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

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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, pheripheral 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, REG3α 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 multicentre, 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/µL and an undetectable viral load. Forty participants will be randomised to receive letermovir (PREVYMIS 480 mg or 2×240 mg 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 clincal centres: (1) at the Chronic Viral Illness Service at the McGill University Health Centre (MUHC), Montreal, QC; (2) Centre de recherche

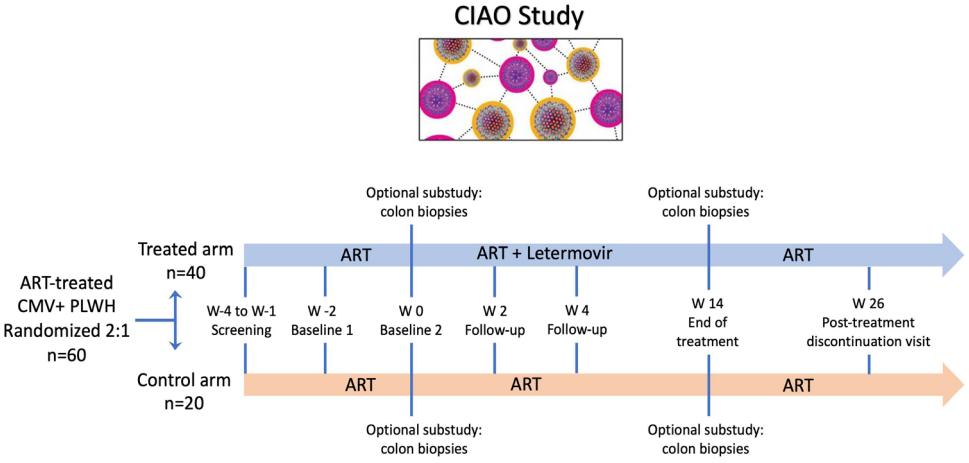

Figure 1 Study flow chart. Visit 1, the screening visit, will take place 1–4 weeks prior to the second baseline visit (week 0, visit 3) and informed consent document will be explained to the participant and will be signed. Two baseline visits will be conducted, the second one being at week 0 and all 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 1×480 mg or 2×240 mg 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	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)**

*CBC, CD4 and CD8 T cell counts, erythrocyte sedimentation rate.
†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).
‡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.
§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).
¶Monocyte and T-cell activation markers include: HLA-DR and CD38. T-cell exhaustion marker: PD-1. Measured by staining and flow cytometry.
¶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).
**qPCR of *Akkermansia muciniphila*, 16S and 18S rDNA sequencing for other members of the microbiota.
††Treatment arm only.
Optional substudy procedure.
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.
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.

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/µL 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 postmenopausal (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: immunemodulatory 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 1×480 mg or 2×240 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 translocation[31] 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-1β, 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 previously[22]). 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.

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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.

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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 Quebec 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 Quebec -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.

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