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Camu Camu effects on microbial translocation and systemic immune activation in ART-treated people living with HIV: protocol of the single-arm non-randomised Camu Camu prebiotic pilot study (CIHR/CTN PT032)

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ABSTRACT (249 words)

Introduction

Despite the success of antiretroviral therapy (ART) in transforming HIV disease into a chronic infection, people living with HIV (PLWH) remain at risk for various non-AIDS inflammatory comorbidities. Risk of non-AIDS comorbidities is associated with gut dysbiosis, epithelial gut damage and subsequent microbial translocation, and increased activation of both circulating CD4+ and CD8+ T-cells. Therefore, in addition to ART, novel gut microbiota-modulating therapies could aid in reducing inflammation and immune activation, gut damage, and microbial translocation. Amongst various gut-modulation strategies under investigation, the Amazonian fruit Camu Camu (CC) presents itself as a prebiotic candidate based on its anti-inflammatory and antioxidant properties in animal models and tobacco smokers.

Method and analysis

A total of 22 PLWH on ART for more than 2 years, with a viral load <50 copies/mL, a CD4+ count >200 and a CD4+/CD8+ ratio <1 (suggesting increased inflammation and risk for non-AIDS comorbidities), will be recruited in a single arm, non-randomized, interventional pilot trial. We will assess tolerance and effect of supplementation with CC in ART-treated PLWH on reducing gut damage, microbial translocation, inflammation, and HIV latent reservoir by various assays.

Ethics and dissemination

The Canadian Institutes of Health Research (CIHR)/Canadian HIV Trials Network (CTN) pilot trial protocol CTNPT032 was approved by the Natural and Non-prescription Health Products Directorate of Health Canada and the research ethics board of the McGill university Health Centre committee (number 2020-5903). Results will be made available as a free access through publications in peer reviewed journals and through the CIHR/CTN website.

Trial registration number

ClinicalTrials.gov: NCT04058392

KEYWORDS

Camu Camu; HIV; Antiretroviral therapy; Prebiotic; Gut microbiota; gut mucosa; Inflammation.

ARTICLE SUMMARY: STRENGTHS AND LIMITATIONS OF THIS STUDY

- Camu camu (CC) is an Amazonian rainforest fruit which has been shown to have anti-inflammatory and gut microbiota-modulating properties in mice.
- The Camu Camu study seeks to confirm mouse model findings on systemic inflammation and immune activation, gut dysbiosis and damage, and subsequent microbial translocation in antiretroviral therapy (ART)-treated people living with HIV (PLWH).
- We hypothesize that treatment with CC will beneficially impact ART-treated PLWH by improving their gut microbiota composition, reducing microbial translocation, reducing inflammation to potentially decreasing latent HIV reservoir size and the risk to develop non-AIDS comorbidities.
- Changes induced by CC treatment will be assessed by plasma markers of gut damage, microbial translocation, inflammation, percentage of activated T-cells, HIV reservoir size and gut bacterial taxa.
- This pilot trial with 22 ART-treated PLWH, will provide sufficient data for future sample size calculations to confirm the effect of CC in more definitive larger studies.

Main text (3899 words)

INTRODUCTION

Antiretroviral therapy inhibits viral replication without eradication.

Antiretroviral therapy (ART) successfully controls Human Immunodeficiency Virus (HIV) infection by inhibiting viral replication and has significantly improved the life expectancy of people living with HIV (PLWH) while eliminating transmission to others. However, ART-treated PLWH remain at risk for developing inflammatory non-AIDS comorbidities such as cardiovascular diseases, fatty liver, neurocognition dysfunction and cancer (1, 2). These non-AIDS comorbidities are associated with persistent immune activation and increase with aging, co-infections like cytomegalovirus (CMV) and viral hepatitis as well as microbial translocation. It has been observed in HIV infection and inflammatory bowel disease that abnormal composition of the gut microbiota called "dysbiosis", alteration of the gut barrier, T-helper (Th) 17 cell dysfunction and microbial translocation lead to systemic inflammation and immune activation contributing to non-AIDS comorbidities (3-9). Long-term HIV control by ART appears to only partially reduce inflammation and poorly replenishes Th17 protective mucosal function, highlighting the importance of research on gut microbiota and the epithelial barrier. Furthermore, despite control of HIV replication, persistent HIV infection in long-lived memory CD4+ T-cells and likely macrophages also contribute to inflammation and microbial translocation, creating a vicious cycle nurturing inflammation. Importantly, the size of the HIV reservoir has been linked to the level of inflammation and immune activation measured in CD8+ T-cells and macrophages (3, 10). Conversely, it remains unknown whether the reduction of inflammation can lead to a decrease in the size of the HIV reservoir.

Gut damage and immune activation

As HIV is not cleared with ART, persistent viral products and inflammation subsequently impair antigen-specific T-cell responses. This overall activation leads to the exhaustion of the immune system, including T-cells. This distinctive feature from other chronic viral infections is relevant as HIV replicates preferentially in Th17 CD4+ T-cells residing in the gut, leading to cell death and mucosa damage (11-13). Microbial products penetrate the damaged gut barrier and pass into the systemic circulation. Such microbial translocation contributes to systemic immune activation,

disease progression, and development of non-AIDS comorbidities (14-17). Markers of bacterial translocation, including lipopolysaccharide (LPS), LPS binding protein (LBP), and soluble CD14 (sCD14) have been correlated with immune activation and disease progression (3, 11, 18, 19). While bacterial translocation is thought to be a major cause of immune activation, we have shown that circulating beta-D-glucan (BDG), a marker of fungal translocation, also contributes to the immune activation in an LPS-independent manner (20, 21).

Although ART suppresses HIV replication to undetectable levels, microbial translocation and Th17 damage remain, contributing to persistent immune activation. Local gut inflammation has been associated with HIV reservoir size (22). Monocytes/macrophages, Dendritic cells, and Natural Killer (NK) cells detect microbial products in the mesenteric lymph nodes and in systemic circulation, secreting pro-inflammatory cytokines (interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF)-α). These cytokines drive CD4+ T-cell activation, leading to elevated expression of the HIV co-receptor C-C Chemokine receptor (CCR) 5 and the gut homing receptor CCR6 (23). Th17 measured by CD4+ T-cells expressing CCR6 have then been shown by our group to be preferentially infected by HIV (12, 13, 24).

Gut microbiota, dysbiosis and immune regulation

The gut microbiota composition and metabolites play an important role in inflammation in obesity, diabetes, cancer, and HIV infection. Its role includes food and metabolite processing, microbial regulation, and immune regulation (25-28). PLWH, compared to uninfected controls, present with a dysbiosis characterized by a lower abundance of Firmicutes and more abundant Proteobacteria in their gut microbiota. In addition lack of *Lactobacilli* in stools is associated with lower CD4+ T-cell count and a higher levels of systemic immune activation (29). Moreover, lower levels of *Akkermansia muciniphila* have been observed in PLWH. Dysbiosis combined with microbial translocation has been linked to non-AIDS comorbidities in HIV-infected individuals and influences CD4+ T-cell recovery on ART as reported by our group and others (3, 14, 18, 30, 31).

***Akkermansia muciniphila* in health and disease**

Akkermansia muciniphila (A. muciniphila) is a gram-negative, strict anaerobe and mucin-degrading bacterium that colonizes the gut of humans and rodents. *A. muciniphila* represents 1-5% of all intestinal bacteria. This bacterium acts as a shield on the gut epithelial barrier and has

been shown to reduce insulin resistance in obese individuals (26, 32-36). Lower abundance of *A. muciniphila* has been found in the feces of patients with inflammatory bowel disease (IBD) and individuals with obesity, when compared to feces of healthy individuals (25, 26). Furthermore, oral administration of *A. muciniphila* to mice fed a high-fat diet alleviates obesity, reduces LPS in the circulation and alleviates insulin resistance (26, 37, 38).

Additionally, patients with lung and renal cell carcinoma responding to anti-PD-1 treatment were more prone to have an elevated abundance of gut *A. muciniphila* compared to non-responders (28). To go beyond association, B. Routy *et al*. transferred the microbiota from responders and non-responders into germ-free mice and observed a tumor response only in mice with a *A. muciniphila* rich human fecal microbiota from the responders (28). Both *in vitro* and *in vivo*, *A. muciniphila* has been shown to increase mucus secretion by goblet cells and gut epithelium integrity contributing to the prevention of other bacterial products from passing into the circulation (35, 38, 39). Moreover, oral administration *A. muciniphila* was shown to successfully elevate key anti-aging and anticancer metabolites primarily in the gut and liver (40).

Based on these encouraging results, different attempts have been made to increase *A. muciniphila* in the gut. Everard *et al*. showed that pasteurized *A. muciniphila* increased mucus thickness, decreased LPS translocation, and reduced metabolic syndrome in obese mice. In contrast, heat-killed *A. muciniphila* did not protect mice from obesity (38). However, such pasteurized strains are costly, difficult to produce and may not last after oral administration.

The Amazonian fruit Camu Camu

Camu Camu (CC), also named *Myriciaria dubia*, is an Amazon rainforest fruit with antioxidant and anti-inflammatory properties. Anhê *et al*. showed that polyphenol-rich cranberry and CC extracts protect mice from diet-induced obesity and intestinal inflammation in association with increased *A. muciniphila* in the gut microbiota (32-35). CC was more efficient at reducing the amount of LPS in plasma than cranberry extract in the diet-induced model of obesity, and it was also found to increase other beneficial microbes in addition to *A. muciniphila*. Other studies have shown that polyphenols could favor *A. muciniphila* in the gut (26, 41, 42). Importantly, CC extracts also decreased C-Reactive protein (CRP), IL-6 and IL-8 in the plasma of healthy tobacco smokers (43). CC is considered a "super fruit" which is widely available in many Canadian health food

stores in either powder or capsule form. CC products have been used as a nutritional supplement that is well tolerated in humans (44).

CC is a fruit rich in polyphenols and has been shown in mouse model of inflammation-related diseases to reduce inflammation and improve gut microbiota with increased *A. muciniphila* and other beneficial bacteria (32-35). However, no studies have been performed to test CC in PLWH. Moreover, PLWH on ART have been shown to exhibit persistent dysbiosis, an altered gut microbiota composition, along with microbial translocation which can cause non-AIDS comorbidities and hamper CD4+ T-cell recovery (3, 14, 18, 30). Therefore, we will evaluate if the polyphenol rich CC can positively affect PLWH on ART in terms of reducing inflammation, improving gut microbiota and potentially decreasing HIV persistent reservoir.

Objective

To determine the feasibility and suitable design of a full-scale study on the effect of Camu Camu in ART-treated PLWH, we designed a non-randomized, single arm, interventional study.

Primary outcomes

The primary outcome of this study will be to evaluate the effect of CC on the reduction of the plasma marker of microbial translocation LPS, assessed using ELISA.

Secondary outcomes

The secondary outcomes of this study will be changes in the following before and after 12 weeks of CC intake, and after 8 weeks of CC discontinuation:

- Safety and tolerability of CC measured by evaluating adverse events, hematology, and serum chemistries over the course of the study. These evaluations will include HIV viral load, glucose levels, a lipid profile and plasma levels of hsCRP and D-dimer.
- Gut barrier integrity markers I-FABP and sST2, measured by ELISA.
- Microbial translocation marker (1-3)-β-D-Glucan (BDG) assessed using the Fungitell assay.
- Pro-inflammatory markers (IL-1β, IL-6, IL-8, IL-18. IP-10, IL-17A and F, IL-22, and soluble CD14) and anti-inflammatory markers (IL-10) assessed by ELISA.

- T-cell and monocyte activation levels assessed by flow cytometry using markers CD38, HLA-DR and PD-1.
- *A. muciniphila* levels in stools using qPCR.
- Microbiota composition and diversity in stools assessed using 16s rDNA sequencing.
- HIV reservoir size in blood assessed by PCR.
- Evaluate intra-patient variability using data from two baseline visits, approximately two weeks apart from each other to confirm reliability of baseline results.

Exploratory outcomes

The exploratory outcomes of this study will be the following:

- Difference in HIV reservoir size from Baseline (Visit 0) to 12 weeks post-CC treatment by TILDA, performed on blood samples.
- Changes in other markers of gut damage (including plasma REG3α (45)), microbial translocation (such as plasma 16S rDNA) and immune activation (T-cell activation, cytokines) in the blood and gut biopsies.

Sub-study outcomes

The sub-study outcomes will be the following:

- Changes in gut mucosa architecture in a subset of participants who will consent to have colon biopsies before and at the end of the 12 weeks of CC treatment.
- Changes in inflammation in gut mucosa biopsy assessed by myeloperoxidase staining before and at the end of the 12 weeks of CC treatment.
- Changes in HIV reservoir size in biopsies using qPCR.
- Association between baseline gut microbiota composition (16S rDNA sequencing), and markers of gut integrity (I-FABP, tissue staining) and inflammation (T-cell activation, inflammatory cytokines).

METHODS AND ANALYSIS

Study design, settings, sample size and recruitment strategy

Trial CTN PT032 is an open label, non-randomized, single arm interventional pilot study (Clinicaltrials.gov NCT04058392); protocol version # 1.3; February 12, 2021. The study sponsor

is the Canadian Institutes of Health Research (CIHR) Canadian HIV Trials Network (CTN). The following study protocol fulfills the requirements of the 2013 Standard Protocol Items: Recommendations for Interventional Trials guidelines (46, 47).

Comparisons and assessment of outcomes will be made through various measures at baseline, during and after CC use (**figure 1 and table 1**).

A total of 22 ART-treated participants living with HIV will be enrolled at the Chronic Viral Illness Service (CVIS) at the McGill University Health Centre (MUHC), Glen Site, Montreal, QC and the Ottawa Hospital, General Campus, Infectious Diseases Clinic, Ottawa, ON, Canada. A convenient sample size of 22 participants was chosen without formal power calculations for this pilot study, based on the Lilac study design (48, 49) and the study by Inoue *et al.* (43). This sample size accounts for an estimated loss to follow-up/non-completion of 10% for the study. It can therefore be estimated that 2 participants may not fully complete the study. There will be an optional colon biopsy sub-study. For logistical reasons, only participants recruited at the Montreal site will be given the option to participate in this sub-study, after giving informed consent to participate in the main study and being shown to be eligible for the main study (after screening). The sub-study will have a separate informed consent form. The obtained data from this study will be used for calculation of sample size for future larger studies.

Participants will be recruited at two above-mentioned centers in Canada. Both participating medical clinics provide care to more than 2000 HIV-infected persons. Teleconferences and face-to-face meetings will be organized between the Qualified Investigators and study staff to help promote patient recruitment and follow-up during the study.

Inclusion criteria

Participants will be eligible for the study if they meet the following criteria: (1) Male or female adults ≥18 years of age; (2) Documented HIV-1 infection by Western Blot, Enzyme Immuno Assay (EIA) or viral load assay; (3) On ART for at least 2 years, and stable ART regimen (same prescription) for at least 3 months to ensure a stabilization of inflammation markers; (4) Persistent undetectable viral load < 50 copies/ml for the past 2 years. One viral blip are allowed if preceded and followed by a HIV viremia below 50 copies/ml; (5) CD4+ count >200 and a CD4+/CD8+ ratio <1, to recruit participants with increased inflammation and risk for non-AIDS comorbidities; (6) Able to communicate adequately in either French or English; (7) Able and willing to provide

written informed consent prior to screening; (8) As the influence of CC on pregnant women is unknown, women of childbearing potential must have a negative serum pregnancy test; (9) 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; (10) Women of non-child-bearing potential as defined as either post-menopausal (12 months of spontaneous amenorrhea and ≥ 45 years of age) or physically incapable of becoming pregnant with documented tubal ligation, hysterectomy or bilateral oophorectomy; (11) Sexually active men with a female partner of childbearing potential must agree to use an approved methods of birth control.

Exclusion criteria

Participants will not be eligible to participate in the study if they meet any of the following conditions: (1) Known allergy/hypersensitivity to Camu Camu; (2) Current AIDS-related event or serious health condition including systemic infections in the last 3 months; (3) Severe systemic diseases (e.g. uncontrolled hypertension, chronic renal failure), or active uncontrolled infections; (4) Co-infection with active Hepatitis B or C Virus; (5) Current use or have used in the past 3 months: immune-modulatory agents, prophylactic antibiotics(41)/antibiotics, proton pump inhibitors, or Morphine as these drugs modulate gut microbiota; (6) Current use of aluminum containing phosphate binders, chemotherapeutics, niacin, anticoagulant and protease inhibitors (including in their ART-regimen) as increased vitamin-C levels can prevent the activity of those molecules; (7) Diagnosis of diabetes mellitus (HbA1c≥6.5%) as defined by the Canadian Clinical Practice Guidelines for the Prevention and Management of Diabetes (50); (8) Frequent use of probiotics or polyphenol-rich prebiotics (e.g. cranberry and CC powders and/or capsules) in the last 12 months; (9) Recent changes in dietary habits, intermittent fasting, chronic constipation or laxative use as these can affect gut microbiota; (10) Psychiatric or cognitive disturbance or any illness that could preclude compliance with the study; (11) Current participation in an experimental therapy study or receipt of experimental therapy within the last 6 months; (12) Women who are planning to become or who are pregnant, or breast-feeding; (13) A score of higher than 8 on a Full AUDIT questionnaire at the screening visit, suggesting an alcohol abuse problem.

Study intervention

Participants will be instructed to take 1000 mg of Camu C™ (provided by Natural Traditions, Canada) once daily administered as two 500 mg oral capsules for 12 weeks. Capsules will be taken at the same time each day with a meal, preferentially breakfast. Camu C™ can be taken with ART as no interactions are expected. The 1000 mg dose is based on the dose given to mice per mean body weight divided by 12.3 as per the Food and Drug Administration (FDA) equation to determine equivalent dosing in human vs. mice, and consistent with Health Canada's recommendations of 1-3 capsules daily (51, 52).

The interaction between CC and other medication is unknown. CC has a high vitamin C concentration and therefore any drug with negative interactions with vitamin C were included in the exclusion criteria. The vitamin C in CC could interact with aluminum in phosphate binders (possible harmful to kidneys); chemotherapeutics (CC antioxidative properties could reduce the chemotherapeutic drug's effect); protease inhibitors (vitamin C might reduce the effect of antiviral drugs containing protease inhibitors); niacins (vitamin C could reduce niacin's effect); and anticoagulants since high doses of vitamin C can reduce responses to some anticoagulants. Hence, participants will be asked to refrain from using Vitamin C supplements during the study.

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.

Adverse events and toxicity management

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

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

When side effects are perceived to be related to Camu C™, the Investigator can use their clinical judgment regarding whether to continue or to discontinue the study medication. If Camu C™ treatment is discontinued, the participant will be scheduled for follow-up visit(s) as required to treat the symptoms or adverse event related to Camu C™ intake.

Clinical and laboratory assessments

Assessment of gut damage, microbial translocation, and inflammation

To evaluate gut epithelial damage, we and others have validated markers that will be measured in the plasma by ELISA before, during and after CC intake (11, 53-55). LPS, a common marker of bacterial translocation (56), soluble Suppression of Tumorigenicity (sST2)(20) and Intestinal-Fatty Acid Binding Protein (I-FABP) will be measured to assess gut barrier integrity. Immune activation markers (sCD14) and pro-(IL-1β, IL-6. IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines will be quantified (57, 58). Activation of monocytes and CD4+ and CD8+ T-cells will be assessed *ex vivo* by flow cytometry with HLA-DR and CD38 staining. CD4+ and CD8+ T-cells will also be assessed for PD-1 expression as a marker of T-cell exhaustion. Plasma will be assayed for beta D-glucan as a marker of fungal infection(21, 58). REG3α and 16S rDNA as well as other markers of microbial translocation and gut damage may be tested in plasma as well (45).

Assessment of microbiota composition

qPCR for *A. muciniphila* will be performed on fecal DNA samples as previously described by our group (48). Gut microbiota composition will be further studied by 16S and 18S rDNA sequencing to determine the impact on other beneficial microbes (e.g., *Barnesiella* and *Turicibacter*) known to respond to CC in the obesity mouse model (48).

Assessment of HIV reservoir size

If differences in microbial translocation and/or inflammation are observed, HIV reservoirs will be quantified in blood and rectal biopsies. HIV DNA (total and integrated) and cell-associated HIV RNA (gag) will be measured in DNA and RNA obtained from isolated CD4+ T-cells from the blood (obtained by negative magnetic selection) and gut biopsies (sorted by flow cytometry). HIV DNA and cell-associated RNA measures will be performed using an ultrasensitive nested qPCR as described previously (59).

In addition, the frequency of cells with inducible proviruses will be measured in isolated CD4+ T-cells from PBMCs using the Tat/rev Induced Limiting Dilution Assay (TILDA) in the laboratory of Dr. Chomont at Baseline Week 0 and End-treatment Week 12 timepoints.

In mucosal biopsies, HIV DNA and RNA will also be quantified and localized by DNA/RNAscope (24, 60).

Assessment of gut mucosa architecture (optional colon biopsy/sub-study)

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)(56). If a diminution in inflammation is noted, myeloperoxidase staining will be performed to allow for the quantification of inflammatory myeloid cells in the gut.

For other analyses, gut cells will be separated from tissues by enzyme digestion using a collagenase-based method as reported previously (61, 62). Briefly, fresh tissue biopsies will be incubated with type II collagenase for 30 minutes at 37°C in a shaking incubator. The resulting lymphocyte suspension will be stained with monoclonal Antibodies (mAbs) against CD3+, CD4+, CD8+, and myeloid markers. The total frequency of activated CD4+ and CD8+ T-cells will be determined by flow cytometry as described above.

Statistical analysis

To examine the change in plasma LPS and soluble CD14 levels relative to baseline, linear mixed effects regression will be used. Time will be considered as a categorical variable in the model to allow flexible modeling of the time trend. All five measurements (two for baseline and three for follow-up visits) will be included as outcome variable in the model. Log transformation of the outcome variable or generalized mixed effects regression will be employed if normality

assumption is not satisfied. Changes in plasma markers, percentage of activated T-cells, HIV reservoir size and bacterial taxa by type in the stools samples relative to baselines will be assessed in the same fashion.

Patient and public involvement

- Initial design of the study was presented to community groups.
- Compliance 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, will not be directly disseminated to study participants.

ETHICS AND DISSEMINATION PLAN

All participants will be given detailed oral and written information about the study. Consent documents 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 must sign an informed consent document that has been approved by a participating center's research ethics board (REB) prior to any procedures being done specifically for the trial. All potential protocol amendments will be submitted to Health Canada and the respective research ethics board of the participating centers. 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.

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 Camu Camu study when attending or presenting at local, national, and international meetings.

CONCLUSION

We hypothesize that treatment with CC will beneficially impact ART-treated PLWH by improving the gut microbiota composition, reducing systemic inflammation and immune action, reducing gut damage and microbial translocation, and potentially decreasing latent HIV reservoir size, thus decreasing the risk in developing non-AIDS comorbidities. This pilot trial with 22 ART-treated PLWH will provide sufficient data for future sample size calculations and set the foundation to assess the impact of CC in larger definitive studies.

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Author statement

Group Authorship/Collaborating Author Names: SI, BF, JO, JL, LR, SB, NS, PLL, TB, NS, MBK, BL, CTC, BR, AM, and JPR, for the Camu Camu Study Group

Contributors: J-PR and SI designed the study, with insights from JO, JL, NS, NC, BR, and AM. BF and SI wrote the manuscript. JL, LR, SB, PLL, TB, NC, MBK, BL, CTC, BR, AM, will participate in data collection and analysis. All authors critically reviewed the manuscript and approved the final version.

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Conflicts of interest

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, Argos Pharmaceuticals from InnaVirVax and has served on the Advisory Board of Theravectys. JBA has performed contract research and/or served on Advisory Boards for Gilead Sciences Canada, Merck Canada, Abbvie, ViiV Healthcare, Bristol Myers Squibb, Janssen and Argos Pharmaceuticals. NC has received research funding from EMD Serono and has served on the Advisory Board of Gilead Sciences Canada. SI is a post-doctoral fellow from the Fonds de recherche du Quebec en santé,

and from the CIHR/CTN. B.L. is supported by a career award LE 250 from Quebec's Ministry of Health for researchers in family medicine. BL has received consultancy fees and/or honoraria from Gilead, Merck, and ViiV, and research funds from Gilead, Merck, and ViiV, support to attend educational conferences from Viiv Healthcare and Gilead.

Patient consent for publication

Not required

Ethics approval

Canadian Institutes of Health Research/Canadian HIV Trials Network (CTN) pilot trial protocol CTNPT032. The study was approved by the Natural and Non-prescription Health Products Directorate of health Canada and the research ethics board of the McGill university Health Centre committee (number 2020-5903) and will be conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000.

Data access statement

The data generated by this study will be available from Dr Routy upon reasonable request after publication.

Table 1: Schedule of Events.

Visit Type	Screening	Study Visits				
		Baseline 1	Baseline 2	Treatment		Follow-up
Visit Window Procedures:	-8 to -1 weeks	Week -2 (±7 days)	Week 0 (Day 0)	Week 4 (±7 days)	Week 12 *(+7 days)*	Week 20 (±7 days)
Visit No.	1	2	3	4	5	6
Informed Consent	X	X				
Eligibility Assessment	X	X	X			
Concomitant Medication	X	X	X	X	X	X
Medical History	X					
Complete Physical Exam and Vital Signs	X					
Targeted Physical Exam and Vital Signs		X	X	X	X	X
Adverse Event Assessment				X	X	X
Serum Pregnancy Test	X	X	X	X	X	X
Hematology*	X	X†	X	X	X	X
Serum Chemistry **	X	X†	X	X	X	X
Serology***	X		X			
HIV-1 Viral Load***	X	X†	X	X	X	X
Immune activation markers/cytokines (ELISA)****		X	X	X	X	X
Monocyte and T-cell activation markers+		X	X	X	X	X
Markers of gut barrier integrity, inflammation, and microbial translocation++		X	X	X	X	X
Size of HIV reservoir in Latently Infected CD4+ T-cells+++		X	X	X	X	X
Stool sample collection and microbiota composition++++		X	X	X	X	X
Alcohol use questionnaire (AUDIT-Full)	X					
Alcohol use questionnaire (AUDIT-C)		X	X	X	X	X
Study Product Dispensation			X			
Study Product Compliance				X	X	
Colon mucosal biopsies#			X		X	

*CBC, CD4 and CD8 T-cell counts, erythrocyte sedimentation rate (ESR).
**Alkaline phosphatase, ALT, Amylase, AST, Bilirubin (total), Creatine kinase, Creatinine, D-dimer, fasting blood glucose, HbA1c, high sensitivity C-reactive protein (hsCRP), Lipase, lipid profile (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), Triglycerides), serum phosphate, Urea
***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 (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokine IL-10. Measured in plasma by ELISA.
+Monocyte and T-cell activation markers include HLA-DR and CD38. T-cell exhaustion marker: PD-1. Measured by staining and flow cytometry.
++Markers of gut barrier integrity, microbial translocation, and inflammation: lipopolysaccharide, soluble ST2, I-FABP (measured in plasma by ELISA).
+++PBMCs will be isolated and then latent CD4 T-cells will be isolated by flow cytometry. HIV viral reservoir in the latent CD4 T-cell population will be measured by nested qPCR. More specific TILDA analysis will be performed on Baseline Week 0 and End-treatment Week 12 samples to assess the HIV viral reservoir (Exploratory analysis).
++++qPCR of A. muciniphila, 16S and 18S rDNA sequencing for other members of the microbiota.
#Optional sub-study procedure.
† Not required when the same tests have been performed at the screening visit within the past 14 days, with the exception of CBC, CD4, CD8 (and serum pregnancy test)

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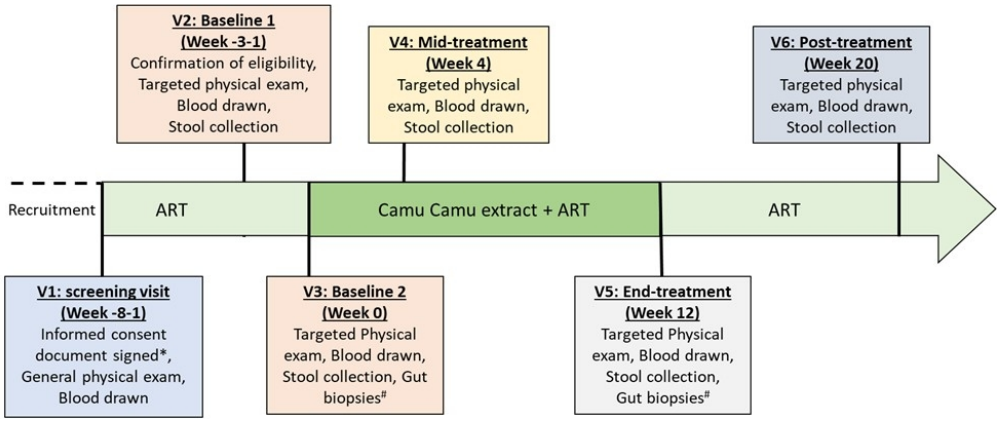
Figure legend

Figure 1: Study flow chart.

Visit 1, the Screening visit, will take place 1-8 weeks prior to the second Baseline visit (Week 0, Visit 3). At the Screening visit the informed consent document will be explained to the participant and will be signed prior to any screening and study activities. Two Baseline visits will be conducted, the second one being at Week 0 and all visits after that will be relative to this Baseline Week 0 Visit (Visit 3, Day 0). Data collected at these two Baseline visits will be directly compared to determine intra-patient variability. Camu Camu treatment will be a single daily dose of 1000 mg (2*500 mg Camu C™ capsules) taken with a meal, at the same time each day for 12 weeks. Treatment and post-treatment visit dates (Visit 4, Week 4 and Visit 6, Week 20) can vary ± 7 days according to participant and/or research team availability. Visit 5 at Week 12 can vary +7 days to ensure the participant has completed 12 weeks of Camu Camu treatment prior to the end-of-treatment visit. See Section 8 Schedule of Events (Table 1) to see more test details.

*The consent form for the optional gut biopsy will also be explained, but consent for this will not be necessary to be part of the main study. The sub-study is only available to participants at the Montreal site.

#Optional gut biopsies will be taken for the sub-study at indicated time points.



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164x68mm (150 x 150 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Camu Camu pilot study (CTN PT032). Isnard et al.

Section/item	Item No	Description	Page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	8
Funding	4	Sources and types of financial, material, and other support	8, 16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	8
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	8
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7

	6b	Explanation for choice of comparators	8,9
Objectives	7	Specific objectives or hypotheses	7,8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8,9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9,10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10,11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10,11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7,8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12,13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	NA
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11,12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	14
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9, table 1
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16,17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11,12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the

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BMJ Open

Camu Camu effects on microbial translocation and systemic immune activation in ART-treated people living with HIV: protocol of the single-arm non-randomised Camu Camu prebiotic pilot study (CIHR/CTN PT032)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-053081.R1
Article Type:	Protocol
Date Submitted by the Author:	15-Nov-2021
Complete List of Authors:	Isnard, Stéphane; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine Fombuena, Brandon; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine Ouyang, Jing; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine Royston, Léna; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine Lin, John; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine Bu, Simeng; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine Sheehan, Nancy; McGill University Health Centre, Department of Medicine Lakatos, Péter; McGill University Bessissow, Talat; McGill University Chomont, Nicolas; Centre de recherche du centre hospitalier de l'université de Montréal, Centre de Recherche du CHUM and Department of microbiology, infectiology and immunology, Université de Montréal Klein, Marina; Research Institute of the McGill University Health Centre Lebouché, Bertrand; McGill University Health Centre, Chronic Viral Illness Service, McGill University Health Centre; McGill University, Department of Family Medicine, McGill University Costiniuk, Cecilia T.; McGill University, Chronic Viral Illness Service Routy, Bertrand; Centre Hospitalier de l'Universite de Montreal Centre de Recherche Marette, André; McGill University Health Centre, Department of Family Medicine; Laval University Routy, Jean-Pierre; Research Institute of the McGill University Health Centre; McGill University Health Centre, Department of Medicine
Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Gastroenterology and hepatology, Immunology (including allergy), Infectious diseases, Nutrition and metabolism
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Inflammatory bowel disease <

	GASTROENTEROLOGY, Nutritional support < GASTROENTEROLOGY, IMMUNOLOGY

Camu Camu effects on microbial translocation and systemic immune activation in ART-treated people living with HIV: protocol of the single-arm non-randomised Camu Camu prebiotic pilot study (CIHR/CTN PT032)

Stéphane Isnard[1,2,3], Brandon Fombuena[1,2], Jing Ouyang[1,2,4], Léna Royston[1,2], John Lin[1,2], Simeng Bu[1,2], Nancy Sheehan[2], Peter L. Lakatos[5], Talat Bessissow[5], Nicolas Chomont[6], Marina B. Klein[1,2], Bertrand Lebouché[1,2,7,8], Cecilia T Costiniuk[1,2], Bertrand Routy[6], André Marette[8,9], Jean Pierre Routy[1,2,11]*, For the Camu Camu Study Group

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ABSTRACT (249 words)

Introduction

Despite the success of antiretroviral therapy (ART) in transforming HIV disease into a chronic infection, people living with HIV (PLWH) remain at risk for various non-AIDS inflammatory comorbidities. Risk of non-AIDS comorbidities is associated with gut dysbiosis, epithelial gut damage and subsequent microbial translocation, and increased activation of both circulating CD4+ and CD8+ T-cells. Therefore, in addition to ART, novel gut microbiota-modulating therapies could aid in reducing inflammation and immune activation, gut damage, and microbial translocation. Amongst various gut-modulation strategies under investigation, the Amazonian fruit Camu Camu (CC) presents itself as a prebiotic candidate based on its anti-inflammatory and antioxidant properties in animal models and tobacco smokers.

Method and analysis

A total of 22 PLWH on ART for more than 2 years, with a viral load <50 copies/mL, a CD4+ count >200 and a CD4+/CD8+ ratio <1 (suggesting increased inflammation and risk for non-AIDS comorbidities), will be recruited in a single arm, non-randomized, interventional pilot trial. We will assess tolerance and effect of supplementation with CC in ART-treated PLWH on reducing gut damage, microbial translocation, inflammation, and HIV latent reservoir by various assays.

Ethics and dissemination

The Canadian Institutes of Health Research (CIHR)/Canadian HIV Trials Network (CTN) pilot trial protocol CTNPT032 was approved by the Natural and Non-prescription Health Products Directorate of Health Canada and the research ethics board of the McGill university Health Centre committee (number 2020-5903). Results will be made available as a free access through publications in peer reviewed journals and through the CIHR/CTN website.

Trial registration number

ClinicalTrials.gov: NCT04058392

KEYWORDS

Camu Camu; HIV; Antiretroviral therapy; Prebiotic; Gut microbiota; gut mucosa; Inflammation.

ARTICLE SUMMARY: STRENGTHS AND LIMITATIONS OF THIS STUDY

- Camu camu (CC) is an Amazonian rainforest fruit which has been shown to have anti-inflammatory and gut microbiota-modulating properties in mice.
- The Camu Camu study seeks to confirm mouse model findings on systemic inflammation and immune activation, gut dysbiosis and damage, and subsequent microbial translocation in antiretroviral therapy (ART)-treated people living with HIV (PLWH).
- We hypothesize that treatment with CC will beneficially impact ART-treated PLWH by improving their gut microbiota composition, reducing microbial translocation, reducing inflammation to potentially decreasing latent HIV reservoir size and the risk to develop non-AIDS comorbidities.
- Changes induced by CC treatment will be assessed by plasma markers of gut damage, microbial translocation, inflammation, percentage of activated T-cells, HIV reservoir size and gut bacterial taxa.
- This pilot trial with 22 ART-treated PLWH, will provide sufficient data for future sample size calculations to confirm the effect of CC in more definitive larger studies.

Main text (3941 words)

INTRODUCTION

Antiretroviral therapy inhibits viral replication without eradication.

Antiretroviral therapy (ART) successfully controls Human Immunodeficiency Virus (HIV) infection by inhibiting viral replication and has significantly improved the life expectancy of people living with HIV (PLWH) while eliminating transmission to others. However, ART-treated PLWH remain at risk for developing inflammatory non-AIDS comorbidities such as cardiovascular diseases, fatty liver, neurocognition dysfunction and cancer (1, 2). These non-AIDS comorbidities are associated with persistent immune activation and increase with aging, co-infections like cytomegalovirus (CMV) and viral hepatitis as well as microbial translocation. It has been observed in HIV infection and inflammatory bowel disease that abnormal composition of the gut microbiota called "dysbiosis", alteration of the gut barrier, T-helper (Th) 17 cell dysfunction and microbial translocation lead to systemic inflammation and immune activation contributing to non-AIDS comorbidities (3-9). Long-term HIV control by ART appears to only partially reduce inflammation and poorly replenishes Th17 protective mucosal function, highlighting the importance of research on gut microbiota and the epithelial barrier. Furthermore, despite control of HIV replication, persistent HIV infection in long-lived memory CD4+ T-cells and likely macrophages also contribute to inflammation and microbial translocation, creating a vicious cycle nurturing inflammation. Importantly, the size of the HIV reservoir has been linked to the level of inflammation and immune activation measured in CD8+ T-cells and macrophages (3, 10). Conversely, it remains unknown whether the reduction of inflammation can lead to a decrease in the size of the HIV reservoir.

Gut damage and immune activation

As HIV is not cleared with ART, persistent viral products and inflammation subsequently impair antigen-specific T-cell responses. This overall activation leads to the exhaustion of the immune system, including T-cells. This distinctive feature from other chronic viral infections is relevant as HIV replicates preferentially in Th17 CD4+ T-cells residing in the gut, leading to cell death and mucosa damage (11-13). Microbial products penetrate the damaged gut barrier and pass into the systemic circulation. Such microbial translocation contributes to systemic immune activation,

disease progression, and development of non-AIDS comorbidities (14-17). Markers of bacterial translocation, including lipopolysaccharide (LPS), LPS binding protein (LBP), and soluble CD14 (sCD14) have been correlated with immune activation and disease progression (3, 11, 18, 19). While bacterial translocation is thought to be a major cause of immune activation, we have shown that circulating beta-D-glucan (BDG), a marker of fungal translocation, also contributes to the immune activation in an LPS-independent manner (20, 21).

Although ART suppresses HIV replication to undetectable levels, microbial translocation and Th17 damage remain, contributing to persistent immune activation. Local gut inflammation has been associated with HIV reservoir size (22). Monocytes/macrophages, Dendritic cells, and Natural Killer (NK) cells detect microbial products in the mesenteric lymph nodes and in systemic circulation, secreting pro-inflammatory cytokines (interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF)-α). These cytokines drive CD4+ T-cell activation, leading to elevated expression of the HIV co-receptor C-C Chemokine receptor (CCR) 5 and the gut homing receptor CCR6 (23). Th17 measured by CD4+ T-cells expressing CCR6 have then been shown by our group to be preferentially infected by HIV (12, 13, 24).

Gut microbiota, dysbiosis and immune regulation

The gut microbiota composition and metabolites play an important role in inflammation in obesity, diabetes, cancer, and HIV infection. Its role includes food and metabolite processing, microbial regulation, and immune regulation (25-28). PLWH, compared to uninfected controls, present with a dysbiosis characterized by a lower abundance of Firmicutes and more abundant Proteobacteria in their gut microbiota. In addition lack of *Lactobacilli* in stools is associated with lower CD4+ T-cell count and a higher levels of systemic immune activation (29). Moreover, lower levels of *Akkermansia muciniphila* have been observed in PLWH. Dysbiosis combined with microbial translocation has been linked to non-AIDS comorbidities in HIV-infected individuals and influences CD4+ T-cell recovery on ART as reported by our group and others (3, 14, 18, 30, 31).

***Akkermansia muciniphila* in health and disease**

Akkermansia muciniphila (A. muciniphila) is a gram-negative, strict anaerobe and mucin-degrading bacterium that colonizes the gut of humans and rodents. *A. muciniphila* represents 1-5% of all intestinal bacteria. This bacterium acts as a shield on the gut epithelial barrier and has

been shown to reduce insulin resistance in obese individuals (26, 32-36). Lower abundance of *A. muciniphila* has been found in the feces of patients with inflammatory bowel disease (IBD) and individuals with obesity, when compared to feces of healthy individuals (25, 26). Furthermore, oral administration of *A. muciniphila* to mice fed a high-fat diet alleviates obesity, reduces LPS in the circulation and alleviates insulin resistance (26, 37, 38).

Additionally, patients with lung and renal cell carcinoma responding to anti-PD-1 treatment were more prone to have an elevated abundance of gut *A. muciniphila* compared to non-responders (28). To go beyond association, B. Routy *et al*. transferred the microbiota from responders and non-responders into germ-free mice and observed a tumor response only in mice with a *A. muciniphila* rich human fecal microbiota from the responders (28). Both *in vitro* and *in vivo*, *A. muciniphila* has been shown to increase mucus secretion by goblet cells and gut epithelium integrity contributing to the prevention of other bacterial products from passing into the circulation (35, 38, 39). Moreover, oral administration *A. muciniphila* was shown to successfully elevate key anti-aging and anticancer metabolites primarily in the gut and liver (40).

Based on these encouraging results, different attempts have been made to increase *A. muciniphila* in the gut. Everard *et al*. showed that pasteurized *A. muciniphila* increased mucus thickness, decreased LPS translocation, and reduced metabolic syndrome in obese mice. In contrast, heat-killed *A. muciniphila* did not protect mice from obesity (38). However, such pasteurized strains are costly, difficult to produce and may not last after oral administration.

The Amazonian fruit Camu Camu

Camu Camu (CC), also named *Myriciaria dubia*, is an Amazon rainforest fruit with antioxidant and anti-inflammatory properties. Anhê *et al*. showed that polyphenol-rich cranberry and CC extracts protect mice from diet-induced obesity and intestinal inflammation in association with increased *A. muciniphila* in the gut microbiota (32-35). CC was more efficient at reducing the amount of LPS in plasma than cranberry extract in the diet-induced model of obesity, and it was also found to increase other beneficial microbes in addition to *A. muciniphila*. Other studies have shown that polyphenols could favor *A. muciniphila* in the gut (26, 41, 42). Importantly, CC extracts also decreased C-Reactive protein (CRP), IL-6 and IL-8 in the plasma of healthy tobacco smokers (43). CC is considered a "super fruit" which is widely available in many Canadian health food

stores in either powder or capsule form. CC products have been used as a nutritional supplement that is well tolerated in humans (44).

CC is a fruit rich in polyphenols and has been shown in mouse model of inflammation-related diseases to reduce inflammation and improve gut microbiota with increased *A. muciniphila* and other beneficial bacteria (32-35). However, no studies have been performed to test CC in PLWH. Moreover, PLWH on ART have been shown to exhibit persistent dysbiosis, an altered gut microbiota composition, along with microbial translocation which can cause non-AIDS comorbidities and hamper CD4+ T-cell recovery (3, 14, 18, 30). Therefore, we will evaluate if the polyphenol rich CC can positively affect PLWH on ART in terms of reducing inflammation, improving gut microbiota and potentially decreasing HIV persistent reservoir.

Objective

To determine the feasibility and suitable design of a full-scale study on the effect of Camu Camu in ART-treated PLWH, we designed a non-randomized, single arm, interventional study.

Primary outcomes

The primary outcome of this study will be to evaluate the effect of CC on the reduction of the plasma marker of microbial translocation LPS, assessed using ELISA.

Secondary outcomes

The secondary outcomes of this study will be changes in the following before and after 12 weeks of CC intake, and after 8 weeks of CC discontinuation:

- Safety and tolerability of CC measured by evaluating adverse events, hematology, and serum chemistries over the course of the study. These evaluations will include HIV viral load, glucose levels, a lipid profile and plasma levels of hsCRP and D-dimer.
- Gut barrier integrity markers I-FABP and sST2, measured by ELISA.
- Microbial translocation marker (1-3)-β-D-Glucan (BDG) assessed using the Fungitell assay.
- Pro-inflammatory markers (IL-1β, IL-6, IL-8, IL-18. IP-10, IL-17A and F, IL-22, and soluble CD14) and anti-inflammatory markers (IL-10) assessed by ELISA.

- T-cell and monocyte activation levels assessed by flow cytometry using markers CD38, HLA-DR and PD-1.
- *A. muciniphila* levels in stools using qPCR.
- Microbiota composition and diversity in stools assessed using 16s rDNA sequencing.
- HIV reservoir size in blood assessed by PCR.
- Evaluate intra-patient variability using data from two baseline visits, approximately two weeks apart from each other to confirm reliability of baseline results.

Exploratory outcomes

The exploratory outcomes of this study will be the following:

- Difference in HIV reservoir size from Baseline (Visit 0) to 12 weeks post-CC treatment by TILDA, performed on blood samples.
- Changes in other markers of gut damage (including plasma REG3α (45)), microbial translocation (such as plasma 16S rDNA) and immune activation (T-cell activation, cytokines) in the blood and gut biopsies.

Sub-study outcomes

The sub-study outcomes will be the following:

- Changes in gut mucosa architecture in a subset of participants who will consent to have colon biopsies before and at the end of the 12 weeks of CC treatment.
- Changes in inflammation in gut mucosa biopsy assessed by myeloperoxidase staining before and at the end of the 12 weeks of CC treatment.
- Changes in HIV reservoir size in biopsies using qPCR.
- Association between baseline gut microbiota composition (16S rDNA sequencing), and markers of gut integrity (I-FABP, tissue staining) and inflammation (T-cell activation, inflammatory cytokines).

METHODS AND ANALYSIS

Study design, settings, sample size and recruitment strategy

Trial CTN PT032 is an open label, non-randomized, single arm interventional pilot study (Clinicaltrials.gov NCT04058392); protocol version # 1.3; February 12, 2021. The study sponsor

is the Canadian Institutes of Health Research (CIHR) Canadian HIV Trials Network (CTN). The following study protocol fulfills the requirements of the 2013 Standard Protocol Items: Recommendations for Interventional Trials guidelines (46, 47).

Comparisons and assessment of outcomes will be made through various measures at baseline, during and after CC use (**figure 1 and table 1**).

A total of 22 ART-treated participants living with HIV will be enrolled at the Chronic Viral Illness Service (CVIS) at the McGill University Health Centre (MUHC), Glen Site, Montreal, QC and the Ottawa Hospital, General Campus, Infectious Diseases Clinic, Ottawa, ON, Canada. A convenient sample size of 22 participants was chosen without formal power calculations for this pilot study, based on the Lilac study design (48, 49) and the study by Inoue *et al.* (43). This sample size accounts for an estimated loss to follow-up/non-completion of 10% for the study. It can therefore be estimated that 2 participants may not fully complete the study. There will be an optional colon biopsy sub-study. For logistical reasons, only participants recruited at the Montreal site will be given the option to participate in this sub-study, after giving informed consent to participate in the main study and being shown to be eligible for the main study (after screening). The sub-study will have a separate informed consent form. The obtained data from this study will be used for calculation of sample size for future larger studies.

Participants will be recruited at two above-mentioned centers in Canada. Both participating medical clinics provide care to more than 2000 HIV-infected persons. Teleconferences and face-to-face meetings will be organized between the Qualified Investigators and study staff to help promote patient recruitment and follow-up during the study.

Recruitment started in November 2020 and is expected to end in January 2022.

Inclusion criteria

Participants will be eligible for the study if they meet the following criteria: (1) Male or female adults ≥18 years of age; (2) Documented HIV-1 infection by Western Blot, Enzyme Immuno Assay (EIA) or viral load assay; (3) On ART for at least 2 years, and stable ART regimen (same prescription) for at least 3 months to ensure a stabilization of inflammation markers; (4) Persistent undetectable viral load < 50 copies/ml for the past 2 years. One viral blip are allowed if preceded and followed by a HIV viremia below 50 copies/ml; (5) CD4+ count >200 and a CD4+/CD8+ ratio <1, to recruit participants with increased inflammation and risk for non-AIDS comorbidities; (6)

Able to communicate adequately in either French or English; (7) Able and willing to provide written informed consent prior to screening; (8) As the influence of CC on pregnant women is unknown, women of childbearing potential must have a negative serum pregnancy test; (9) 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; (10) Women of non-child-bearing potential as defined as either post-menopausal (12 months of spontaneous amenorrhea and ≥ 45 years of age) or physically incapable of becoming pregnant with documented tubal ligation, hysterectomy or bilateral oophorectomy; (11) Sexually active men with a female partner of childbearing potential must agree to use an approved methods of birth control.

Exclusion criteria

Participants will not be eligible to participate in the study if they meet any of the following conditions: (1) Known allergy/hypersensitivity to Camu Camu; (2) Current AIDS-related event or serious health condition including systemic infections in the last 3 months; (3) Severe systemic diseases (e.g. uncontrolled hypertension, chronic renal failure), or active uncontrolled infections; (4) Co-infection with active Hepatitis B or C Virus; (5) Current use or have used in the past 3 months: immune-modulatory agents, prophylactic antibiotics(41)/antibiotics, proton pump inhibitors, or Morphine as these drugs modulate gut microbiota; (6) Current use of aluminum containing phosphate binders, chemotherapeutics, niacin, anticoagulant and protease inhibitors (including in their ART-regimen) as increased vitamin-C levels can prevent the activity of those molecules; (7) Diagnosis of diabetes mellitus (HbA1c≥6.5%) as defined by the Canadian Clinical Practice Guidelines for the Prevention and Management of Diabetes (50); (8) Frequent use of probiotics or polyphenol-rich prebiotics (e.g. cranberry and CC powders and/or capsules) in the last 12 months; (9) Recent changes in dietary habits, intermittent fasting, chronic constipation or laxative use as these can affect gut microbiota; (10) Psychiatric or cognitive disturbance or any illness that could preclude compliance with the study; (11) Current participation in an experimental therapy study or receipt of experimental therapy within the last 6 months; (12) Women who are planning to become or who are pregnant, or breast-feeding; (13) A score of higher than 8 on a Full AUDIT questionnaire at the screening visit, suggesting an alcohol abuse problem.

Study intervention

Participants will be instructed to take 1000 mg of Camu C™ (provided by Natural Traditions, Canada) once daily administered as two 500 mg oral capsules for 12 weeks. Capsules will be taken at the same time each day with a meal, preferentially breakfast. Camu C™ can be taken with ART as no interactions are expected. The 1000 mg dose is based on the dose given to mice per mean body weight divided by 12.3 as per the Food and Drug Administration (FDA) equation to determine equivalent dosing in human vs. mice, and consistent with Health Canada's recommendations of 1-3 capsules daily (51, 52).

The interaction between CC and other medication is unknown. CC has a high vitamin C concentration and therefore any drug with negative interactions with vitamin C were included in the exclusion criteria. The vitamin C in CC could interact with aluminum in phosphate binders (possible harmful to kidneys); chemotherapeutics (CC antioxidative properties could reduce the chemotherapeutic drug's effect); protease inhibitors (vitamin C might reduce the effect of antiviral drugs containing protease inhibitors); niacins (vitamin C could reduce niacin's effect); and anticoagulants since high doses of vitamin C can reduce responses to some anticoagulants. Hence, participants will be asked to refrain from using Vitamin C supplements during the study.

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.

Adverse events and toxicity management

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

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

When side effects are perceived to be related to Camu C™, the Investigator can use their clinical judgment regarding whether to continue or to discontinue the study medication. If Camu C™ treatment is discontinued, the participant will be scheduled for follow-up visit(s) as required to treat the symptoms or adverse event related to Camu C™ intake.

Clinical and laboratory assessments

Assessment of gut damage, microbial translocation, and inflammation

To evaluate gut epithelial damage, we and others have validated markers that will be measured in the plasma by ELISA before, during and after CC intake (11, 53-55). LPS, a common marker of bacterial translocation (56), soluble Suppression of Tumorigenicity (sST2)(20) and Intestinal-Fatty Acid Binding Protein (I-FABP) will be measured to assess gut barrier integrity. Immune activation markers (sCD14) and pro-(IL-1β, IL-6. IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines will be quantified (57, 58). Activation of monocytes and CD4+ and CD8+ T-cells will be assessed *ex vivo* by flow cytometry with HLA-DR and CD38 staining. CD4+ and CD8+ T-cells will also be assessed for PD-1 expression as a marker of T-cell exhaustion. Plasma will be assayed for beta D-glucan as a marker of fungal infection(21, 58). REG3α and 16S rDNA as well as other markers of microbial translocation and gut damage may be tested in plasma as well (45).

Assessment of microbiota composition

qPCR for *A. muciniphila* will be performed on fecal DNA samples as previously described by our group (48). Gut microbiota composition will be further studied by 16S and 18S rDNA sequencing to determine the impact on other beneficial microbes (e.g., *Barnesiella* and *Turicibacter*) known to respond to CC in the obesity mouse model (48).

Assessment of HIV reservoir size

If differences in microbial translocation and/or inflammation are observed, HIV reservoirs will be quantified in blood and rectal biopsies. HIV DNA (total and integrated) and cell-associated HIV RNA (gag) will be measured in DNA and RNA obtained from isolated CD4+ T-cells from the blood (obtained by negative magnetic selection) and gut biopsies (sorted by flow cytometry). HIV DNA and cell-associated RNA measures will be performed using an ultrasensitive nested qPCR as described previously (59).

In addition, the frequency of cells with inducible proviruses will be measured in isolated CD4+ T-cells from PBMCs using the Tat/rev Induced Limiting Dilution Assay (TILDA) in the laboratory of Dr. Chomont at Baseline Week 0 and End-treatment Week 12 timepoints.

In mucosal biopsies, HIV DNA and RNA will also be quantified and localized by DNA/RNAscope (24, 60).

Assessment of gut mucosa architecture (optional colon biopsy/sub-study)

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)(56). If a diminution in inflammation is noted, myeloperoxidase staining will be performed to allow for the quantification of inflammatory myeloid cells in the gut.

For other analyses, gut cells will be separated from tissues by enzyme digestion using a collagenase-based method as reported previously (61, 62). Briefly, fresh tissue biopsies will be incubated with type II collagenase for 30 minutes at 37°C in a shaking incubator. The resulting lymphocyte suspension will be stained with monoclonal Antibodies (mAbs) against CD3+, CD4+, CD8+, and myeloid markers. The total frequency of activated CD4+ and CD8+ T-cells will be determined by flow cytometry as described above.

Statistical analysis

To examine the change in plasma LPS and soluble CD14 levels relative to baseline, linear mixed effects regression will be used. Time will be considered as a categorical variable in the model to allow flexible modeling of the time trend. All five measurements (two for baseline and three for follow-up visits) will be included as outcome variable in the model. Log transformation of the outcome variable or generalized mixed effects regression will be employed if normality

assumption is not satisfied. Changes in plasma markers, percentage of activated T-cells, HIV reservoir size and bacterial taxa by type in the stools samples relative to baselines will be assessed in the same fashion. Demographics including age, sex, and sexual practice will be included in multivariable analyses as they have been shown to influence microbiota composition and immune activation in ART-treated PLWH.(29, 63)

Patient and public involvement

- Initial design of the study was presented to community groups.
- Compliance 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, will not be directly disseminated to study participants.

ETHICS AND DISSEMINATION PLAN

All participants will be given detailed oral and written information about the study. Consent documents 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 must sign an informed consent document that has been approved by a participating center's research ethics board (REB) prior to any procedures being done specifically for the trial. All potential protocol amendments will be submitted to Health Canada and the respective research ethics board of the participating centers. 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.

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 Camu Camu study when attending or presenting at local, national, and international meetings.

CONCLUSION

We hypothesize that treatment with CC will beneficially impact ART-treated PLWH by improving the gut microbiota composition, reducing systemic inflammation and immune action, reducing gut damage and microbial translocation, and potentially decreasing latent HIV reservoir size, thus decreasing the risk in developing non-AIDS comorbidities. This pilot trial with 22 ART-treated PLWH will provide sufficient data for future sample size calculations and set the foundation to assess the impact of CC in larger definitive studies.

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Author statement

Group Authorship/Collaborating Author Names: SI, BF, JO, JL, LR, SB, NS, PLL, TB, NS, MBK, BL, CTC, BR, AM, and JPR, for the Camu Camu Study Group

Contributors: J-PR and SI designed the study, with insights from JO, LR, JL, NS, NC, BR, and AM. BF and SI wrote the manuscript. JL, LR, SB, PLL, TB, NC, MBK, BL, CTC, BR, AM, will participate in data collection and analysis. All authors critically reviewed the manuscript and approved the final version.

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Conflicts of interest

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, Argos Pharmaceuticals from InnaVirVax and has served on the Advisory Board of Theravectys. JBA has performed contract research and/or served on Advisory Boards for Gilead Sciences Canada, Merck Canada, Abbvie, ViiV Healthcare, Bristol Myers Squibb, Janssen and Argos Pharmaceuticals. NC has received research funding from EMD Serono and has served on the Advisory Board of Gilead Sciences Canada. SI is a post-doctoral fellow from the Fonds de recherche du Quebec en santé,

and from the CIHR/CTN. B.L. is supported by a career award LE 250 from Quebec's Ministry of Health for researchers in family medicine. B.L. has received consultancy fees and/or honoraria from Gilead, Merck, and ViiV, and research funds from Gilead, Merck, and ViiV, support to attend educational conferences from Viiv Healthcare and Gilead. L.R. is a post-doctoral fellow supported by the Swiss National Science Foundation.

Patient consent for publication

Not required

Ethics approval

Canadian Institutes of Health Research/Canadian HIV Trials Network (CTN) pilot trial protocol CTNPT032. The study was approved by the Natural and Non-prescription Health Products Directorate of health Canada and the research ethics board of the McGill university Health Centre committee (number 2020-5903) and will be conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000.

Data access statement

The data generated by this study will be available from Dr Routy upon reasonable request after publication.

Table 1: Schedule of Events.

Visit Type	Screening	Study Visits				
		Baseline 1	Baseline 2	Treatment		Follow-up
Visit Window Procedures:	-8 to -1 weeks	Week -2 (±7 days)	Week 0 (Day 0)	Week 4 (±7 days)	Week 12 *(+7 days)*	Week 20 (±7 days)
Visit No.	1	2	3	4	5	6
Informed Consent	X	X				
Eligibility Assessment	X	X	X			
Concomitant Medication	X	X	X	X	X	X
Medical History	X					
Complete Physical Exam and Vital Signs	X					
Targeted Physical Exam and Vital Signs		X	X	X	X	X
Adverse Event Assessment				X	X	X
Serum Pregnancy Test	X	X	X	X	X	X
Hematology*	X	X†	X	X	X	X
Serum Chemistry **	X	X†	X	X	X	X
Serology***	X		X			
HIV-1 Viral Load***	X	X†	X	X	X	X
Immune activation markers/cytokines (ELISA)****		X	X	X	X	X
Monocyte and T-cell activation markers+		X	X	X	X	X
Markers of gut barrier integrity, inflammation, and microbial translocation++		X	X	X	X	X
Size of HIV reservoir in Latently Infected CD4+ T-cells+++		X	X	X	X	X
Stool sample collection and microbiota composition++++		X	X	X	X	X
Alcohol use questionnaire (AUDIT-Full)	X					
Alcohol use questionnaire (AUDIT-C)		X	X	X	X	X
Study Product Dispensation			X			
Study Product Compliance				X	X	
Colon mucosal biopsies#			X		X	

*CBC, CD4 and CD8 T-cell counts, erythrocyte sedimentation rate (ESR).
**Alkaline phosphatase, ALT, Amylase, AST, Bilirubin (total), Creatine kinase, Creatinine, D-dimer, fasting blood glucose, HbA1c, high sensitivity C-reactive protein (hsCRP), Lipase, lipid profile (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), Triglycerides), serum phosphate, Urea
***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 (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokine IL-10. Measured in plasma by ELISA.
+Monocyte and T-cell activation markers include HLA-DR and CD38. T-cell exhaustion marker: PD-1. Measured by staining and flow cytometry.
++Markers of gut barrier integrity, microbial translocation, and inflammation: lipopolysaccharide, soluble ST2, I-FABP (measured in plasma by ELISA).
+++PBMCs will be isolated and then latent CD4 T-cells will be isolated by flow cytometry. HIV viral reservoir in the latent CD4 T-cell population will be measured by nested qPCR. More specific TILDA analysis will be performed on Baseline Week 0 and End-treatment Week 12 samples to assess the HIV viral reservoir (Exploratory analysis).
++++qPCR of A. muciniphila, 16S and 18S rDNA sequencing for other members of the microbiota.
#Optional sub-study procedure.
† Not required when the same tests have been performed at the screening visit within the past 14 days, with the exception of CBC, CD4, CD8 (and serum pregnancy test)

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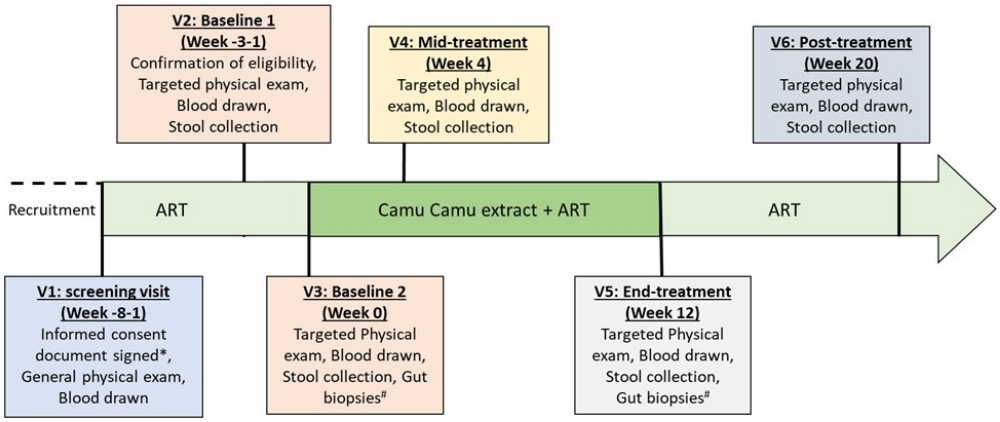
Figure legend

Figure 1: Study flow chart.

Visit 1, the Screening visit, will take place 1-8 weeks prior to the second Baseline visit (Week 0, Visit 3). At the Screening visit the informed consent document will be explained to the participant and will be signed prior to any screening and study activities. Two Baseline visits will be conducted, the second one being at Week 0 and all visits after that will be relative to this Baseline Week 0 Visit (Visit 3, Day 0). Data collected at these two Baseline visits will be directly compared to determine intra-patient variability. Camu Camu treatment will be a single daily dose of 1000 mg (2*500 mg Camu C™ capsules) taken with a meal, at the same time each day for 12 weeks. Treatment and post-treatment visit dates (Visit 4, Week 4 and Visit 6, Week 20) can vary ± 7 days according to participant and/or research team availability. Visit 5 at Week 12 can vary +7 days to ensure the participant has completed 12 weeks of Camu Camu treatment prior to the end-of-treatment visit. See Section 8 Schedule of Events (Table 1) to see more test details.

*The consent form for the optional gut biopsy will also be explained, but consent for this will not be necessary to be part of the main study. The sub-study is only available to participants at the Montreal site.

#Optional gut biopsies will be taken for the sub-study at indicated time points.



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#Optional gut biopsies will be taken for the sub-study at indicated time points.

164x68mm (150 x 150 DPI)

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Camu Camu pilot study (CTN PT032). Isnard et al.

Section/item	Item No	Description	Page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	8
Funding	4	Sources and types of financial, material, and other support	8, 16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	8
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	8
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7

	6b	Explanation for choice of comparators	8,9
Objectives	7	Specific objectives or hypotheses	7,8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8,9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9,10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10,11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10,11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7,8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12,13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	NA
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11,12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	14
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9, table 1
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16,17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11,12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the

protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Camu Camu effects on microbial translocation and systemic immune activation in ART-treated people living with HIV: protocol of the single-arm non-randomised Camu Camu prebiotic pilot study (CIHR/CTN PT032)

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Primary Subject Heading:	HIV/AIDS
Secondary Subject Heading:	Gastroenterology and hepatology, Immunology (including allergy), Infectious diseases, Nutrition and metabolism
Keywords:	HIV & AIDS < INFECTIOUS DISEASES, Inflammatory bowel disease <

	GASTROENTEROLOGY, Nutritional support < GASTROENTEROLOGY, IMMUNOLOGY

Camu Camu effects on microbial translocation and systemic immune activation in ART-treated people living with HIV: protocol of the single-arm non-randomised Camu Camu prebiotic pilot study (CIHR/CTN PT032)

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ABSTRACT (249 words)

Introduction

Despite the success of antiretroviral therapy (ART) in transforming HIV disease into a chronic infection, people living with HIV (PLWH) remain at risk for various non-AIDS inflammatory comorbidities. Risk of non-AIDS comorbidities is associated with gut dysbiosis, epithelial gut damage and subsequent microbial translocation, and increased activation of both circulating CD4+ and CD8+ T-cells. Therefore, in addition to ART, novel gut microbiota-modulating therapies could aid in reducing inflammation and immune activation, gut damage, and microbial translocation. Amongst various gut-modulation strategies under investigation, the Amazonian fruit Camu Camu (CC) presents itself as a prebiotic candidate based on its anti-inflammatory and antioxidant properties in animal models and tobacco smokers.

Method and analysis

A total of 22 PLWH on ART for more than 2 years, with a viral load <50 copies/mL, a CD4+ count >200 and a CD4+/CD8+ ratio <1 (suggesting increased inflammation and risk for non-AIDS comorbidities), will be recruited in a single arm, non-randomized, interventional pilot trial. We will assess tolerance and effect of supplementation with CC in ART-treated PLWH on reducing gut damage, microbial translocation, inflammation, and HIV latent reservoir by various assays.

Ethics and dissemination

The Canadian Institutes of Health Research (CIHR)/Canadian HIV Trials Network (CTN) pilot trial protocol CTNPT032 was approved by the Natural and Non-prescription Health Products Directorate of Health Canada and the research ethics board of the McGill university Health Centre committee (number 2020-5903). Results will be made available as a free access through publications in peer reviewed journals and through the CIHR/CTN website.

Trial registration number

ClinicalTrials.gov: NCT04058392

KEYWORDS

Camu Camu; HIV; Antiretroviral therapy; Prebiotic; Gut microbiota; gut mucosa; Inflammation.

ARTICLE SUMMARY: STRENGTHS AND LIMITATIONS OF THIS STUDY

- Camu camu (CC) is an Amazonian rainforest fruit which has been shown to have anti-inflammatory and gut microbiota-modulating properties in mice.
- The Camu Camu study seeks to confirm mouse model findings on systemic inflammation and immune activation, gut dysbiosis and damage, and subsequent microbial translocation in antiretroviral therapy (ART)-treated people living with HIV (PLWH).
- We hypothesize that treatment with CC will beneficially impact ART-treated PLWH by improving their gut microbiota composition, reducing microbial translocation, reducing inflammation to potentially decreasing latent HIV reservoir size and the risk to develop non-AIDS comorbidities.
- Changes induced by CC treatment will be assessed by plasma markers of gut damage, microbial translocation, inflammation, percentage of activated T-cells, HIV reservoir size and gut bacterial taxa.
- This pilot trial with 22 ART-treated PLWH, will provide sufficient data for future sample size calculations to confirm the effect of CC in more definitive larger studies.

Main text (3999 words)

INTRODUCTION

Antiretroviral therapy inhibits viral replication without eradication.

Antiretroviral therapy (ART) successfully controls Human Immunodeficiency Virus (HIV) infection by inhibiting viral replication and has significantly improved the life expectancy of people living with HIV (PLWH) while eliminating transmission to others. However, ART-treated PLWH remain at risk for developing inflammatory non-AIDS comorbidities such as cardiovascular diseases, fatty liver, neurocognition dysfunction and cancer (1, 2). These non-AIDS comorbidities are associated with persistent immune activation and increase with aging, co-infections like cytomegalovirus (CMV) and viral hepatitis as well as microbial translocation. It has been observed in HIV infection and inflammatory bowel disease that abnormal composition of the gut microbiota called "dysbiosis", alteration of the gut barrier, T-helper (Th) 17 cell dysfunction and microbial translocation lead to systemic inflammation and immune activation contributing to non-AIDS comorbidities (3-9). Long-term HIV control by ART appears to only partially reduce inflammation and poorly replenishes Th17 protective mucosal function, highlighting the importance of research on gut microbiota and the epithelial barrier. Furthermore, despite control of HIV replication, persistent HIV infection in long-lived memory CD4+ T-cells and likely macrophages also contribute to inflammation and microbial translocation, creating a vicious cycle nurturing inflammation. Importantly, the size of the HIV reservoir has been linked to the level of inflammation and immune activation measured in CD8+ T-cells and macrophages (3, 10). Conversely, it remains unknown whether the reduction of inflammation can lead to a decrease in the size of the HIV reservoir.

Gut damage and immune activation

As HIV is not cleared with ART, persistent viral products and inflammation subsequently impair antigen-specific T-cell responses. This overall activation leads to the exhaustion of the immune system, including T-cells. This distinctive feature from other chronic viral infections is relevant as HIV replicates preferentially in Th17 CD4+ T-cells residing in the gut, leading to cell death and mucosa damage (11-13). Microbial products penetrate the damaged gut barrier and pass into the systemic circulation. Such microbial translocation contributes to systemic immune activation,

disease progression, and development of non-AIDS comorbidities (14-17). Markers of bacterial translocation, including lipopolysaccharide (LPS), LPS binding protein (LBP), and soluble CD14 (sCD14) have been correlated with immune activation and disease progression (3, 11, 18, 19). While bacterial translocation is thought to be a major cause of immune activation, we have shown that circulating beta-D-glucan (BDG), a marker of fungal translocation, also contributes to the immune activation in an LPS-independent manner (20, 21).

Although ART suppresses HIV replication to undetectable levels, microbial translocation and Th17 damage remain, contributing to persistent immune activation. Local gut inflammation has been associated with HIV reservoir size (22). Monocytes/macrophages, Dendritic cells, and Natural Killer (NK) cells detect microbial products in the mesenteric lymph nodes and in systemic circulation, secreting pro-inflammatory cytokines (interleukin (IL)-1β, IL-8, tumor necrosis factor (TNF)-α). These cytokines drive CD4+ T-cell activation, leading to elevated expression of the HIV co-receptor C-C Chemokine receptor (CCR) 5 and the gut homing receptor CCR6 (23). Th17 measured by CD4+ T-cells expressing CCR6 have then been shown by our group to be preferentially infected by HIV (12, 13, 24).

Gut microbiota, dysbiosis and immune regulation

The gut microbiota composition and metabolites play an important role in inflammation in obesity, diabetes, cancer, and HIV infection. Its role includes food and metabolite processing, microbial regulation, and immune regulation (25-28). PLWH, compared to uninfected controls, present with a dysbiosis characterized by a lower abundance of Firmicutes and more abundant Proteobacteria in their gut microbiota. In addition lack of *Lactobacilli* in stools is associated with lower CD4+ T-cell count and a higher levels of systemic immune activation (29). Moreover, lower levels of *Akkermansia muciniphila* have been observed in PLWH. Dysbiosis combined with microbial translocation has been linked to non-AIDS comorbidities in HIV-infected individuals and influences CD4+ T-cell recovery on ART as reported by our group and others (3, 14, 18, 30, 31).

***Akkermansia muciniphila* in health and disease**

Akkermansia muciniphila (A. muciniphila) is a gram-negative, strict anaerobe and mucin-degrading bacterium that colonizes the gut of humans and rodents. *A. muciniphila* represents 1-5% of all intestinal bacteria. This bacterium acts as a shield on the gut epithelial barrier and has

been shown to reduce insulin resistance in obese individuals (26, 32-36). Lower abundance of *A. muciniphila* has been found in the feces of patients with inflammatory bowel disease (IBD) and individuals with obesity, when compared to feces of healthy individuals (25, 26). Furthermore, oral administration of *A. muciniphila* to mice fed a high-fat diet alleviates obesity, reduces LPS in the circulation and alleviates insulin resistance (26, 37, 38).

Additionally, patients with lung and renal cell carcinoma responding to anti-PD-1 treatment were more prone to have an elevated abundance of gut *A. muciniphila* compared to non-responders (28). To go beyond association, B. Routy *et al*. transferred the microbiota from responders and non-responders into germ-free mice and observed a tumor response only in mice with a *A. muciniphila* rich human fecal microbiota from the responders (28). Both *in vitro* and *in vivo*, *A. muciniphila* has been shown to increase mucus secretion by goblet cells and gut epithelium integrity contributing to the prevention of other bacterial products from passing into the circulation (35, 38, 39). Moreover, oral administration *A. muciniphila* was shown to successfully elevate key anti-aging and anticancer metabolites primarily in the gut and liver (40).

Based on these encouraging results, different attempts have been made to increase *A. muciniphila* in the gut. Everard *et al*. showed that pasteurized *A. muciniphila* increased mucus thickness, decreased LPS translocation, and reduced metabolic syndrome in obese mice. In contrast, heat-killed *A. muciniphila* did not protect mice from obesity (38). However, such pasteurized strains are costly, difficult to produce and may not last after oral administration.

The Amazonian fruit Camu Camu

Camu Camu (CC), also named *Myriciaria dubia*, is an Amazon rainforest fruit with antioxidant and anti-inflammatory properties. Anhê *et al*. showed that polyphenol-rich cranberry and CC extracts protect mice from diet-induced obesity and intestinal inflammation in association with increased *A. muciniphila* in the gut microbiota (32-35). CC was more efficient at reducing the amount of LPS in plasma than cranberry extract in the diet-induced model of obesity, and it was also found to increase other beneficial microbes in addition to *A. muciniphila*. Other studies have shown that polyphenols could favor *A. muciniphila* in the gut (26, 41, 42). Importantly, CC extracts also decreased C-Reactive protein (CRP), IL-6 and IL-8 in the plasma of healthy tobacco smokers (43). CC is considered a "super fruit" which is widely available in many Canadian health food

stores in either powder or capsule form. CC products have been used as a nutritional supplement that is well tolerated in humans (44).

CC is a fruit rich in polyphenols and has been shown in mouse model of inflammation-related diseases to reduce inflammation and improve gut microbiota with increased *A. muciniphila* and other beneficial bacteria (32-35). However, no studies have been performed to test CC in PLWH. Moreover, PLWH on ART have been shown to exhibit persistent dysbiosis, an altered gut microbiota composition, along with microbial translocation which can cause non-AIDS comorbidities and hamper CD4+ T-cell recovery (3, 14, 18, 30). Therefore, we will evaluate if the polyphenol rich CC can positively affect PLWH on ART in terms of reducing inflammation, improving gut microbiota and potentially decreasing HIV persistent reservoir.

Objective

To determine the feasibility and suitable design of a full-scale study on the effect of Camu Camu in ART-treated PLWH, we designed a non-randomized, single arm, interventional study.

Primary outcomes

The primary outcome of this study will be to evaluate the effect of CC on the reduction of the plasma marker of microbial translocation LPS, assessed using ELISA.

Secondary outcomes

The secondary outcomes of this study will be changes in the following before and after 12 weeks of CC intake, and after 8 weeks of CC discontinuation:

- Safety and tolerability of CC measured by evaluating adverse events, hematology, and serum chemistries over the course of the study. These evaluations will include HIV viral load, glucose levels, a lipid profile and plasma levels of hsCRP and D-dimer.
- Gut barrier integrity markers I-FABP and sST2, measured by ELISA.
- Microbial translocation marker (1-3)-β-D-Glucan (BDG) assessed using the Fungitell assay.
- Pro-inflammatory markers (IL-1β, IL-6, IL-8, IL-18. IP-10, IL-17A and F, IL-22, and soluble CD14) and anti-inflammatory markers (IL-10) assessed by ELISA.

- T-cell and monocyte activation levels assessed by flow cytometry using markers CD38, HLA-DR and PD-1.
- *A. muciniphila* levels in stools using qPCR.
- Microbiota composition and diversity in stools assessed using 16s rDNA sequencing.
- HIV reservoir size in blood assessed by PCR.
- Evaluate intra-patient variability using data from two baseline visits, approximately two weeks apart from each other to confirm reliability of baseline results.

Exploratory outcomes

The exploratory outcomes of this study will be the following:

- Difference in HIV reservoir size from Baseline (Visit 0) to 12 weeks post-CC treatment by TILDA, performed on blood samples.
- Changes in other markers of gut damage (including plasma REG3α (45)), microbial translocation (such as plasma 16S rDNA) and immune activation (T-cell activation, cytokines) in the blood and gut biopsies.

Sub-study outcomes

The sub-study outcomes will be the following:

- Changes in gut mucosa architecture in a subset of participants who will consent to have colon biopsies before and at the end of the 12 weeks of CC treatment.
- Changes in inflammation in gut mucosa biopsy assessed by myeloperoxidase staining before and at the end of the 12 weeks of CC treatment.
- Changes in HIV reservoir size in biopsies using qPCR.
- Association between baseline gut microbiota composition (16S rDNA sequencing), and markers of gut integrity (I-FABP, tissue staining) and inflammation (T-cell activation, inflammatory cytokines).

METHODS AND ANALYSIS

Study design, settings, sample size and recruitment strategy

Trial CTN PT032 is an open label, non-randomized, single arm interventional pilot study (Clinicaltrials.gov NCT04058392); protocol version # 1.3; February 12, 2021. The study sponsor

is the Canadian Institutes of Health Research (CIHR) Canadian HIV Trials Network (CTN). The following study protocol fulfills the requirements of the 2013 Standard Protocol Items: Recommendations for Interventional Trials guidelines (46, 47).

Comparisons and assessment of outcomes will be made through various measures at baseline, during and after CC use (**figure 1 and table 1**).

A total of 22 ART-treated participants living with HIV will be enrolled at the Chronic Viral Illness Service (CVIS) at the McGill University Health Centre (MUHC), Glen Site, Montreal, QC and the Ottawa Hospital, General Campus, Infectious Diseases Clinic, Ottawa, ON, Canada. A convenient sample size of 22 participants was chosen without formal power calculations for this pilot study, based on the Lilac study design (48, 49) and the study by Inoue *et al.* (43). This sample size accounts for an estimated loss to follow-up/non-completion of 10% for the study. It can therefore be estimated that 2 participants may not fully complete the study. There will be an optional colon biopsy sub-study. For logistical reasons, only participants recruited at the Montreal site will be given the option to participate in this sub-study, after giving informed consent to participate in the main study and being shown to be eligible for the main study (after screening). The sub-study will have a separate informed consent form. The obtained data from this study will be used for calculation of sample size for future larger studies.

Participants will be recruited at two above-mentioned centers in Canada. Both participating medical clinics provide care to more than 2000 HIV-infected persons. Teleconferences and face-to-face meetings will be organized between the Qualified Investigators and study staff to help promote patient recruitment and follow-up during the study.

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 recorded. Previous use of ART drugs and other medication will also be documented.

Recruitment started in November 2020 and is expected to end in January 2022.

Inclusion criteria

Participants will be eligible for the study if they meet the following criteria: (1) Male or female adults ≥18 years of age; (2) Documented HIV-1 infection by Western Blot, Enzyme Immuno Assay (EIA) or viral load assay; (3) On ART for at least 2 years, and stable ART regimen (same

prescription) for at least 3 months to ensure a stabilization of inflammation markers; (4) Persistent undetectable viral load < 50 copies/ml for the past 2 years. One viral blip are allowed if preceded and followed by a HIV viremia below 50 copies/ml; (5) CD4+ count >200 and a CD4+/CD8+ ratio <1, to recruit participants with increased inflammation and risk for non-AIDS comorbidities; (6) Able to communicate adequately in either French or English; (7) Able and willing to provide written informed consent prior to screening; (8) As the influence of CC on pregnant women is unknown, women of childbearing potential must have a negative serum pregnancy test; (9) 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; (10) Women of non-child-bearing potential as defined as either post-menopausal (12 months of spontaneous amenorrhea and ≥ 45 years of age) or physically incapable of becoming pregnant with documented tubal ligation, hysterectomy or bilateral oophorectomy; (11) Sexually active men with a female partner of childbearing potential must agree to use an approved methods of birth control.

Exclusion criteria

Participants will not be eligible to participate in the study if they meet any of the following conditions: (1) Known allergy/hypersensitivity to Camu Camu; (2) Current AIDS-related event or serious health condition including systemic infections in the last 3 months; (3) Severe systemic diseases (e.g. uncontrolled hypertension, chronic renal failure), or active uncontrolled infections; (4) Co-infection with active Hepatitis B or C Virus; (5) Current use or have used in the past 3 months: immune-modulatory agents, prophylactic antibiotics(41)/antibiotics, proton pump inhibitors, or Morphine as these drugs modulate gut microbiota; (6) Current use of aluminum containing phosphate binders, chemotherapeutics, niacin, anticoagulant and protease inhibitors (including in their ART-regimen) as increased vitamin-C levels can prevent the activity of those molecules; (7) Diagnosis of diabetes mellitus (HbA1c≥6.5%) as defined by the Canadian Clinical Practice Guidelines for the Prevention and Management of Diabetes (50); (8) Frequent use of probiotics or polyphenol-rich prebiotics (e.g. cranberry and CC powders and/or capsules) in the last 12 months; (9) Recent changes in dietary habits, intermittent fasting, chronic constipation or laxative use as these can affect gut microbiota; (10) Psychiatric or cognitive disturbance or any illness that could preclude compliance with the study; (11) Current participation in an experimental therapy study or receipt of experimental therapy within the last 6 months; (12) Women who are

planning to become or who are pregnant, or breast-feeding; (13) A score of higher than 8 on a Full AUDIT questionnaire at the screening visit, suggesting an alcohol abuse problem.

Study intervention

Participants will be instructed to take 1000 mg of Camu C™ (provided by Natural Traditions, Canada) once daily administered as two 500 mg oral capsules for 12 weeks. Capsules will be taken at the same time each day with a meal, preferentially breakfast. Camu C™ can be taken with ART as no interactions are expected. The 1000 mg dose is based on the dose given to mice per mean body weight divided by 12.3 as per the Food and Drug Administration (FDA) equation to determine equivalent dosing in human vs. mice, and consistent with Health Canada's recommendations of 1-3 capsules daily (51, 52).

The interaction between CC and other medication is unknown. CC has a high vitamin C concentration and therefore any drug with negative interactions with vitamin C were included in the exclusion criteria. The vitamin C in CC could interact with aluminum in phosphate binders (possible harmful to kidneys); chemotherapeutics (CC antioxidative properties could reduce the chemotherapeutic drug's effect); protease inhibitors (vitamin C might reduce the effect of antiviral drugs containing protease inhibitors); niacins (vitamin C could reduce niacin's effect); and anticoagulants since high doses of vitamin C can reduce responses to some anticoagulants. Hence, participants will be asked to refrain from using Vitamin C supplements during the study.

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.

Adverse events and toxicity management

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

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

When side effects are perceived to be related to Camu C™, the Investigator can use their clinical judgment regarding whether to continue or to discontinue the study medication. If Camu C™ treatment is discontinued, the participant will be scheduled for follow-up visit(s) as required to treat the symptoms or adverse event related to Camu C™ intake.

Clinical and laboratory assessments

Assessment of gut damage, microbial translocation, and inflammation

To evaluate gut epithelial damage, we and others have validated markers that will be measured in the plasma by ELISA before, during and after CC intake (11, 53-55). LPS, a common marker of bacterial translocation (56), soluble Suppression of Tumorigenicity (sST2)(20) and Intestinal-Fatty Acid Binding Protein (I-FABP) will be measured to assess gut barrier integrity. Immune activation markers (sCD14) and pro-(IL-1β, IL-6. IL-8, TNF-α) and anti-inflammatory (IL-10) cytokines will be quantified (57, 58). Activation of monocytes and CD4+ and CD8+ T-cells will be assessed *ex vivo* by flow cytometry with HLA-DR and CD38 staining. CD4+ and CD8+ T-cells will also be assessed for PD-1 expression as a marker of T-cell exhaustion. Plasma will be assayed for beta D-glucan as a marker of fungal infection(21, 58). REG3α and 16S rDNA as well as other markers of microbial translocation and gut damage may be tested in plasma as well (45).

Assessment of microbiota composition

qPCR for *A. muciniphila* will be performed on fecal DNA samples as previously described by our group (48). Gut microbiota composition will be further studied by 16S and 18S rDNA sequencing to determine the impact on other beneficial microbes (e.g., *Barnesiella* and *Turicibacter*) known to respond to CC in the obesity mouse model (48).

Assessment of HIV reservoir size

If differences in microbial translocation and/or inflammation are observed, HIV reservoirs will be quantified in blood and rectal biopsies. HIV DNA (total and integrated) and cell-associated HIV RNA (gag) will be measured in DNA and RNA obtained from isolated CD4+ T-cells from the blood (obtained by negative magnetic selection) and gut biopsies (sorted by flow cytometry). HIV DNA and cell-associated RNA measures will be performed using an ultrasensitive nested qPCR as described previously (59).

In addition, the frequency of cells with inducible proviruses will be measured in isolated CD4+ T-cells from PBMCs using the Tat/rev Induced Limiting Dilution Assay (TILDA) in the laboratory of Dr. Chomont at Baseline Week 0 and End-treatment Week 12 timepoints.

In mucosal biopsies, HIV DNA and RNA will also be quantified and localized by DNA/RNAscope (24, 60).

Assessment of gut mucosa architecture (optional colon biopsy/sub-study)

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)(56). If a diminution in inflammation is noted, myeloperoxidase staining will be performed to allow for the quantification of inflammatory myeloid cells in the gut.

For other analyses, gut cells will be separated from tissues by enzyme digestion using a collagenase-based method as reported previously (61, 62). Briefly, fresh tissue biopsies will be incubated with type II collagenase for 30 minutes at 37°C in a shaking incubator. The resulting lymphocyte suspension will be stained with monoclonal Antibodies (mAbs) against CD3+, CD4+, CD8+, and myeloid markers. The total frequency of activated CD4+ and CD8+ T-cells will be determined by flow cytometry as described above.

Statistical analysis

To examine the change in plasma LPS and soluble CD14 levels relative to baseline, linear mixed effects regression will be used. Time will be considered as a categorical variable in the model to allow flexible modeling of the time trend. All five measurements (two for baseline and three for follow-up visits) will be included as outcome variable in the model. Log transformation of the outcome variable or generalized mixed effects regression will be employed if normality assumption is not satisfied. Changes in plasma markers, percentage of activated T-cells, HIV reservoir size and bacterial taxa by type in the stools samples relative to baselines will be assessed in the same fashion. Demographics including age, sex, sexual practice and HIV history data will be included in multivariable analyses as they have been shown to influence microbiota composition and immune activation in ART-treated PLWH (29, 63).

Patient and public involvement

- Initial design of the study was presented to community groups.
- Compliance 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, will not be directly disseminated to study participants.

ETHICS AND DISSEMINATION PLAN

All participants will be given detailed oral and written information about the study. Consent documents 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 must sign an informed consent document that has been approved by a participating center's research ethics board (REB) prior to any procedures being done specifically for the trial. All potential protocol amendments will be submitted to Health Canada and the respective research ethics board of the participating centers. 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.

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 Camu Camu study when attending or presenting at local, national, and international meetings.

CONCLUSION

We hypothesize that treatment with CC will beneficially impact ART-treated PLWH by improving the gut microbiota composition, reducing systemic inflammation and immune action, reducing gut damage and microbial translocation, and potentially decreasing latent HIV reservoir size, thus decreasing the risk in developing non-AIDS comorbidities. This pilot trial with 22 ART-treated PLWH will provide sufficient data for future sample size calculations and set the foundation to assess the impact of CC in larger definitive studies.

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Author statement

Group Authorship/Collaborating Author Names: SI, BF, JO, JL, LR, SB, NS, PLL, TB, NS, MBK, BL, CTC, BR, AM, and JPR, for the Camu Camu Study Group

Contributors: J-PR and SI designed the study, with insights from JO, LR, JL, NS, NC, BR, and AM. BF and SI wrote the manuscript. JL, LR, SB, PLL, TB, NC, MBK, BL, CTC, BR, AM, will participate in data collection and analysis. All authors critically reviewed the manuscript and approved the final version.

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Conflicts of interest

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, Argos Pharmaceuticals from InnaVirVax and has served on the Advisory Board of Theravectys. JBA has performed contract research and/or served on Advisory Boards for Gilead Sciences Canada, Merck Canada, Abbvie, ViiV Healthcare, Bristol Myers Squibb, Janssen and Argos Pharmaceuticals. NC has received research funding from EMD Serono and has served on the Advisory Board of Gilead Sciences Canada. SI is a post-doctoral fellow from the Fonds de recherche du Quebec en santé,

and from the CIHR/CTN. B.L. is supported by a career award LE 250 from Quebec's Ministry of Health for researchers in family medicine. B.L. has received consultancy fees and/or honoraria from Gilead, Merck, and ViiV, and research funds from Gilead, Merck, and ViiV, support to attend educational conferences from Viiv Healthcare and Gilead. L.R. is a post-doctoral fellow supported by the Swiss National Science Foundation.

Patient consent for publication

Not required

Ethics approval

Canadian Institutes of Health Research/Canadian HIV Trials Network (CTN) pilot trial protocol CTNPT032. The study was approved by the Natural and Non-prescription Health Products Directorate of health Canada and the research ethics board of the McGill university Health Centre committee (number 2020-5903) and will be conducted in accordance with the Declaration of Helsinki of 1975, as revised in 2000.

Data access statement

The data generated by this study will be available from Dr Routy upon reasonable request after publication.

Table 1: Schedule of Events.

Visit Type	Screening	Study Visits				
		Baseline 1	Baseline 2	Treatment		Follow-up
Visit Window Procedures:	-8 to -1 weeks	Week -2 (±7 days)	Week 0 (Day 0)	Week 4 (±7 days)	Week 12 *(+7 days)*	Week 20 (±7 days)
Visit No.	1	2	3	4	5	6
Informed Consent	X	X				
Eligibility Assessment	X	X	X			
Concomitant Medication	X	X	X	X	X	X
Medical History	X					
Complete Physical Exam and Vital Signs	X					
Targeted Physical Exam and Vital Signs		X	X	X	X	X
Adverse Event Assessment				X	X	X
Serum Pregnancy Test	X	X	X	X	X	X
Hematology*	X	X†	X	X	X	X
Serum Chemistry **	X	X†	X	X	X	X
Serology***	X		X			
HIV-1 Viral Load***	X	X†	X	X	X	X
Immune activation markers/cytokines (ELISA)****		X	X	X	X	X
Monocyte and T-cell activation markers+		X	X	X	X	X
Markers of gut barrier integrity, inflammation, and microbial translocation++		X	X	X	X	X
Size of HIV reservoir in Latently Infected CD4+ T-cells+++		X	X	X	X	X
Stool sample collection and microbiota composition++++		X	X	X	X	X
Alcohol use questionnaire (AUDIT-Full)	X					
Alcohol use questionnaire (AUDIT-C)		X	X	X	X	X
Study Product Dispensation			X			
Study Product Compliance				X	X	
Colon mucosal biopsies#			X		X	

*CBC, CD4 and CD8 T-cell counts, erythrocyte sedimentation rate (ESR).
**Alkaline phosphatase, ALT, Amylase, AST, Bilirubin (total), Creatine kinase, Creatinine, D-dimer, fasting blood glucose, HbA1c, high sensitivity C-reactive protein (hsCRP), Lipase, lipid profile (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), Triglycerides), serum phosphate, Urea
***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 (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokine IL-10. Measured in plasma by ELISA.
+Monocyte and T-cell activation markers include HLA-DR and CD38. T-cell exhaustion marker: PD-1. Measured by staining and flow cytometry.
++Markers of gut barrier integrity, microbial translocation, and inflammation: lipopolysaccharide, soluble ST2, I-FABP (measured in plasma by ELISA).
+++PBMCs will be isolated and then latent CD4 T-cells will be isolated by flow cytometry. HIV viral reservoir in the latent CD4 T-cell population will be measured by nested qPCR. More specific TILDA analysis will be performed on Baseline Week 0 and End-treatment Week 12 samples to assess the HIV viral reservoir (Exploratory analysis).
++++qPCR of A. muciniphila, 16S and 18S rDNA sequencing for other members of the microbiota.
#Optional sub-study procedure.
† Not required when the same tests have been performed at the screening visit within the past 14 days, with the exception of CBC, CD4, CD8 (and serum pregnancy test)

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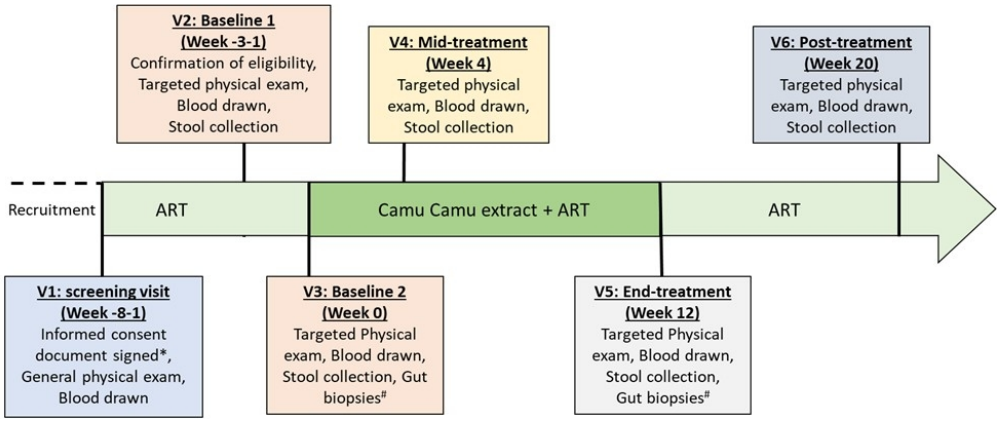
Figure legend

Figure 1: Study flow chart.

Visit 1, the Screening visit, will take place 1-8 weeks prior to the second Baseline visit (Week 0, Visit 3). At the Screening visit the informed consent document will be explained to the participant and will be signed prior to any screening and study activities. Two Baseline visits will be conducted, the second one being at Week 0 and all visits after that will be relative to this Baseline Week 0 Visit (Visit 3, Day 0). Data collected at these two Baseline visits will be directly compared to determine intra-patient variability. Camu Camu treatment will be a single daily dose of 1000 mg (2*500 mg Camu C™ capsules) taken with a meal, at the same time each day for 12 weeks. Treatment and post-treatment visit dates (Visit 4, Week 4 and Visit 6, Week 20) can vary ± 7 days according to participant and/or research team availability. Visit 5 at Week 12 can vary +7 days to ensure the participant has completed 12 weeks of Camu Camu treatment prior to the end-of-treatment visit. See Section 8 Schedule of Events (Table 1) to see more test details.

*The consent form for the optional gut biopsy will also be explained, but consent for this will not be necessary to be part of the main study. The sub-study is only available to participants at the Montreal site.

#Optional gut biopsies will be taken for the sub-study at indicated time points.



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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Camu Camu pilot study (CTN PT032). Isnard et al.

Section/item	Item No	Description	Page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	8
Funding	4	Sources and types of financial, material, and other support	8, 16
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	8
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	8
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7

	6b	Explanation for choice of comparators	8,9
Objectives	7	Specific objectives or hypotheses	7,8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8,9
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	9
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9,10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10,11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	10,11
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	12
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	7,8
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Figure 1, table 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	9
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	NA
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12,13
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	13

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	NA
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	NA
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11,12
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	

Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	14
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	9, table 1
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	16,17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	11,12
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	14
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	14
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	NA

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the

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