this study, it will be used outside of its approved label claims, in an experimental manner.

Prevymis® will not be provided to you after the study is completed. The goal of this study is to understand CMV and how it affects inflammation and the gut rather than testing letermovir itself. Larger studies will be required to establish if letermovir should be prescribed lifelong in people living with HIV. If you withdraw from the study without completing the study, we will ask you to return any unused study drug.

STUDY VISITS AND PROCEDURES

The study visits and procedures are summarized in Table 1 and described below.

Screening Visit: Week -8 to Week -2 (1 hour 30 minutes)

Up to six weeks prior to being enrolled into the study, you will be asked to come to the clinic for a screening visit. You will undergo the following procedures to determine if you are eligible to take part in this study.

- You will be asked to sign this informed consent form if you agree to take part in this study
- You will be asked about demographic information and your medical history including
 - Age, education, ethnicity, sexual preference
 - Past and current illnesses
 - Medications or supplements that you are currently taking or have taken recently, including a review of ART history
 - Review an acceptable method of contraception
- You will undergo a complete physical examination including measurement of your vital signs (blood pressure, temperature, respiratory rate and heart rate) and weight and height

- A blood sample (about 20 ml or 1½ tablespoons) will be collected for routine blood work:
 - to determine if you are CMV positive
 - to measure the amount of HIV in your blood;
 - to determine CD4 and CD8 T cell counts;
 - to measure the level of your blood cells
 - to perform serum chemistry tests, including tests to see how your liver and kidneys are working and the level of fats in your blood (e.g. cholesterol) and to test for diabetes
 - to test for Hepatitis B and C
 - Hepatitis B and C: by signing this consent, you agree for the study team to perform these tests. The study doctor or study staff will tell you if the test results are positive. Positive result(s) testing are reportable to local health authorities in Quebec. If you have active Hepatitis B or C, you may not be able to participate in the study but you will be linked to a specialized doctor for appropriate care.
 - to test for pregnancy (for women of childbearing potential)
- Study staff will administer a questionnaire about your alcohol use
- You will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours before your next visit

The study doctor will review the information collected about you and your test results and if you meet the eligibility criteria to take part in the study you will be contacted to attend a Baseline visit.

To prepare for your study visits we ask that you:

- Do not take any non-prescription drugs in the 48 hours before each study visit
- Do not use marijuana products in the 24 hours before each study visit
- Do not consume alcohol in the 24 hours before each study visit, except for one drink (optional) at the previous night's meal

Baseline and Follow-up Visits (Week -2, Week 0, Week 2, Week 4, Week 14, and Week 26)

If you are eligible and agree to take part in this study, you will be asked to return to the clinic at the start of the study (**Week -2**), and subsequently at **Week 0** (*start of treatment visit*), **Week 2** (*on treatment visit*), **Week 4** (*on treatment visit*), **Week 14** (*end of treatment visit*) and **Week 26** (*final study visit*). If you are randomized in the letermovir group, you will be asked to take this drug for 14 weeks starting at Week 0 and you must continue to take your anti-HIV drugs during the whole study period (e.g. from Week -2 to Week 26). If you are randomized in the control group, you will be asked to take your anti-HIV drugs only for the whole study period. Each study visit will take approximately 30 minutes to 1 hour.

Please remember to bring your stool sample (collected in the 24 hours prior to the study visit) at Week -2, Week 0, Week 2, Week 4, Week 14 and Week 26.

During the Baseline and Follow-up visits, you will undergo the following procedures:

- You will be asked to confirm your ongoing consent.
- Starting at Week 0, you will be provided with a supply of study drug, letermovir, sufficient to last until your next study visit.
- At Week 0, you will be given a daily Dosing Diary to help you to remember and track taking your study drug.

- You will be asked about any changes in health and any medications (including prescription drugs, (e.g., anti-HIV ART), non-prescription medications), acceptable method of contraception, supplements, vitamins, cannabis products, and street drug use you are taking or have taken.
- Adverse event(s) will be assessed.
- Study staff will administer a questionnaire about your alcohol use and differences in bowel movements during the study.
- You will undergo a targeted physical examination including measurement of vital signs and weight.
- A blood sample (about 20 ml or 1½ tablespoons) will be collected for routine blood work:
 - to measure the amount of HIV in your blood;
 - to measure the amount of CMV in your blood;
 - to determine CD4 and CD8 T cell counts;
 - to measure the number and percentage of your blood cells;
 - to perform serum chemistry tests, including tests to see how your liver and kidneys are working and to measure the level of fats in your blood (e.g. cholesterol);
 - to test for Hepatitis B and C, and cytomegalovirus infections (Week 0);
 - to test for pregnancy (for women of childbearing potential).
- A blood sample (about 100 ml or 7 tablespoons) will be collected for analysis of your immune system (e.g. biomarkers, inflammatory markers)
- Study staff will collect the stool sample from you. This sample will be used to assess the effect of letermovir on the types of bacteria in your gut. You will be provided with a stool collection kit and instructions for collecting a stool sample in the 24 hours before your next visit.
- At Week 2, Week 4 and Week 14 study staff will administer a questionnaire about your compliance to the study medication and study staff will count the pills you have not taken.
Please remember to bring your Prevmis® bottles and Dosing Diary to the Week 2, Week 4 and Week 14 visits.

The research team will consult your medical file to collect information related to your medical history and record relevant data for this research project. We will also access your personal medical data in the provincial database/registries of the provincial database Dossier Santé Québec (DSQ) to be able to link data collected from this study. Newly diagnosed Hepatitis B and C infections will be notified to the local health authorities in Quebec as nationally notifiable diseases. This information will confirm medical diagnoses, medications and in the event of death, details on the cause of death as well as any tests and exams done before the event.

Taking letermovir (Prevmis®)

At Week 0, Week 2, and Week 4, you will be given a supply of **Prevmis®** sufficient to last until your next study visit, and instructions on how to take it. Begin taking the study medication on the day you receive it and continue to take the study medication as instructed. Remember to promptly record each dose of Prevmis you take in the daily Dosing Diary provided.

Visit Scheduling

To ensure adequate follow-up during your study participation, the study staff will contact you by a way that is agreed upon by you and the study staff (e.g., email/phone) during the Screening visit. If you miss a study visit, clinic staff will try to contact you to reschedule your visit. Please contact the study staff if you are unable to attend a scheduled appointment so that the visit can be rescheduled. Clinical research staff trying to contact you will be careful to maintain your

privacy and will not tell anyone, without your permission, about your participation in this HIV study.

Table 1: Schedule of Visits and Procedures

Visit Type	Screening	Study Visits					
		Baseline 1	Baseline 2	Treatment			Post-treatment
Visit Window	Week -4 to -1 (±7 days)	Week -2 (±7 days)	Week 0 (Day 0)	Week 2 (±3 days)	Week 4 (±3 days)	Week 14 (±7 days)	Week 26 (±7 days)
Procedures:							
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 and Vital Signs	X						
Targeted Physical Exam and Vital Signs		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),	X						
Alcohol use (AUDIT-C),		X	X	X	X	X	X
Bristol score questionnaire,	X	X	X	X	X	X	X
Provision of Dosing Diary			X				
Study Product Dispensation			X	X	X		
Study Product Compliance				X	X	X	
Collection of Dosing Diary				X	X	X	

Results of routine blood work, including measurements of HIV viral load and CD4 T-cell counts, will be communicated to you in “real time” (without delay) so that you and your doctor can act quickly on the information.

POTENTIAL RISKS AND/OR DISCOMFORTS

Taking part in this study has risks. Some of these risks we know about. There is also a possibility of risks that we do not know about and have not been seen in humans to date. Please call the study doctor or study staff if you have any side effects, even if you do not think it has anything to do with this study.

Risks associated with letermovir

The most frequently reported side effects of letermovir are:

- nausea (1.6%),
- vomiting (0.8%), and
- abdominal pain (0.6%).

These symptoms are usually mild, transient, and reversible after discontinuation of the product. Moreover, in rare cases, cardiac arrhythmia (tachycardia) has also been reported. If you feel any palpitation, shortness of breath or chest pain, you should immediately contact your physician or an emergency unit.

Risks associated with blood draws

Less than 1% of individuals who have their blood taken experience any side effects; these side effects may include discomfort, bleeding, or bruising where the needle enters the body.

Reproductive risks

Limited information is available regarding the safety of letermovir in pregnant or breast-feeding women. For this reason, women who are pregnant, are planning to become pregnant, or lactating women will be excluded from this study. If you become pregnant during your participation in this clinical study, letermovir should be discontinued right away. **Please contact the study doctor or study staff immediately if you think you have become pregnant during the study.**

Participants in this study will need to follow the following guidelines for prevention of pregnancy.

Birth Control: 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 and 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.

Drug-drug interactions

Some drugs will interact with letermovir and should be avoided during the study. For a complete list, see "STUDY EXCLUSION CRITERIA".

Alcohol Consumption

Alcoholic beverage consumption has been linked to increased inflammation and a leaky gut in HIV-infected population. A leaky gut is a condition in which bacteria and toxins are able to "leak" through the intestinal wall. Alcoholic beverages should be avoided, or limited to one per day (preferably less than daily use), during the study. Regular or excessive use may mask any positive effects of letermovir on reducing inflammation and could potentially increase the risk of diarrhea. An alcohol use questionnaire (AUDIT) will be conducted during screening to exclude those with potential alcohol abuse problems. At subsequent study visits, a shortened alcohol use questionnaire will be used to monitor differences in alcohol consumption between participants in case this becomes a differentiating/group-defining factor.

POTENTIAL BENEFITS

We do not know whether taking part in this study will benefit you. What we do learn from this study may benefit other people living with HIV in the future.

RETURN OF RESULTS

At the end of the study, you will be told when study results will be available and how to learn about them. Individual research results from sample analyses will not be returned to you or to your physicians.

INCIDENTAL FINDINGS

Significant incidental findings are unexpected findings made during the course of the study that may affect your current or future wellbeing or that of your family members. If, in the course of this study, we discover evidence of a significant finding we will communicate this to you and to a health professional of your choice. You may then use this information to make a decision about continuing to participate in the study.

ALTERNATIVE TO BEING IN THE STUDY

If you do not want to take part in this study that combines ART with letermovir treatment, the current standard of care for a person infected by HIV is ART alone. You do not need to be in this study to receive treatment for HIV. If you decide not to enter this study, there may be other choices available. Please ask the study doctor to discuss these options with you. You will be advised of any changes to the HIV treatment guidelines that occur during the course of this study.

CONFIDENTIALITY

During your participation in this study, the study doctor and their team will collect and record information about you in a study file. They will only collect information required to meet the scientific goals of the study.

The study file may include information from your medical chart, including your identity, concerning your past and present state of health, your lifestyle, as well as the results of the tests, exams, and procedures that you will undergo during this research project. Your research file could also contain other information, such as your name, address, phone number, health plan number, sex, date of birth and ethnic origin. All personal information obtained during this study will be kept strictly confidential. The study doctor will store your study data for 15 years in accordance with Health Canada requirements, after which time it will be destroyed.

All the information collected during the research project will remain strictly confidential to the extent provided by law. You will only be identified by a code number. The key to the code linking your name to your study file will be kept under lock and key by the study doctor. The study doctor will forward your coded data to the Sponsor-Investigator or their representatives for the exclusive objectives of this study. This data will be stored for 15 years and then destroyed.

To ensure your safety, a copy of this information and consent form will be placed in your medical chart. As a result, any person or company to whom you give access to your medical chart will have access to the information in this form.

The study data may be published or shared during scientific meetings; however, it will not be possible to identify you.

Your medical charts may be examined by a person mandated by Canadian authorities, such as Health Canada, as well as by representatives of the study sponsor-investigator, the institution, or the Research Ethics Board for the purposes of monitoring the study, ensuring patient safety, and assessing compliance with applicable regulations. All these individuals and organizations adhere to policies on confidentiality.

You have the right to consult your study file in order to verify the information gathered, and to have it corrected if necessary.

COSTS

There will be no cost to you for participating in this study. All clinical and professional services, diagnostic and laboratory tests that are part of this study will be provided to you at no cost. If you are randomized in the letermovir group, the study medication, letermovir will be supplied to you at no cost. You will continue to obtain your other anti-HIV treatment by prescription; this medication will be paid for either by a government drug program or by your own insurance.

COMPENSATION

You will receive an amount of \$60.00 per study visit, for a total of 7 visits, for a total amount of \$420.00 for costs and inconveniences incurred during this research study. If you withdraw from the study, or are withdrawn before it is completed, you will receive compensation proportional to the number of visits you have completed.

SHOULD YOU SUFFER ANY HARM

Should you suffer harm of any kind following administration of the study medication, letermovir, or following any other procedure related to the research study, you will receive the appropriate care and services required by your state of health.

By agreeing to participate in this research study, you are not waiving any of your legal rights, nor discharging the study doctor, the sponsor-investigator, or the institution of their civil and professional responsibilities.

VOLUNTARY PARTICIPATION AND STUDY WITHDRAWAL

Your participation in this research project is voluntary. Therefore, you may refuse to participate. You may also withdraw from the project at any time, without giving any reason, by informing the study doctor or a member of the research team.

Your decision not to participate in the study, or to withdraw from it, will have no impact on the quality of care and services to which you are otherwise entitled, or on your relationship with the study doctor or clinical team.

The study doctor, the Research Ethics Board, the funding agency, Health Canada, or the Sponsor-Investigator may terminate your participation in the study without your consent, or terminate the entire study. This may happen if new findings or information indicate that participation is no longer in your best interest, if you do not follow study instructions, or if there are administrative reasons to terminate the study. In addition, your participation in the study could be terminated by your doctor due to study medication side effects, failure to take the study product as indicated, failure to come for study visits or termination of the study. If you are asked to leave the study, the reasons for this will be explained to you.

However, for safety reasons, before you withdraw from the study, we ask that you attend the clinic for a safety follow-up visit. You will be asked about any side effects you may have experienced, any medication you are taking or have taken. You will be asked to return any unused letermovir. You may be asked to provide a blood sample for routine blood work.

During the course of the study, you will be kept informed of any new treatments or findings that may influence your decision to continue participation in the study.

If you withdraw or are withdrawn from the study, the information and biological material already collected for the study will be stored, analyzed and used to ensure the integrity of the study.

Any new findings that could influence your decision to stay in the research study will be shared with you as soon as possible.

STORAGE AND USE OF BIOLOGICAL SAMPLES

Your blood and stool samples contain several types of cells that will not be possible to analyze immediately after collection. These expensive analytical tests will be performed in batches with samples from other participants. Your collected samples will be stored under the supervision of Drs. L. Royston, S. Isnard and J.-P. Routy at the Research Institute of the McGill University Health Centre, Glen site, 1001 Decarie Blvd, Montreal, QC, H4A 3J1. Samples will be labelled with a study code and no identifying information. If any of your samples are not needed for the analyses, the storage period for your leftover samples will be 10 years, after which they will be destroyed. During this period, you can ask Dr. Royston or Dr. Routy to withdraw your stored samples. Only the co-investigators and collaborators who will perform the analyses will have access to your samples. Blood samples will be used only for the purposes of this HIV research study. No genetic nor additional tests will be performed outside of the scope of the study without additional consent. Products extracted from your blood or stool samples may be sent to Dr. Marette's lab in Quebec City, QC, or Dr. Chomont's lab in Montreal, QC, for analysis. Those samples will be labelled with a study code and without any identifying information.

FUNDING OF THE RESEARCH PROJECT

This study is being funded by the Canadian HIV Trials Network (CTN) and by Merck Canada Investigator Studies Program (MISP). Merck will be providing the study drug in-kind during the whole study. The study will be run by Dr Jean-Pierre Routy. The sponsor-investigator has received funding for conducting this research study.

CLINICAL TRIAL REGISTRATION

A description of this clinical trial will be available on <http://www.ClinicalTrials.gov>. This Website will not include information that can identify you. At most, the Website will include a summary of the results. You can search this Website at any time.

CONTACT INFORMATION

If you have questions or if you have a problem you think may be related to your participation in this clinical research study or you would like to withdraw, you may communicate with Dr Jean-Pierre Routy during working hours at **514-843-2090** or with the study coordinator at **514-934-1934 Ext. 34240**. After working hours, call 514 934-1934, Ext. 53333 and ask for the physician-on-call for the McGill University Health Centre.

If you have questions concerning your rights as a research participant and wish to discuss them with someone not connected to the clinical research study, please contact the **Ombudsman of the McGill University Health Centre at (514) 934-1934 extension 35655**.

CONTROL OF ETHICAL ASPECTS OF THE RESEARCH PROJECT

The Research Ethics Board of the McGill University Health Centre has given ethics approval to this research study and is responsible for its ongoing ethics oversight at all participating institutions in the health and social services network in Quebec.

Study Title: Influence of a 3-month letermovir treatment on gut inflammation in ART-treated HIV-infected persons in an open labelled controlled randomized study**PARTICIPANT INFORMED CONSENT
SIGNATURE PAGE****Signature of the participant:**

I have reviewed the information and consent form. Both the research study and the information and consent form were explained to me. My questions were answered, and I was given sufficient time to make a decision. After reflection, I consent to participate in this research study in accordance with the conditions stated above, understanding that I may withdraw my participation at any time.

I authorize the research study team to have access to my medical record for the purposes of this study.

Name of Participant (printed)	Signature of Participant	Date of consent dd mmm yyyy

Signature of the person obtaining consent:

I have explained the research study and the terms of this information and consent form to the research participant, and I answered all his/her questions.

Name of person obtaining consent (printed)	Study Role of the person obtaining consent
Signature of person obtaining consent	Date of consent dd mmm yyyy