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Oral cannabinoids in people living with HIV on effective antiretroviral therapy—CTN PT028: Study protocol for a pilot randomized trial to assess safety, tolerability and effect on immune activation

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Abstract:

Introduction: Despite antiretroviral therapy(ART), people living with HIV have higher rates of non-infectious chronic diseases. These conditions are driven by relatively high levels of inflammation persisting on ART compared to uninfected individuals. Chronic inflammation also contributes to HIV persistence during ART. Cannabis when taken orally may represent a way to reduce inflammation and strengthen immune responses. Before planning large interventional studies, it is important to ensure that cannabis taken orally is safe and well-tolerated in people living with HIV. We propose to conduct a pilot randomized trial to examine the safety and tolerability of cannabis oils containing tetrahydrocannabinol(THC) and cannabidiol(CBD) consumed orally in people living with HIV. We will also measure inflammatory markers, markers of HIV persistence in peripheral blood cells and changes in the gastrointestinal microbiome.

Methods and Analysis: Twenty-six people living with HIV having undetectable viral load for at least three years will be randomized to receive TN-TC11LM(THC:CBD in 1:1 ratio) or TN-TC19LM(THC:CBD in 1:9 ratio) capsules daily for 12 weeks. Safety and tolerability of these capsules will be assessed through haematological, hepatic and renal blood tests, face-to-face interviews and questionnaires. Proportions of participants without any signs of significant

toxicity(Grades 0-2 scores on the WHO toxicity scale) and who complete the study, as well as scores on quality of life and mood will be examined using descriptive statistics. The effects on inflammatory markers, markers of peripheral blood reservoir size and effect on the composition of the gastrointestinal microbiome will be assessed before and after study completion.

Ethics and Dissemination: This study has been approved by the Research Institute of the McGill University Health Centre. A Data Safety Monitor will review safety information at regular intervals. The final manuscript will be submitted to an open-access journal within 6 months of study completion. This trial is registered with clinicaltrials.gov(NCT03550352).

Strengths and limitations of this study

- Will provide important preliminary data of cannabinoids on safety and tolerability in people living with HIV

- The first and only randomized clinical trial to date which will examine the effects of oral cannabinoids on markers of inflammation and HIV reservoir, as well as the gut microbiome

- Identified by Community Advisory Committee for people living with HIV as a key interest of study and study commencement coincides with cannabis legalization in Canada

- The number of participants should provide insight into the degree of variability for continuous outcomes we are measuring and should help guide future sample size calculations for larger studies

Background

Despite antiretroviral therapy (ART), persistent immune activation is associated with increased risk of non-opportunistic complications in people living with HIV such as cardiovascular, pulmonary, renal and hepatic events[1 2]. HIV pathogenesis and persistence appear to be related to chronic inflammation and immune activation[3], driven by microbial translocation of bacterial products across the gut mucosa[4-6]. Even when ART is initiated in the primary or early phase of HIV, gut integrity is not fully restored[7]. Furthermore, there is recent evidence to suggest that inflammatory features of the enteric microbiota, and not just increased permeability alone, is driving chronic inflammation in people living with HIV. Indeed, some studies have shown correlations between specific enteric bacteria and immune activation markers in gut and blood[8 9]. Persistent immune activation may also contribute to the persistence of HIV during ART[10-15]. HIV reservoirs are the reason why HIV remains an incurable infection. Although HIV may also persist in myeloid cells, CD4+ T cells are the best-characterized and the most abundant reservoirs[16-18].

Attenuation of immune activation and levels of inflammation may portend several therapeutic benefits to people living with HIV and novel strategies are needed to achieve this goal. Having both anti-inflammatory and anti-fibrotic properties[19], cannabis may represent a

feasible method to reduce immune activation and enhance immune profile. When consumed orally, notably soluble in oil, it may also temper inflammation at the gastrointestinal mucosa[20]. This, in turn, may hasten the progression of non-opportunistic complications associated with HIV. Furthermore, cannabis use, mainly through smoking, is already extremely common amongst PLWHIV. A study conducted by our team involving 1072 participants in the Canadian Co-infection Cohort Study (CTN 222), a multi-centered longitudinal study of HIV-Hepatitis C co-infected individuals, revealed that 53% of individuals had smoked marijuana in the past 6 months[21]. A clearer understanding of the safety, tolerability and feasibility of using cannabis in a clinical trial is an important first step before designing interventional studies to ameliorate specific conditions of importance to people living with HIV. Furthermore, as cannabis use will be legalized in Canada July 2018, understanding its safety profile is very important.

Pharmacology and medical properties of cannabis

Cannabinoids, found in the hemp plant *Cannabis sativa*, have been recognized for centuries for their analgesic, anticonvulsant, bronchodilatory, sedative, hypnotic and antispasmodic properties[22 23]. Their biological activity is conferred by cannabinoid receptors CB1 and CB2 through activation of heterodimeric G-proteins that function as signaling and

regulatory proteins to operate or modulate intracellular signaling pathways[24 25]. While CB1 receptors are expressed predominantly in the central nervous system, they are also found in the lung, liver and kidneys. The endocannabinoid system also plays a key role in the gastrointestinal tract's neural and molecular control mechanisms. Indeed, the endocannabinoid system plays a role in normal physiological functions of the gastrointestinal tract including motility, gut–brain-mediated fat intake, hunger signaling, inflammation and gut permeability[26]. Furthermore, there is some evidence for and interactions between the endocannaboid system and the gut microbiota[26]. In lean mice who received 4 weeks of a cannabinoid-receptor agonist HU-210, plasma lipopolysaccharides (LPS) were significantly increased[26 27]. When obese mice with disrupted gastrointestinal musoca were treated with rimonabant, an inverse CB1 agonist, for nearly 2 weeks, reductions in plasma LPS were observed[26 28]. Furthermore, improvements in localization of tight junction proteins, occluding and zonula occludens-1 were measured, suggestive of improvement in endothelial barrier function[26 28].

The presence of cannabinoid receptors in the central nervous system accounts for the psychoactive effects of cannabis[24 25]. In contrast, CB2 receptors are abundant on immune cells including T and B cells, natural killer cells, monocytes and neutrophils as well as the liver[24 29]. Two primary active constituents found in hemp plants include Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Δ9-THC is a partial agonist at both CB1 and CB2[3 30 31] while CBD is

thought to mediate its effects through a variety of signaling mechanisms and act as a negative allosteric modulator at CB1[32 33].

Cannabis is perhaps best known for its effect on stimulating appetite via CB1 receptor activity and was historically used for this purpose in AIDS wasting syndrome. A variety of randomized controlled trials have shown that smoked cannabis is efficacious in the management of chronic pain, including in painful HIV-associated sensory neuropathy[34-36]. In animal models, Δ9-THC prevents atherosclerosis by inhibiting macrophage migration into atheromas through CB2 activation[37 38]. Endocannabinoids may also play a role in liver disease, where CB1 receptors are present in endothelial cells and hepatocytes and CB2 receptors are distributed in Kupffer cells[39 40]. CB1 receptors have an important role in non-alcoholic fatty liver disease and alcoholic liver disease while anti-inflammatory action of CB2 receptors can be useful in inflammatory liver disease. CB2 receptors show antifibrinogenic properties and administration of its agonists in fibrotic rats resulted in improvement in liver fibrosis, decreased inflammation and increased apoptosis of hepatic myofibroblasts[40-44].

Cannabis, the immune system and HIV

In vitro **studies**

Cannabinoids have been shown to inhibit productive HIV infection in primary human T cells and a CB2 antagonist blocked this effect[45]. Interference with the signal transduction of chemokine receptor CXCR4 is thought to lead to reduced F-actin accumulation. This in turn prevents movement of viral pre-integration complexes to the nucleus. It has also been postulated that CB2 agonists may inhibit T cell activation induced by anti-CD3/anti-CD28[45]. Along with reduced HIV production, immunological effects of cannabinoids include induction of immunosuppressive cytokines including IL-10, TGF-β and inhibition of IL-2 which stimulates T-cell division and expansion. They have also been shown to decrease adhesion capacity of leukocytes to extravasate into sites of inflammation[46-48]. Apoptosis and induction of Treg may potentially add to the pathways of cannabinoid-mediated immunosuppression[49].

Animal studies

Mice treated with Δ9-THC experience persistent inhibition of IL-2 production even 7 days after treatment[50]. Rhesus macaques who were administered Δ9-THC for 28 days prior to Simian Immunodeficiency virus (SIV) inoculation had reduced mortality and reduced SIV viral load in the cerebrospinal fluid and plasma. In another study involving macaques infected with

SIV for 17 months of chronic THC administration[51], Δ9-THC resulted in trends towards a decreased viral load. The lack of statistically significance in some parameters can be attributed to the small number of animals in the vehicle vs. the Δ9-THC groups (n=4)[30].

Human studies

To date, seven randomized controlled studies have examined the medicinal use of cannabis for reducing morbidity and mortality in people living with HIV[52]. Interventions included smoked marijuana or hashish, ingested marijuana or hashish or ingested THC (dronabinol or other synthetic cannabinoid). Studies ranged from 21-84 days. Primary outcomes included HIV-related mortality (all-cause), morbidity (including type and duration of episodes of opportunistic infections, malignancies, incidence of AIDS, and hospital admissions). Secondary outcomes included change in appetite, nausea, mood, pain, quality of life, body composition, hematological and nutritional markers, cognitive function, respiratory function and effect on pharmacokinetics of antiretrovirals and development of dependence or adverse sociological effects. The use of cannabis posed challenges for blinding due to the psychoactive effects[52].

Only 1 study involving 62 patients examined the effects of cannabinoids on immune function and parameters associated with HIV infection. Participants in this study were assigned

to either a 3.95% THC marijuana cigarette, a 2.5 mg dronabinol capsule or a place capsule three times per day for 21 days. For the marijuana group, there was a statistically significant increase in CD4 counts from baseline vs. placebo and for the dronabinol group there was a trend towards statistical significance. Neither CD4 nor CD8 cell counts were adversely affected, and the pharmacokinetic component of the study did not reveal any clinically significant interactions that would require dose adjustments of protease inhibitors[22]. Adverse effects across studies included concentration difficulties, fatigue, sleepiness or sedation, increased duration of sleep, reduced salivation and thirst which improved upon discontinuation. The authors of a systematic review on the use of cannabis for reducing morbidity and mortality in HIV concluded that 1) evidence for substantial effects on morbidity and mortality is currently limited and 2) evidence for safety and efficacy of cannabis is lacking. Studies have been of short duration, in small numbers of patients and have focused on short-term measures of efficacy[52]. Furthermore, no study examined the effects of cannabinoids on inflammatory markers or HIV reservoir markers through a randomized trial.

More recently, Manuzak *et al.* published an observational study assessing the effect of cannabis use on peripheral immune cell frequency, activation, and function in 198 people living with HIV[53]. Individuals were grouped into heavy, medium, or occasional cannabis users or noncannabis users as determined by the quantify cannabis metabolite 11-nor-carboxy-

tetrahydrocannabinol (THCCOOH) detected in plasma by mass spectrometry. They found that persons with heavy cannabis use had lower frequencies of HLA-DR+CD38+CD4+ and CD8+ T-cell frequencies compared to people living with HIV[53]. Furthermore, heavy cannabis use was associated with decreased frequencies of pro-inflammatory intermediate (CD14++CD16+) and non-classical (CD14+CD16+) monocyte subsets[53]. They also documented a reduction in antigen-producing cells secreting pro-inflammatory cytokines IL-23 and tumor necrosis factor (TNF)-α[53]. Rizzo *et al.* also demonstrated that levels of circulating CD16 monocytes and interferon-gamma-induced protein (IP)-10 from people living with HIV who either were or were not cannabis users[54]. Lower levels of CD16+ monocytes and plasma IP-10 were found in cannabis users compared to non-cannabis users[54]. However, this study did not quantify the level of cannabis exposure in the two groups. Although these studies demonstrated favorable associations between inflammation and cannabis use, it must be borne in mind that both of these studies were observational only. As these studies were not randomized controlled trials, it is possible that people living with HIV who used cannabis in these studies differed in other significant ways from PLWHIV who did not use cannabis.

Study rationale

Cannabis may hold many potential therapeutic benefits for people living with HIV due to its promising anti-inflammatory and anti-fibrotic effects. Before adequately-powered interventional studies can be designed to study cannabis as a therapy for specific conditions associated with chronic inflammation and fibrosis, a key first step will be to demonstrate that cannabinoid use in a clinical trial is feasible and that they have a favorable safety and tolerability profile. As such, we propose a proof-of-concept pilot study to examine the feasibility, safety and tolerability of cannabinoid oils consumed orally in people living with HIV on effective ART. As a secondary objective, we will examine the effect of cannabinoid oils on immune profiles, including levels inflammatory markers associated with HIV disease progression and frequencies of activated and senescent CD4 and CD8 T-cells. Frequencies of regulatory T cells and various subsets of Th17 will also be assessed. Finally, an exploratory objective will be to study the effect of cannabinoid oils on markers of HIV persistence and the composition of the gastrointestinal microbiome.

We propose to use combination therapy of THC:CBD oils in capsule format (TN-TC11LM and TN-TC19LM capsules) ingested orally to examine these outcomes. Although research to date involving HIV/SIV has examined THC, data from *in vitro*, animal and human studies suggests that CBD has favorable anti-inflammatory effects and the combination of CBD with THC increases tolerability[55-59].

Methods/Design

Study design

This is a randomized, open-label, interventional study (the Canadian HIV Trials Network (CTN) number PT028) whereby capsules containing CBD:THC oils are consumed for 12 weeks to assess safety and tolerability. Their ability to reduce immune activation (as determined by percentage of activated CD8+CD38+HLA-DR+ T-cells), size of the peripheral HIV reservoir and change in gastrointestinal microbiome composition will also be examined. Participants will continue to take their ART treatments as prescribed throughout the study.

Setting

Recruitment of participants will occur at the Chronic Viral Illness Service (CVIS), Royal Victoria Hospital (Glen campus) of the McGill University Health Centre (MUHC), the largest academic HIV clinic in Canada.

Recruitment and enrollment

Study staff at the CVIS will conduct chart reviews of prospective people living with HIV ahead of their clinic visits to determine which persons have had suppressed viral load for at least 3 years on ART. The patient chart will be flagged, and if the treating HIV physician believes the person to be suitable for the study, the physician or study staff will approach potential trial participants at their clinic visit. The trial staff will inform the patients about the trial and invite him or her for eligibility screening and possible trial enrolment. Participant eligibility will be documented and written informed consent obtained for eligible patients by the study coordinator. The study coordinator will systematically document all individuals who have been approached for the study in addition to reasons for acceptance and refusal to participate in the study. Individuals who wish to discuss their participation in the study with their treating physician and or family and friends will be have the opportunity to do and may enroll a their next scheduled clinic visit. Following enrolment, participants will be followed during the study by the principal investigator and study coordinator at the CVIS.

Inclusion criteria

Eligible participants must meet the following criteria within 4 weeks prior to beginning the cannabinoid capsules to be considered eligible for study entry: 1) documented HIV infection

by Western blot, enzyme immunoassay or viral load assay; 2) aged 18 or older; 3) viral load <40 copies/mL for at least the last 3 years (maximum 2 blips <500 copies/ml allowed); 4) no cannabinoid use for at least 1 month prior to enrolment with negative baseline cannabinoid urine screen; 5) able to communicate in either English for French.

Exclusion criteria

Individuals who meet any of the following criteria will be ineligible to participate: 1) using cannabinoid-containing products outside of the study or within 4 weeks of study commencement; 2) pregnant, breastfeeding or planning to become pregnant during the course of the study; 3) enrolled in a separate study involving administration of medication, vitamin, supplement or herbal product; 4) active intravenous drug use; 5) active substance dependence; 6) prior history of hypersensitivity to cannabis or cannabis-containing products; 7) known or suspected allergy to sunflower lecithin oil; 8) active opportunistic infection or malignant condition; 9) unintentional weight loss of 10 % or more of body weight in the last 6 months; 10) unstable angina or acute cardiac event in the past year; 11) active psychiatric disorder or history of psychiatric depression (other than mild depression or anxiety); 12) on antipsychotic medication; 13) known or suspected family history of schizophrenia or severe personality

disorder; 14) serious cardiovascular disease such as ischemic heart disease, arrhythmias, poorly

controlled hypertension, or severe heart failure; 15) anemia (Hemoglobin <100 g/L); 16) active

liver disease or unexplained persistent elevations of serum transaminases; 17) Co-infection with

Hepatitis B or C (positive HBsAg or positive anti-HBc antibodies with a detectable HBV DNA

viral load or positive anti HCV antibodies with a detectable HCV RNA viral load); 18) alanine

aminotransferase (ALT) or Aspartate aminotransferase (AST) or alkaline phosphatase >2.5 x

upper limit of normal (ULN); 19) opportunistic infection in the last month as determined by the

treating physician; 20) renal dysfunction; 21) unstable psychological or psychiatric condition as

determined by the treating physician; 22) holding employment which requires operation of heavy

machinery or which requires undergoing drug screening (ie, pilot or police officer); 23)

concurrent use within the past 8 week of anabolic hormones, prednisone, IL-2 or other agents

known to alter immune function.

Study intervention

The study medications are TN-TC11LM and TN-TC19LM capsules which contain THC:CBD in

a ratio of 1:1 (2.5 mg/2.5 mg) and 1:9 (5 mg/45 mg), respectively. Participants will be advised to

gradually increase the number of capsules they take based on the suggested titration scheme

presented in Tables 1 and 2, until a daily maximum is reached. These maximum amounts are comprised of 10 capsules of TN-TC11LM (25 mg THC/25 mg CBD total per day) or 3 capsules of TN-TC19LM (15 mg THC: 135 mg CBD for TN-CT19L) per day. These doses were selected as in a clinical trial for neuropathic pain, doses equivalent to 2.5 mg of THC were well-tolerated[34]. More recently, among patients (ages 2-55 years) with the Lennox-Gastaut syndrome, cannabidiol at a dose of 10 or 20 mg per kilogram per day resulted in greater reductions in the frequency of drop seizures than placebo and was well-tolerated overall other than for an increase liver aminotransferase concentrations[60]. Due to person-to-person variability in the ability to metabolize and tolerate cannabinoids [61], we have opted for patients to titrate their dose of medication to a range where they are comfortable as the titration method of dosing has proven successful in other clinical trials involving cannabinoids[34].

Table 1: Recommended titration schedule for Group 1 – Low CBD dose TN-TC11LM oral capsules

Weeks	Daily Dose	Number of capsules
1	5 mg THC/ 5 mg CBD	1 capsule twice daily, taken orally
2	10 mg THC/ 10 mg CBD	2 capsules twice daily, taken orally (4 capsules per day)
3	15 mg THC/ 15 mg CBD	2 capsule three times daily, taken orally (6 capsules per day)
4	20 mg THC/ 20 mg CBD	2 capsules four times daily, taken orally (8 capsules per day)
5-12	25 mg THC/25 mg CBD	2 capsules 5 times daily, taken orally (10 capsules per day)

Group 1: Low CBD dose TN-TC11LM oral capsules (2.5 mg THC/2.5 mg CBD capsules). This group will be advised to start by taking 1 capsule twice daily for 1 week (5 mg THC/5 mg CBD) and increase the number of capsules as tolerated to a maximum of 10 capsules daily by weeks 5-12 (25 mg THC/25 mg CBD total per day). Participants will record the times and dates of all capsules consumed in a logbook.

Table 2: Suggested titration schedule for Group 2 – High CBD dose TN-TC19LM oral capsules

Weeks	Daily Dose	Number of capsules
1	5 mg THC/ 45 mg CBD	1 capsule daily, taken orally
2	10 mg THC/ 90 mg CBD	1 capsule twice daily, taken orally (2 capsules per day)
3	15 mg THC/ 135mg CBD	1 capsule three times daily, taken orally (3 capsules daily)
4	20 mg THC/ 180 mg CBD	1 capsule four times daily, taken orally (4 capsules)
5-12	25 mg THC/ 225 mg CBD	1 capsule five times daily, taken orally (5 capsules)

Group 2: High CBD dose TN-TC19LM (5 mg THC/45 mg CBD capsules). This group will be advised to start by taking 1 capsule once daily for 1 week (5 mg THC/45 mg CBD) and increase the number of capsules as tolerated to a maximum of 10 capsules daily by week 5(25 mg THC/225 mg CBD total). Participants will record the times and dates of all capsules consumed in a log book.

Randomization

After eligibility is confirmed and written informed consent obtained, participants will be randomized to either TN-TC11LM (group 1) or TN-TC19LM (group 2) capsules which contain THC:CBD in a ratio of 1:1 and 1:9, respectively. Prior to study commencement, a statistician unassociated with the study will develop a randomization scheme using SAS and input into a password-protected web-based randomization system. Variable block sizes of 2 and 4 will be used. Participants will be assigned to either Group 1 vs. Group 2 based on the pre-designated allocation code. As this is an unblinded study, participants and study staff will be aware of the group to which the participant has been randomized. A computerized audit trail will track date and time of allocation, patient study identification number and treatment allocation. The randomization group will be recorded in the study log, which will be accessible to the sponsor/medical manager and study coordinator.

Measurements

At the screening visit, clinical information will be collected from each participant including age, ethnicity, list of current medications, dosage, date of treatment initiation, psychiatric disorders, duration of HIV infection, current ART regimen and duration of ART

regimen months, ART history, CD4+ T cells count within the past 3 months, nadir CD4+ T cells count, CD4/CD8 ratio, duration of plasma viral load suppression and any preexisting medical conditions, signs or symptoms. Information will also be collected on whether the individual consumed cannabis in the past, the form(s) in which it was consumed, frequency of use and reasons for use.

Clinical parameters

Scheduled visits will occur to monitor safety and tolerability, as per the visit schedule depicted in Table 3. Visits will include physical exam with vital signs, weight, occurrence of adverse events (AEs and concomitant medications) and the presence of common symptoms associated with cannabinoids including dizziness, nausea, headaches, appetite or mood changes. At visits, blood for some or all of the following will be collected: CD4+ T cells count, CD8+ T cells, CD4/CD8 ratio, plasma viral load, Complete Blood Count (CBC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, urea, creatinine and blood glucose, T-cell activation and inflammatory markers and testing for syphilis if the participant tested positive during the 4 weeks prior to beginning consuming the study capsules. A stool sample for analysis of the bacterial and fungal

microbiome assessment will also be collected prior to beginning the study capsules. Participants will be enrolled in the study for up to 15 weeks but will consume capsules for a period of 12 weeks. Participants will undergo screening tests and eligibility assessment within 4 weeks prior to initiating study capsules. Participants will then undergo assessments after the first week of capsule consumption and every 2 weeks thereafter. A second stool sample for bacterial and fungal microbiome analysis will be collected during the final week of capsule consumption. The final visit will occur 2 weeks after study drug cessation.

Table 3. Schedule of Visits and Procedures

Visit Type	Screening	Visit 1 - Baseline 1	Visit 2 - Baseline 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 – End of Tx Visit	Visit 10 – Final Study Visit
Visit Window	-4 to -1 weeks	W -1	D1, W0	D1, W1 (± 4 days)	D1, W2 (± 4 days)	D1, W4 (± 4 days)	D1, W6 (± 4 days)	D1, W8 (± 4 days)	D1, W10 (± 4 days)	D1, W12 (± 4 days)	D1, W14 (± 4 days)
Procedures:											
Eligibility criteria assessment	X										
Informed Consent	X										
Medical History	X										
Pregnancy Test (urine)[1]	X										
Cannabinoids Screen (urine)	X										
Hepatitis B, C and syphilis	X[3]										
Randomization			X								
Physical Exam			X	X	X	X	X	X	X	X	X
Hematology and chemistry profiles[2]	X			X	X	X	X	X	X	X	
Viral load, CD4 and CD8	X				X		X			X	
Immune activation and inflammatory markers			X	X	X		X			X	X
HIV reservoir assays		X	X	X	X		X	X		X	X
Nasal swab and stool specimen for microbiome assessment		X								X	
WHOQOLHIV-BREF scale			X				X			X	
EQ-5D questionnaire			X				X			X	
POMS questionnaire			X				X[5]			X	
Study medication dispensed			X	X	X	X	X	X	X		
Study Drug Compliance[4]				X	X	X	X	X	X	X	
ART Compliance[4]		X	X	X	X	X	X	X	X	X	X
Alcohol Intake		X	X	X	X	X	X	X	X	X	X
Adverse Events				X	X	X	X	X	X	X	X

Visit Type	Screening	Visit 1 - Baseline 1	Visit 2 - Baseline 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 – End of Tx Visit	Visit 10 – Final Study Visit
Visit Window	-4 to -1 weeks	W -1	D1, W0	D1, W1 (± 4 days)	D1, W2 (± 4 days)	D1, W4 (± 4 days)	D1, W6 (± 4 days)	D1, W8 (± 4 days)	D1, W10 (± 4 days)	D1, W12 (± 4 days)	D1, W14 (± 4 days)
Procedures:											
Concomitant Medications/Therapies	X	X	X	X	X	X	X	X	X	X	X

[1] If urine test is positive, perform serum pregnancy test.

[2] Complete blood count; AST, ALT, ALP, total bilirubin, urea, creatinine and blood glucose.

[3] If participant tests positive for Hepatitis B and C, he/she will no longer be eligible for the study. If participant tests positive for syphilis, he or she will be treated for syphilis according to clinical care guidelines and will still be eligible to participate in the study. The need for syphilis treatment and follow-up testing (usually in 6 and 12 months) will be discussed between the Sponsor and the investigator at the CVIS. It will be up to the investigator to ensure proper follow-up and management of the syphilis, as this is part of standard of care.

[4] Assessed by reviewing log book provided to each participant

[5] Review individual POMS questionnaires completed at Visits 2 and 6 with each participant

Medication adherence

Participants will be asked to keep a log book in which they enter the number of TN-TC11LM or TN-TC19LM capsules consumed, the time, as well any adverse effects they noted and the timing of these adverse effects relative to capsule intake. Furthermore, individuals will be asked to record whether or not they took their ART that day or whether any doses were missed. Participants will be asked to bring their log books with them to study visits and the coordinator will photocopy this information.

Quality of life and mood assessment

Questionnaires measuring quality of life (World Health Organization Quality of Life HIV-BEF (WHOQOLHIV-BREF) and the EQ-5D) and mood (Profile of Mood States (POMS) will be administered at baseline, midway through the study and at the end of the study, as outlined in Table 3. WHOQOLHIV-BREF consists of 31 items which measure the following domains: physical health, psychological health, social relationships and environment. It is a shorter version of the original instrument (WHOQOL) and is more convenient for use in clinical trials, taking approximately 10 minutes to complete. EQ-5D is a descriptive questionnaire examining 5 dimensions: 1) mobility, 2) self-care, 3) usual activities, 4) pain/discomfort, and 5)

anxiety/depression. Each dimension has 5 levels: No problem, slight problems, moderate problems, severe problems and extreme problems. The participant indicates the state of his/her health by ticking the box most appropriate to the statement in each of the 5 dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the 5 dimensions can be combined into a 5-digit number that describes the participant's health state. It takes about 10 minutes to complete. The Profile of Mood States (POMS) questionnaire measures the following 6 factors: 1) Tension-Anxiety, 2) Anger-Hostility, 3) Fatigue-Inertia, 4) Depression-Dejection, 5) Vigor-Activity, and 6) Confusion-Bewilderment. It is very sensitive to non-clinical changes in mood states and takes approximately 5 minutes to complete. These questionnaires will be administered by a trained research coordinator.

Research hypothesis

THC:CBD oils consumed orally – as TN-TC11LM and TN-TC19LM oral capsules – will be safe and well-tolerated in PLWHIV. They will also be associated with a reduction in markers of inflammation, reduction in frequency of activated T cells and reduction in HIV reservoir size.

Study outcome measures

The primary objective is to evaluate the safety and tolerability of TN-TC11LM and TN-TC19LM oral capsules in PLWHIV on effective ART. The primary between group comparison is the percentage of participants without any signs of significant toxicity; percentage oparticipants who are able to complete the study and scores on the WHOQOLHIV-BREF Scale, EQ-D5 and POMS questionnaires from week 0 to week 12 are secondary outcomes that will also be compared between groups. The secondary objective is to determine the effect of TN-TC11LM and TN-TC19LM oral capsules on frequency of activated T-cells and markers of inflammation association with HIV disease progression. Exploratory objectives are to determine the effect of TN-TC11LM and TN-TC19LM oral capsules on 1) the size of the peripheral HIV reservoir 2) the composition of the gastrointestinal bacterial and fungal microbiome.

Safety and tolerability

Safety will be assessed at regular intervals (Table 3) by vital signs and adverse effects (AE) monitoring, as reported by the participant and actively sought at each study visit by the coordinator or physician. Biological safety will be evaluated by hematology, biochemistry and other clinical, laboratory or other diagnostic tests done on participants during the course of the study. Lab results for all participants for assessed safety variables will be reviewed by the trial investigator. The Data safety monitor will also review safety information. Toxicity of TN-

TC11LM and TN-TC19LM will be assessed using the World Health Organization (WHO) toxicity scale. All AEs, regardless of the grade, will be documented and it will be noted whether or not these symptoms were already present at baseline. Any AEs that occur during the study will be evaluated by the trial investigators and grade 3 and 4 AEs will be recorded on the CRFs. If required, blood specimens will be collected for hematology and biochemistry tests. Participants having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the trial investigator. The trial investigator will report ongoing AEs at the completion of the clinical study to the primary treating physician at the CVIS who will determine the need for and provide standard medical care. The trial investigator will ensure that the event is satisfactorily resolved or that no additional follow-up is needed. Any participant who discontinues the study for an unresolved clinically significant AE will be followed until satisfactory clinical resolution is achieved and the AE recorded on the case report form (CRF), regardless of severity grading. AEs that may be related to TN-TC11LM and TN-TC19LM will be managed by dose reduction of TN-TC11LM and TN-TC19LM. TN-TC11LM and TN-TC19LM will be discontinued permanently in the event of any life-threatening AEs.

T-cell subsets and Immune activation

The frequency of different CD4+ and CD8+ T cell subsets will be defined using multi-parameter flow cytometry (BD Fortessa X-20) in peripheral blood. The expressions of CD3, CD4 and/or CD8, CD45RA, CCR7 and CD27 will be used to measure the frequency of naïve (CD45RA$^+$CCR7$^+$CD27$^+$), central memory (CD45RA$^-$CCR7$^+$CD27$^+$), transitional memory (CD45RA$^-$CCR7$^-$CD27$^+$), effector memory (CD45RA$^-$CCR7$^-$CD27$^-$) and terminally differentiated (CD45RA$^+$CCR7$^-$CD27$^-$) cells. Regulatory T (Treg) cells will be defined as CD3$^+$CD4$^+$CD25^{high}FoxP3^{high}CD127^{low} cells. Expression of the CD39 and CD73 ectoenzymes involved in Treg-mediated immunosuppression and HIV disease progression (via the adenosine pathway) will be also assessed [62] [63]. Various subsets of Th cells (T helper) will be defined as Th1 (CD45RA$^-$CCR6$^-$CCR4$^-$CXCR3$^+$), Th2 (CD45RA$^-$CCR6$^-$CCR4$^+$CXCR3$^-$), Th17 (CD45RA$^-$CCR6$^+$CCR4$^+$CXCR3$^-$) and Th1/Th17 (CD45RA$^-$CCR6$^+$CCR4$^-$CXCR3$^+$). Levels of CD8+ and CD4+ T cell immune activation (CD38/HLA-DR co-expression) and senescence (CD28$^-$CD57$^+$) will be also assessed on all T cell subsets. These markers will be assessed at week 0, 1, 2, 6, 12 and 14.

Inflammatory markers assessments

Plasma levels of various inflammatory markers including interferon-α, interleukin (IL)-1β, IL-6, IL-10, IL-17, TGF- β, interferon-gamma-induced protein (IP)-10, will be assessed via Luminex (Millipore) and levels of d-dimer, C-reactive protein, and markers of microbial translocation lipopolysaccharide and sCD14 will be assessed by ELISA in batch from blood drawn at weeks 0, 1, 2, 6, 12 and 14.

Peripheral blood HIV reservoir size

Blood for HIV reservoir assessment will be collected at 2 time points prior to cannabis initiation (1 week prior to cannabinoid capsule initiation and immediately prior to cannabinoid capsule initiation) to account for normal fluctuations in baseline levels of HIV persistence markers. Subsequently, virological measures will be done at weeks 1, 2, 6, 8, 12, 14. CD4+ T cells isolated form PBMCs by magnetic negative selection. To capture all infected cells, the frequency of cells harbouring total and integrated HIV DNA will be measured using well-established assays on a total of 500,000 cells (sensitivity of 1 copy/reaction)[17 64]. As most of the HIV genomes are defective, the recently developed "tat/rev induced limiting dilution assay" (TILDA), which provides a more functional measurement of the HIV reservoir[65], will be employed. To assess if residual levels of viral replication may occur, we will measure 2-LTR

circles, which are proposed to be a surrogate marker for ongoing HIV replication during ART[64]

[66 67]. Specifically, this combination of assays will indicate if cannabis has an impact on the size

of the total reservoir (DNA), the functional reservoir (TILDA) and ongoing viral replication (2-

LTR circles). Measurements will be performed in batch.

Gastrointestinal Microbiome Composition

A stool sample without preservative will be collected from each participant at the

beginning of the study prior to consuming the capsules and during the final week (week 12) of

capsule consumption. Specimens will be stored at -80°C until analyzed in batch, as previously

described[68]. Bacterial DNA will be extracted with PCR amplified targeting of the 16S rRNA

gene using universal primers which flank the V3-V4 region of the 16S gene modified with the

addition of TruSeq Illumina adapters, also as previously outlined[69]. Internal Transcribed Spacer

for Fungal DNA extraction will be used for fungi. PCR amplification, PCR amplicon

quantification and sequencing will be performed as previously described[68 69].

Sample size and statistical analyses

In this proof-of-concept study, we are exploring a phenomenon with little *in vivo* data and with a limited study budget. For this reason we have chosen a convenience sample of 26 participants, 13 per arm, without formal power calculations. This number of individuals will enable us to assess feasibility (willingness of patients to participate, attend study visits and complete questionnaires, numbers of drop-out participants) as well as safety and tolerability. The data obtained will help to guide future sample size calculations for future studies. Although the small number of participants may result in wide confidence intervals for adverse events, this number of participants should give us an idea about the degree of variability for continuous outcomes we are measuring.

For the primary endpoint, the proportions of participants without any signs of significant toxicity (Grades 0-2 scores on the WHO toxicity scale), proportions of participants who complete the study and scores on the WHOQOLHIV-BREF, EQ-5D and POMS questionnaires will be examined using descriptive statistics. We will also compare these proportions for Group 1 vs. Group 2 using a Fisher's exact test. For Quality of Life and mood measures, we will use analysis of covariance with 12 week score as outcome and baseline score as covariate and treatment as independent variable. With regards to the POMS questionnaire, we will consider only overall scores (and not sub-scores) due to the small sample size which would make comparisons of the sub-scale inappropriate.

Immune activation levels for Groups 1 vs. Group 2 at week 12 will be compared using analysis of co-variance with adjustment for the week 0 activation levels. The mean change and associated 95% confidence interval will be reported for each of the secondary endpoints described above. Furthermore, the study drug treatment period will be compared to the baseline period with each arm. If the treatment effect is similar within the two arms, then an analysis of the treatment effect will be pooled over the two arms, using analysis of covariance. The change is immune activation levels following discontinuation of study drug (i.e., from week 12 to week 14) will be reported as a mean with corresponding 95% confidence interval. A 50% reduction will be considered significant[70].

Analogous analyses will be conduction for reservoir assessments other outcome measures listed above as endpoints. A signed rank (non-parametric) test will be used to compare number of copies of total and integrated DNA at baseline 2 vs. at week 12. Group differences in the change of HIV reservoir size from baseline to 12 weeks will be assessed by the Mann-Whitney *U* test. Wilcoxon signed-rank test will be used to compare the HIV reservoir and inflammatory markers in blood samples of the same patient from baseline to 12 weeks. At least a 2-fold decrease in frequency of infected cells in both groups from baseline to 12 weeks of treatment will be considered significant [71]. Microbiome composition will be described with regards to the

frequencies of microorganisms families for the groups at baseline and then at 12 weeks of treatment. Due to the exploratory nature of this objective, no formal statistics will be applied.

Data management

All clinical data and electronic files will be stored in the secure environment of the CVIS of the MUHC.   All data published will be anonymized. Only researchers affiliated with the study will have access to participant data.   Study progress and safety will be evaluated in an ongoing fashion by the principal and co-investigators.   The study will be monitored for safety and ongoing progress by a standing Data and Safety Monitoring Committee (DSMC) of clinicians and methodologists established by the Canadian HIV Trials Network. The committee meets every six months or as needed.

Storage of biological specimens

All biological specimens will be stored at the CVIS of the MUHC for analysis in the current trial and for future use in additional studies.

Ethics and dissemination

Written informed consent will be obtained from all study participants. The study protocol and informed consent have been approved by the Research Ethics Board of the McGill University Health Centre (MUHC-2018-4336) and is in the process of being reviewed by Health Canada's Therapeutic Product Directorate. The study will be conducted in accordance with the application Health Canada regulations, International Conference on Harmonization guidelines on current Good Clinical Practice and the Declaration of Helsinki. Patient enrollment for this trial is anticipated to begin August 2018.

Regardless of outcome, trial results will be disseminated through scientific peer review publication, international and national conferences and the CTN according the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) and CONSORT (Consolidated Standards of Reporting Trials) guidelines for transparent reporting of trials[72 73]. CTC will be responsible for initially drafting the manuscripts and professional writers will not be used for any of the publications. Authorship will be determined based on criteria defined by the International Journal of Medical Editors[74]. We aim to write the manuscript of the final results within 6 months of completing the study. Participants who have been involved in the trial will be given the option of having a summary of results sent to them.

Patient and Public Involvement

The CTN Community Advisory Committee (CAC) was involved in the peer review process of this study proposal, deemed that the research questions addressed were of very high priority to people living with HIV and voted for the funding of this study. The CAC's critiques of the initial proposal were taken into account in the revised proposal. Two members of the CAC (SM and EM) were involved in finalizing the study design, inclusion/exclusion criteria, outcome measures and monitoring plans and are formal study investigators and co-authors. Preliminary and final results of the study will be shared with community members, patient participant and the public at bi-annual CTN meetings, through the CTN newsletter and on the CTN website in addition to the annual Canadian Association of HIV Research meeting.

Discussion

Since the advent of ART, people living with HIV now have a longevity which approaches that of their HIV-uninfected counterparts but have a higher burden of non-communicable comorbidities including cardiovascular, pulmonary, renal and hepatic diseases[1 2]. Heightened inflammation in people living with HIV despite ART is believed to be the driving before behind the increased rates of non-infectious comorbidities. Similarly, chronic immune activation fosters HIV persistence[10-15]. As cannabinoids possesses both anti-inflammatory and

anti-fibrotic properties[19], cannabinoids may represent a feasible method to reduce immune activation and enhance immune profile. This, in turn, may hasten the progression of non-opportunistic complications associated with HIV.

In this pilot study, our primary objective is to assess the safety and tolerability of TN-TC11LM and TN-TC91LM taken by people living with HIV on suppressive ART. We hypothesize that these agents will be safe and well tolerated in people living with HIV, given that similar products are safe and well-tolerated in other populations. Sativex® is currently licensed as an adjunctive treatment for symptomatic relived of spasticity in adult patients with Multiple Sclerosis (MS) who have not responded adequately to other therapy[75-77]. It is an oromucosal spray containing CBD and THC in1:1 ratio. Marinol®, which is a synththetic THC-containing capsule, is currently used for the treatment of anorexia associated with weight loss in persons with Acquired Immunodeficiency Syndrome (AIDS) and nausea and vomiting associated with cancer chemotherapy in patients with insufficient response to conventional antiemetics[78]. It has been estimated that 5,472 patients have been exposed to Sativex® and there have been no safety concerns identified and the product remains well-tolerated. The primary safety concerns of both Sativex® and Marinol® are consistent with the known pharmacological activity of cannabinoids. The primary safety concerns associated with Sativex® included abuse potential, cardiovascular effects and central nervous system adverse effects[79]. Although there is some evidence to suggest

that individuals can develop "cannabis use disorder", individuals do not develop the same

extremes of behavior as observed with other drugs of abuse[80]. In two clinical trials, nabiximols

such as Sativex® have been used in two clinical trials whereby treatment was abruptly ceased to

study whether withdrawal symptoms would develop[81] [82] In both studies, no withdrawal

syndromes were observed[83]. Cannabinoids have cardiovascular effects that include tachycardia

and fluctuations in blood pressure, including episodes of postural hypotension. Therefore these

agents should not be used in patients with serious cardiovascular disease, such as ischemic heart

disease, arrhythmias, poorly controlled hypertension or severe heart failure. THC has complex

effects on the CNS and should not be used in patients with a personal or strong family history of

psychosis. Examples of such conditions include schizophrenia and affective psychosis since

symptoms of these disorders may be aggravated by cannabinoids. In multiple sclerosis (MS)

patients receiving Sativex® in clinical studies, psychiatric-related adverse effects included

disorientation, depression including depressed mood, dissociation, euphoric mood, hallucination,

hallucinations (auditory and visual), illusions, paranoia, suicidal ideation and delusional

perception[84].   Interestingly, there is some evidence to demonstrated that CBD may actually

improve psychotic symptoms in persons suffering from schizophrenia[85].

For Marinol®, which contains only THC, the most frequency reported adverse effects

experienced by patients with AIDS during placebo controlled clinical trials involved CNS and

were reported in 33% of patients receiving Marinol®. About 25% reported a CNS adverse event during the first 2 weeks and about 4% reported such an event each week for the next 6 weeks thereafter[86]. By combining CBD with THC, we anticipate that tolerability will be greatly enhanced. When combined with THC, CBD reduces the risk for many adverse effects [85]. Furthermore, individuals will be instructed to titrate up the dose based on their own tolerability and reduce the dose if they experience any undesirable effects.   Furthermore, due to the extremely low levels of CB1 receptors in the brainstem[87], death due to overdosing on cannabis or cannabinoids alone has never been described.

The study medications and doses were chosen after a lengthy review of the existing literature and discussion with experts in the field of pain management. A high degree of inter-individual variability in metabolism following administration of cannabinoids is observed due to polymorphisms in cytochrome isoenzymes [61]. Given that the therapeutic doses of cannabinoids are highly variable between individuals, a dose titration schedules are usually recommended. When used to treat specific conditions, persons may be told to increase the dose until they achieve adequate symptom relief without adverse effects. This method was observed to work well when used in the first cohort study on the long-term safety of medicinal cannabis for non-cancer chronic pain in seven Canadian clinics[34].

Given this is a pilot study, a convenience sample of 26 participants was selected without formal power calculation. If this study demonstrated that TN-TC11LM and/or TN-TC91LM are safe and tolerable in people living with HIV and can reduce systemic inflammation, future studies will be performed to address the potential of these agents to ameliorate specific conditions in people living with HIV. Should future studies be conducted, data generated from this trial will assist with power calculations. Similar to a study conducted by members of our group on the ability of Niaspan® (extended-release niacin) to reduce immune activation, as determined by percentage of activated CD8+ CD38+ HLA-DR+ T-cells, we decided that a 50% reduction in activated CD8+ CD38+ HLA-DR+ T-cells would be considered significant[70]. This is based on previous reports indicating that a 10-fold difference exists between uninfected healthy controls and treated aviremic HIV-infected individuals in level of activated CD8+ CD38+ HLA-DR+ T-cells[88 89]. All of the inflammatory mediators we selected for this study are known to drive immune activation[1 6 90].

In addition, we decided to make our objective examining the ability of TN-TC11LM and TN-TC91LM capsules to reduce the HIV reservoir, through the reduction of systemic inflammation, an exploratory objective. It is unclear if 3 months of treatment will be long enough to produce any meaningful reduction in the size of the HIV reservoir. Furthermore, it is unclear what reduction in reservoir size is required to have a meaningful effect on clinical

outcomes. As mentioned earlier, we will consider a 50% decrease in the number of HIV-infected cells at baseline vs. week 12 to be a significant reduction in the reservoir, based on a study by *Hill et al* [71]. To our knowledge, there is no other randomized clinical trial examining the effect of cannabinoids on inflammation and HIV reservoir size in people living with HIV.

Our study is unique in being the first randomized trial in the world to examine the association between ingestion of precise quantities of cannabinoids and effect on inflammation and peripheral HIV reservoir size. It is also noteworthy that we chose not to have a placebo arm as the effects of psychoactive effects of THC would be difficult to camouflage. Furthermore, we chose to use oral formulations of cannabinoids so that we could precisely control the dose ingested by participants. When cannabis is smoked or vaped, there is variability in the methods and duration of inhalation used by participants which can influence dosage of cannabis ingested. The oral administration option also removes undesirable pulmonary effects such as symptoms wheezing or breathlessness in addition to inhalation of toxic chemicals [91]. Of special interest to our group is the recent discovery that administration of oral cannabis with lipids leads to high levels of cannabinoids in the intestinal lymphatic system and prominent immunomodulation, as demontrated by *Zgair et al*.[20] This finding is especially important given the prominent role of the mesenteric lymph nodes and gut to HIV persistence[7]. If oral cannabinoids can modify gut

microbiome and the enteric immune system favorably, larger clinical trials could be conducted to examine this phenomena in further detail.

The Canadian government has declared that cannabis' regulatory status will change from being an illegal substance to that of a legal substance in July 2018. Cannabis' change in regulatory status will likely stimulate more discussion amongst patients and physicians and thus physicians need to be informed about the potential risks and benefits of cannabis use. The change in the regulatory landscape will likely also foster more research into cannabis' therapeutic potential. We hope that this study will be a stimulus towards more open discussion between patients and their physicians and that it will reduce stigma associated with cannabinoids use. We also hope that this study will be the cornerstone for future studies investigating the therapeutic benefits of cannabis in PLWHIV and its potential not only at the individual level but also at the population level in the form of harm reduction strategies.

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Authors' contributions

CTC conceived and designed the study, drafted the grant and the protocol manuscript, will organize and supervise trial implementation and will be responsible for trial management. She will also be responsible for trial managements, staff training and supervision. MW and MAJ contributed to study design and participated in grant writing. BL, JPR, JC, MJB and MK will participate in study implementation. MJB provided input on questionnaires while CTC and MAJ provided immunological expertise and NC provided expertise related to the

HIV reservoir. SM and EM contributed to study design. CTC, MAJ and NC designed the experiments. JS contributed to the statistical analysis plan. CTC, MAJ and NC designed the experiments. All authors participated in refinement of the study methods, critical reviewed the manuscript drafts and approved the final manuscript. The CTN provides regulatory support. CTC and BL are Fonds de recherche du Québec-Santé (FRQ-S) chercheur-boursier-clinicien Junior 1. BL holds a rategy for Patient-Oriented Research (SPOR) Mentorship Chair in Innovative Clinical Trials. JPR holds the Louis Lowenstein Chair in Hematology and Oncology at McGill University. MAJ is holder of a Tier 2 Canada Research Chair in immunovirology.

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Competing interests: Tilray Inc. is supplying the study medications free of charge. All elements of the study are being undertaken independently of Tilray Inc. The authors declare there are no conflicts of interests.

Data sharing: There are no data yet to share.

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

Section/item	Item No	Description	Addressed on 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	Abstract p 4___
	2b	All items from the World Health Organization Trial Registration Data Set	_____p.3-4
Protocol version	3	Date and version identifier	Abstract /cover letter_
Funding	4	Sources and types of financial, material, and other support	___50_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_____1, 49,50____
	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	_____49, 50_____

1

| | 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) | _____4,24, 51, cover letter |

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	_13, 14_____
	6b	Explanation for choice of comparators	18, 19_____
Objectives	7	Specific objectives or hypotheses	23,24_____
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)	Abstract, 18, 19_

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	15__
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)	16,17_
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	18
	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)	25,26
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	25,26
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	25,26

2

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	24,25	
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)	Table 3	
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	29	
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Cover letter, already advertised by Canadian HIV Trials Network newsletter and website, meetings involving community	

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	19,20	
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	19, 20	
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	19,20	

3

Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	20,21 (non-blind)	
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	20, 21 (non-blind)	

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	20,21	
	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	22	
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	22, 31, 32	
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	29,30,31	
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	29,30,31	
	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)	N/A	

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	Cover letter, 25	

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		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	25, Health Canada, Canadian HIV Trials Network
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	25, 33, Health Canada, CTN
Auditing		23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	33
Ethics and dissemination				
Research ethics approval		24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	32
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)	32
Consent or assent		26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	32
		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	21 (study coordinator)
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	N/A at this time
Declaration of interests		28	Financial and other competing interests for principal investigators for the overall trial and each study site	31, 32, 50
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	31, 32
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	25

5

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	32
	31b	Authorship eligibility guidelines and any intended use of professional writers	32,33
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not determined at this time

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__Available upon request_
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	N/A (no plans for additional studies at this time)

*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.

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Oral cannabinoids in people living with HIV on effective antiretroviral therapy—CTN PT028: Study protocol for a pilot randomized trial to assess safety, tolerability and effect on immune activation

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Manuscripts

Oral cannabinoids in people living with HIV on effective antiretroviral therapy—CTN PT028: Study protocol for a pilot randomized trial to assess safety, tolerability and effect on immune activation

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Abstract:

Introduction: Despite antiretroviral therapy(ART), people living with HIV have higher rates of non-infectious chronic diseases. These conditions are driven by relatively high levels of inflammation persisting on ART compared to uninfected individuals. Chronic inflammation also contributes to HIV persistence during ART. Cannabis when taken orally may represent a way to reduce inflammation and strengthen immune responses. Before planning large interventional studies, it is important to ensure that cannabis taken orally is safe and well-tolerated in people living with HIV. We propose to conduct a pilot randomized trial to examine the safety and tolerability of cannabis oils containing tetrahydrocannabinol(THC) and cannabidiol(CBD) consumed orally in people living with HIV. We will also measure inflammatory markers, markers of HIV persistence in peripheral blood cells and changes in the gastrointestinal microbiome.

Methods and Analysis: Twenty-six people living with HIV having undetectable viral load for at least three years will be randomized to receive TN-TC11LM(THC:CBD in 1:1 ratio) or TN-TC19LM(THC:CBD in 1:9 ratio) capsules daily for 12 weeks. Safety and tolerability of these capsules will be assessed through haematological, hepatic and renal blood tests, face-to-face interviews and questionnaires. Proportions of participants without any signs of significant

toxicity(Grades 0-2 scores on the WHO toxicity scale) and who complete the study, as well as scores on quality of life and mood will be examined using descriptive statistics. The effects on inflammatory markers, markers of peripheral blood reservoir size and effect on the composition of the gastrointestinal microbiome will be assessed before and after study completion.

Ethics and Dissemination: This study has been approved by the Research Institute of the McGill University Health Centre. A Data Safety Monitor will review safety information at regular intervals. The final manuscript will be submitted to an open-access journal within 6 months of study completion. This trial is registered with clinicaltrials.gov(NCT03550352).

Strengths and limitations of this study

- Randomized clinical trial design involving oral consumption of capsules containing 2 different ratios of cannabinoids

- The capsules used will contain both Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), thus improving tolerability

- The use of oral capsules containing precise amounts of cannabinoids, rather than smoked cannabis, ensures more predictable dosage administration and avoids the harmful pulmonary effects associated with smoking

- Effects of the intervention on quality of life, cognition and mood in addition to biological outcomes are being examined

- The number of participants should provide insight into the degree of variability for continuous outcomes and should guide future sample size calculations for larger studies

Background

Despite antiretroviral therapy (ART), persistent immune activation is associated with increased risk of non-opportunistic complications in people living with HIV such as cardiovascular, pulmonary, renal and hepatic events[1][2]. HIV pathogenesis and persistence appear to be related to chronic inflammation and immune activation[3], driven by microbial translocation of bacterial products across the gut mucosa[4-6]. Even when ART is initiated in the primary or early phase of HIV, gut integrity is not fully restored[7]. Furthermore, there is recent evidence to suggest that inflammatory features of the enteric microbiota, and not just increased permeability alone, is driving chronic inflammation in people living with HIV. Indeed, some studies have shown correlations between specific enteric bacteria and immune activation markers in gut and blood[8][9]. Persistent immune activation may also contribute to the persistence of HIV during ART[10-15]. HIV reservoirs are the reason why HIV remains an incurable infection. Although HIV may also persist in myeloid cells, CD4+ T cells are the best-characterized and the most abundant reservoirs[16-18].

Attenuation of immune activation and levels of inflammation may portend several therapeutic benefits to people living with HIV and novel strategies are needed to achieve this goal. Having both anti-inflammatory and anti-fibrotic properties[19], cannabis may represent a

feasible method to reduce immune activation and enhance immune profile. When consumed orally, notably soluble in oil, it may also temper inflammation at the gastrointestinal mucosa[20]. This, in turn, may hasten the progression of non-opportunistic complications associated with HIV. Furthermore, cannabis use, mainly through smoking, is already extremely common amongst PLWHIV. A study conducted by our team involving 1072 participants in the Canadian Co-infection Cohort Study (CTN 222), a multi-centered longitudinal study of HIV-Hepatitis C co-infected individuals, revealed that 53% of individuals had smoked marijuana in the past 6 months[21]. A clearer understanding of the safety, tolerability and feasibility of using cannabis in a clinical trial is an important first step before designing interventional studies to ameliorate specific conditions of importance to people living with HIV. Furthermore, as cannabis use will be legalized in Canada July 2018, understanding its safety profile is very important.

Pharmacology and medical properties of cannabis

Cannabinoids, found in the hemp plant *Cannabis sativa*, have been recognized for centuries for their analgesic, anticonvulsant, bronchodilatory, sedative, hypnotic and antispasmodic properties[22][23]. Their biological activity is conferred by cannabinoid receptors CB1 and CB2 through activation of heterodimeric G-proteins that function as signaling and

regulatory proteins to operate or modulate intracellular signaling pathways[24] [25]. While CB1

receptors are expressed predominantly in the central nervous system, they are also found in the

lung, liver and kidneys. The endocannabinoid system also plays a key role in the gastrointestinal

tract's neural and molecular control mechanisms. Indeed, the endocannabinoid system plays a

role in normal physiological functions of the gastrointestinal tract including motility, gut–brain-

mediated fat intake, hunger signaling, inflammation and gut permeability[26]. Furthermore, there is

some evidence for and interactions between the endocannaboid system and the gut microbiota[26].

In lean mice who received 4 weeks of a cannabinoid-receptor agonist HU-210, plasma

lipopolysaccharides (LPS) were significantly increased[26] [27]. When obese mice with disrupted

gastrointestinal musoca were treated with rimonabant, an inverse CB1 agonist, for nearly 2

weeks, reductions in plasma LPS were observed[26] [28]. Furthermore, improvements in localization

of tight junction proteins, occluding and zonula occludens-1 were measured, suggestive of

improvement in endothelial barrier function[26] [28].

The presence of cannabinoid receptors in the central nervous system accounts for the

psychoactive effects of cannabis[24] [25]. In contrast, CB2 receptors are abundant on immune cells

including T and B cells, natural killer cells, monocytes and neutrophils as well as the liver[24] [29].

Two primary active constituents found in hemp plants include Δ9-tetrahydrocannabinol (THC)

and cannabidiol (CBD). Δ9-THC is a partial agonist at both CB1 and CB2[3] [30] [31] while CBD is

thought to mediate its effects through a variety of signaling mechanisms and act as a negative allosteric modulator at CB1[32 33].

Cannabis is perhaps best known for its effect on stimulating appetite via CB1 receptor activity and was historically used for this purpose in AIDS wasting syndrome. A variety of randomized controlled trials have shown that smoked cannabis is efficacious in the management of chronic pain, including in painful HIV-associated sensory neuropathy[34-36]. In animal models, Δ9-THC prevents atherosclerosis by inhibiting macrophage migration into atheromas through CB2 activation[37 38]. Endocannabinoids may also play a role in liver disease, where CB1 receptors are present in endothelial cells and hepatocytes and CB2 receptors are distributed in Kupffer cells[39 40]. CB1 receptors have an important role in non-alcoholic fatty liver disease and alcoholic liver disease while anti-inflammatory action of CB2 receptors can be useful in inflammatory liver disease. CB2 receptors show antifibrinogenic properties and administration of its agonists in fibrotic rats resulted in improvement in liver fibrosis, decreased inflammation and increased apoptosis of hepatic myofibroblasts[40-44].

Cannabis, the immune system and HIV

In vitro **studies**

Cannabinoids have been shown to inhibit productive HIV infection in primary human T cells and a CB2 antagonist blocked this effect[45]. Interference with the signal transduction of chemokine receptor CXCR4 is thought to lead to reduced F-actin accumulation. This in turn prevents movement of viral pre-integration complexes to the nucleus. It has also been postulated that CB2 agonists may inhibit T cell activation induced by anti-CD3/anti-CD28[45]. Along with reduced HIV production, immunological effects of cannabinoids include induction of immunosuppressive cytokines including IL-10, TGF-β and inhibition of IL-2 which stimulates T-cell division and expansion. They have also been shown to decrease adhesion capacity of leukocytes to extravasate into sites of inflammation[46-48]. Apoptosis and induction of Treg may potentially add to the pathways of cannabinoid-mediated immunosuppression[49].

Animal studies

Mice treated with Δ9-THC experience persistent inhibition of IL-2 production even 7 days after treatment[50]. Rhesus macaques who were administered Δ9-THC for 28 days prior to Simian Immunodeficiency virus (SIV) inoculation had reduced mortality and reduced SIV viral load in the cerebrospinal fluid and plasma. In another study involving macaques infected with SIV for 17 months of chronic THC administration[51], Δ9-THC resulted in trends towards a

decreased viral load. The lack of statistically significance in some parameters can be attributed to the small number of animals in the vehicle vs. the Δ9-THC groups (n=4)[30].

Human studies

To date, seven randomized controlled studies have examined the medicinal use of cannabis for reducing morbidity and mortality in people living with HIV[52]. Interventions included smoked marijuana or hashish, ingested marijuana or hashish or ingested THC (dronabinol or other synthetic cannabinoid). Studies ranged from 21-84 days. Primary outcomes included HIV-related mortality (all-cause), morbidity (including type and duration of episodes of opportunistic infections, malignancies, incidence of AIDS, and hospital admissions). Secondary outcomes included change in appetite, nausea, mood, pain, quality of life, body composition, hematological and nutritional markers, cognitive function, respiratory function and effect on pharmacokinetics of antiretrovirals and development of dependence or adverse sociological effects. The use of cannabis posed challenges for blinding due to the psychoactive effects[52].

Only 1 study involving 62 patients examined the effects of cannabinoids on immune function and parameters associated with HIV infection. Participants in this study were assigned to either a 3.95% THC marijuana cigarette, a 2.5 mg dronabinol capsule or a place capsule three

times per day for 21 days. For the marijuana group, there was a statistically significant increase in CD4 counts from baseline vs. placebo and for the dronabinol group there was a trend towards statistical significance. Neither CD4 nor CD8 cell counts were adversely affected, and the pharmacokinetic component of the study did not reveal any clinically significant interactions that would require dose adjustments of protease inhibitors[22]. Adverse effects across studies included concentration difficulties, fatigue, sleepiness or sedation, increased duration of sleep, reduced salivation and thirst which improved upon discontinuation. The authors of a systematic review on the use of cannabis for reducing morbidity and mortality in HIV concluded that 1) evidence for substantial effects on morbidity and mortality is currently limited and 2) evidence for safety and efficacy of cannabis is lacking. Studies have been of short duration, in small numbers of patients and have focused on short-term measures of efficacy[52]. Furthermore, no study examined the effects of cannabinoids on inflammatory markers or HIV reservoir markers through a randomized trial.

More recently, Manuzak *et al.* published an observational study assessing the effect of cannabis use on peripheral immune cell frequency, activation, and function in 198 people living with HIV[53]. Individuals were grouped into heavy, medium, or occasional cannabis users or noncannabis users as determined by the quantify cannabis metabolite 11-nor-carboxy-tetrahydrocannabinol (THCCOOH) detected in plasma by mass spectrometry.   They found that

persons with heavy cannabis use had lower frequencies of HLA-DR+CD38+CD4+ and CD8+ T-cell frequencies compared to people living with HIV[53]. Furthermore, heavy cannabis use was associated with decreased frequencies of pro-inflammatory intermediate (CD14++CD16+) and non-classical (CD14+CD16+) monocyte subsets[53]. They also documented a reduction in antigen-producing cells secreting pro-inflammatory cytokines IL-23 and tumor necrosis factor (TNF)-α[53]. Rizzo *et al.* also demonstrated that levels of circulating CD16 monocytes and interferon-gamma-induced protein (IP)-10 from people living with HIV who either were or were not cannabis users[54]. Lower levels of CD16+ monocytes and plasma IP-10 were found in cannabis users compared to non-cannabis users[54]. However, this study did not quantify the level of cannabis exposure in the two groups. Although these studies demonstrated favorable associations between inflammation and cannabis use, it must be borne in mind that both of these studies were observational only. As these studies were not randomized controlled trials, it is possible that people living with HIV who used cannabis in these studies differed in other significant ways from PLWHIV who did not use cannabis.

Study rationale

Cannabis may hold many potential therapeutic benefits for people living with HIV due to its promising anti-inflammatory and anti-fibrotic effects. Before adequately-powered interventional studies can be designed to study cannabis as a therapy for specific conditions associated with chronic inflammation and fibrosis, a key first step will be to demonstrate that cannabinoid use in a clinical trial is feasible and that they have a favorable safety and tolerability profile. As such, we propose a proof-of-concept pilot study to examine the feasibility, safety and tolerability of cannabinoid oils consumed orally in people living with HIV on effective ART. As a secondary objective, we will examine the effect of cannabinoid oils on immune profiles, including levels inflammatory markers associated with HIV disease progression and frequencies of activated and senescent CD4 and CD8 T-cells. Frequencies of regulatory T cells and various subsets of Th17 will also be assessed. Finally, an exploratory objective will be to study the effect of cannabinoid oils on markers of HIV persistence and the composition of the gastrointestinal microbiome.

We propose to use combination therapy of THC:CBD oils in capsule format (TN-TC11LM and TN-TC19LM capsules) ingested orally to examine these outcomes. Although research to date involving HIV/SIV has examined THC, data from *in vitro*, animal and human studies suggests that CBD has favorable anti-inflammatory effects and the combination of CBD with THC increases tolerability[55-59].

Methods/Design

Study design

This is a randomized, open-label, interventional study (the Canadian HIV Trials Network (CTN) number PT028) whereby capsules containing CBD:THC oils are consumed for 12 weeks to assess safety and tolerability. Their ability to reduce immune activation (as determined by percentage of activated CD8+CD38+HLA-DR+ T-cells), size of the peripheral HIV reservoir and change in gastrointestinal microbiome composition will also be examined. Participants will continue to take their ART treatments as prescribed throughout the study.

Setting

Recruitment of participants will occur at the Chronic Viral Illness Service (CVIS), Royal Victoria Hospital (Glen campus) of the McGill University Health Centre (MUHC), the largest academic HIV clinic in Canada.

Recruitment and enrollment

Study staff at the CVIS will conduct chart reviews of prospective people living with HIV ahead of their clinic visits to determine which persons have had suppressed viral load for at least 3 years on ART. The patient chart will be flagged, and if the treating HIV physician believes the person to be suitable for the study, the physician or study staff will approach potential trial participants at their clinic visit. The trial staff will inform the patients about the trial and invite him or her for eligibility screening and possible trial enrolment. Participant eligibility will be documented and written informed consent obtained for eligible patients by the study coordinator. The study coordinator will systematically document all individuals who have been approached for the study in addition to reasons for acceptance and refusal to participate in the study. Individuals who wish to discuss their participation in the study with their treating physician and or family and friends will be have the opportunity to do and may enroll a their next scheduled clinic visit. Following enrolment, participants will be followed during the study by the principal investigator and study coordinator at the CVIS.

Inclusion criteria

Eligible participants must meet the following criteria within 4 weeks prior to beginning the cannabinoid capsules to be considered eligible for study entry: 1) documented HIV infection

by Western blot, enzyme immunoassay or viral load assay; 2) aged 18 or older; 3) viral load <40 copies/mL for at least the last 3 years (maximum 2 blips <500 copies/ml allowed); 4) no cannabinoid use for at least 1 month prior to enrolment with negative baseline cannabinoid urine screen; 5) able to communicate in either English for French. We will attempt to enrol equal proportions of men who have sex with men (MSM) and heterosexual individuals.

Exclusion criteria

Individuals who meet any of the following criteria will be ineligible to participate: 1) using cannabinoid-containing products outside of the study or within 4 weeks of study commencement; 2) pregnant, breastfeeding or planning to become pregnant during the course of the study; 3) enrolled in a separate study involving administration of medication, vitamin, supplement or herbal product; 4) active intravenous drug use; 5) active substance dependence; 6) prior history of hypersensitivity to cannabis or cannabis-containing products; 7) known or suspected allergy to sunflower lecithin oil; 8) active opportunistic infection or malignant condition; 9) unintentional weight loss of 10 % or more of body weight in the last 6 months; 10) unstable angina or acute cardiac event in the past year; 11) active psychiatric disorder or history of psychiatric depression (other than mild depression or anxiety); 12) on antipsychotic

medication; 13) known or suspected family history of schizophrenia or severe personality disorder; 14) serious cardiovascular disease such as ischemic heart disease, arrhythmias, poorly controlled hypertension, or severe heart failure; 15) anemia (Hemoglobin <100 g/L); 16) active liver disease or unexplained persistent elevations of serum transaminases; 17) Co-infection with Hepatitis B or C (positive HBsAg or positive anti-HBc antibodies with a detectable HBV DNA viral load or positive anti HCV antibodies with a detectable HCV RNA viral load); 18) alanine aminotransferase (ALT) or Aspartate aminotransferase (AST) or alkaline phosphatase >2.5 x upper limit of normal (ULN); 19) opportunistic infection in the last month as determined by the treating physician; 20) renal dysfunction; 21) unstable psychological or psychiatric condition as determined by the treating physician; 22) holding employment which requires operation of heavy machinery or which requires undergoing drug screening (ie, pilot or police officer); 23) concurrent use within the past 8 week of anabolic hormones, prednisone, IL-2 or other agents known to alter immune function.

The investigators will review potential participants' current medication lists at the screening visit. If any concomitant therapy interacts with the study medication, and if this therapy cannot be substituted, that participant will not be eligible to enroll in the study. Although some HIV antiretroviral drugs/pharmacokinetic "boosters" are metabolized by the CYP 1A2 and CYP3A4 (e.g., ritonavir and cobicistat-boosted protease inhibitors), suggesting that a drug

interaction may occur, in real practice at the Chronic Viral Illness Service no clinically significant drug interactions have been observed in patients reporting heavy cannabis use. Therefore, individuals will not be precluded to participate based on their specific antiretroviral regimen.

Study intervention

The study medications are TN-TC11LM and TN-TC19LM capsules which contain THC:CBD in a ratio of 1:1 (2.5 mg/2.5 mg) and 1:9 (5 mg/45 mg), respectively. These study drugs are being provided by Tilray and the active pharmaceutical ingredients are extracted from the cannabis plant and purified according to pharmaceutical standards (>98%). Participants will be advised to gradually increase the number of capsules they take based on the suggested titration scheme presented in Tables 1 and 2, until a daily maximum is reached. These maximum amounts are comprised of 10 capsules of TN-TC11LM (25 mg THC/25 mg CBD total per day) or 3 capsules of TN-TC19LM (15 mg THC: 135 mg CBD for TN-CT19L) per day. These doses were selected as in a clinical trial for neuropathic pain, doses equivalent to 2.5 mg of THC were well-tolerated[34]. More recently, among patients (ages 2-55 years) with the Lennox-Gastaut syndrome, cannabidiol at a dose of 10 or 20 mg per kilogram per day resulted in greater reductions in the

frequency of drop seizures than placebo and was well-tolerated overall other than for an increase

liver aminotransferase concentrations[60]. Due to person-to-person variability in the ability to

metabolize and tolerate cannabinoids [61], we have opted for patients to titrate their dose of

medication to a range where they are comfortable as the titration method of dosing has proven

successful in other clinical trials involving cannabinoids[34].

Table 1: Recommended titration schedule for Group 1 – Low CBD dose TN-TC11LM oral capsules

Weeks	Daily Dose	Number of capsules
1	5 mg THC/ 5 mg CBD	1 capsule twice daily, taken orally
2	10 mg THC/ 10 mg CBD	2 capsules twice daily, taken orally (4 capsules per day)
3	15 mg THC/ 15 mg CBD	2 capsule three times daily, taken orally (6 capsules per day)
4	20 mg THC/ 20 mg CBD	2 capsules four times daily, taken orally (8 capsules per day)
5-12	25 mg THC/25 mg CBD	2 capsules 5 times daily, taken orally (10 capsules per day)

Group 1: Low CBD dose TN-TC11LM oral capsules (2.5 mg THC/2.5 mg CBD capsules). This group will be advised to start by taking 1 capsule twice daily for 1 week (5 mg THC/5 mg CBD) and increase the number of capsules as tolerated to a maximum of 10 capsules daily by weeks 5-12 (25 mg THC/25 mg CBD total per day). Participants will record the times and dates of all capsules consumed in a logbook.

Table 2: Suggested titration schedule for Group 2 – High CBD dose TN-TC19LM oral capsules

Weeks	Daily Dose	Number of capsules
1	5 mg THC/ 45 mg CBD	1 capsule daily, taken orally
2	10 mg THC/ 90 mg CBD	1 capsule twice daily, taken orally (2 capsules per day)
3	15 mg THC/ 135mg CBD	1 capsule three times daily, taken orally (3 capsules daily)
4	20 mg THC/ 180 mg CBD	1 capsule four times daily, taken orally (4 capsules)
5-12	25 mg THC/ 225 mg CBD	1 capsule five times daily, taken orally (5 capsules)

Group 2: High CBD dose TN-TC19LM (5 mg THC/45 mg CBD capsules). This group will be advised to start by taking 1 capsule once daily for 1 week (5 mg THC/45 mg CBD) and increase the number of capsules as tolerated to a maximum of 10 capsules daily by week 5(25 mg THC/225 mg CBD total). Participants will record the times and dates of all capsules consumed in a log book.

Randomization

After eligibility is confirmed and written informed consent obtained, participants will be randomized to either TN-TC11LM (group 1) or TN-TC19LM (group 2) capsules which contain THC:CBD in a ratio of 1:1 and 1:9, respectively. Prior to study commencement, a statistician unassociated with the study will develop a randomization scheme using SAS and input into a password-protected web-based randomization system. Variable block sizes of 2 and 4 will be used. Participants will be assigned to either Group 1 vs. Group 2 based on the pre-designated allocation code. As this is an unblinded study, participants and study staff will be aware of the group to which the participant has been randomized. A computerized audit trail will track date and time of allocation, patient study identification number and treatment allocation. The randomization group will be recorded in the study log, which will be accessible to the sponsor/medical manager and study coordinator.

Measurements

At the screening visit, clinical information will be collected from each participant including age, ethnicity, sexual orientation, list of current medications, dosage, date of treatment

initiation, any antimicrobials taken in the previous 6 months, psychiatric disorders, duration of HIV infection, current ART regimen and duration of ART regimen months, ART history, CD4+ T cells count within the past 3 months, nadir CD4+ T cells count, CD4/CD8 ratio, duration of plasma viral load suppression and any preexisting medical conditions, signs or symptoms. Information will also be collected on whether the individual consumed cannabis in the past, the form(s) in which it was consumed, frequency of use and reasons for use.

Clinical parameters

Scheduled visits will occur to monitor safety and tolerability, as per the visit schedule depicted in Table 3. Visits will include physical exam with vital signs, weight, occurrence of adverse events (AEs and concomitant medications) and the presence of common symptoms associated with cannabinoids including dizziness, nausea, headaches, appetite or mood changes. At visits, blood for some or all of the following will be collected: CD4+ T cells count, CD8+ T cells, CD4/CD8 ratio, plasma viral load, Complete Blood Count (CBC), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin, urea, creatinine and blood glucose, T-cell activation and inflammatory markers and testing for syphilis if the participant tested positive during the 4 weeks prior to beginning

consuming the study capsules. A stool sample for analysis of the bacterial and fungal microbiome assessment will also be collected prior to beginning the study capsules. Participants will be enrolled in the study for up to 15 weeks but will consume capsules for a period of 12 weeks. Participants will undergo screening tests and eligibility assessment within 4 weeks prior to initiating study capsules. Participants will then undergo assessments after the first week of capsule consumption and every 2 weeks thereafter. A second stool sample for bacterial and fungal microbiome analysis will be collected during the final week of capsule consumption. The final visit will occur 2 weeks after study drug cessation.

Table 3. Schedule of Visits and Procedures

Visit Type	Screening	Visit 1 - Baseline 1	Visit 2 - Baseline 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 – End of Tx Visit	Visit 10 – Final Study Visit
Visit Window	-4 to -1 weeks	W -1	D1, W0	D1, W1 (± 4 days)	D1, W2 (± 4 days)	D1, W4 (± 4 days)	D1, W6 (± 4 days)	D1 (± 4 days)	D1, W10 (± 4 days)	D1, W12 (± 4 days)	D1, W14 (± 4 days)
Procedures:											
Eligibility criteria assessment	X										
Informed Consent	X										
Medical History	X										
Pregnancy Test (urine)[1]	X										
Cannabinoids Screen (urine)	X										
Hepatitis B, C and syphilis	X[3]										
Randomization			X								
Physical Exam			X	X	X	X	X	X	X	X	X
Hematology and chemistry profiles[2]	X			X	X	X	X	X	X	X	
Viral load, CD4 and CD8	X				X		X			X	
Immune activation and inflammatory markers			X	X	X		X			X	X
HIV reservoir assays		X	X	X	X		X	X		X	X
Nasal swab and stool specimen for microbiome assessment		X								X	
WHOQOLHIV-BREF scale			X				X			X	
EQ-5D questionnaire			X				X			X	
POMS questionnaire			X				X[5]			X	
Study medication dispensed			X	X	X	X	X	X	X		
Study Drug Compliance[4]				X	X	X	X	X	X	X	
ART Compliance[4]		X	X	X	X	X	X	X	X	X	X
Alcohol Intake		X	X	X	X	X	X	X	X	X	X
Adverse Events				X	X	X	X	X	X	X	X

Visit Type	Screening	Visit 1 - Baseline 1	Visit 2 - Baseline 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9 – End of Tx Visit	Visit 10 – Final Study Visit
Visit Window	-4 to -1 weeks	W -1	D1, W0	D1, W1 (± 4 days)	D1, W2 (± 4 days)	D1, W4 (± 4 days)	D1, W6 (± 4 days)	D1, W8 (± 4 days)	D1, W10 (± 4 days)	D1, W12 (± 4 days)	D1, W14 (± 4 days)
Procedures:											
Concomitant Medications/Therapies	X	X	X	X	X	X	X	X	X	X	X

[1] **If urine test is positive, perform serum pregnancy test.**

[2] **Complete blood count; AST, ALT, ALP, total bilirubin, urea, creatinine and blood glucose.**

[3] **If participant tests positive for Hepatitis B and C, he/she will no longer be eligible for the study. If participant tests positive for syphilis, he or she will be treated for syphilis according to clinical care guidelines and will still be eligible to participate in the study. The need for syphilis treatment and follow-up testing (usually in 6 and 12 months) will be discussed between the Sponsor and the investigator at the CVIS. It will be up to the investigator to ensure proper follow-up and management of the syphilis, as this is part of standard of care.**

[4] **Assessed by reviewing log book provided to each participant**

[5] **Review individual POMS questionnaires completed at Visits 2 and 6 with each participant**

Medication adherence

Participants will be asked to keep a log book in which they enter the number of TN-TC11LM or TN-TC19LM capsules consumed, the time, as well any adverse effects they noted and the timing of these adverse effects relative to capsule intake. Furthermore, individuals will be asked to record whether or not they took their ART that day or whether any doses were missed. Participants will be asked to bring their log books with them to study visits and the coordinator will photocopy this information.

Quality of life and mood assessment

Questionnaires measuring quality of life (World Health Organization Quality of Life HIV-BEF (WHOQOLHIV-BREF) and the EQ-5D) and mood (Profile of Mood States (POMS) will be administered at baseline, midway through the study and at the end of the study, as outlined in Table 3. WHOQOLHIV-BREF consists of 31 items which measure the following domains: physical health, psychological health, social relationships and environment. It is a shorter version of the original instrument (WHOQOL) and is more convenient for use in clinical trials, taking approximately 10 minutes to complete. EQ-5D is a descriptive questionnaire examining 5 dimensions: 1) mobility, 2) self-care, 3) usual activities, 4) pain/discomfort, and 5)

anxiety/depression. Each dimension has 5 levels: No problem, slight problems, moderate problems, severe problems and extreme problems. The participant indicates the state of his/her health by ticking the box most appropriate to the statement in each of the 5 dimensions. This decision results in a 1-digit number that expresses the level selected for that dimension. The digits for the 5 dimensions can be combined into a 5-digit number that describes the participant's health state. It takes about 10 minutes to complete. The Profile of Mood States (POMS) questionnaire measures the following 6 factors: 1) Tension-Anxiety, 2) Anger-Hostility, 3) Fatigue-Inertia, 4) Depression-Dejection, 5) Vigor-Activity, and 6) Confusion-Bewilderment. It is very sensitive to non-clinical changes in mood states and takes approximately 5 minutes to complete. These questionnaires will be administered by a trained research coordinator.

Research hypothesis

THC:CBD oils consumed orally – as TN-TC11LM and TN-TC19LM oral capsules – will be safe and well-tolerated in PLWHIV. They will also be associated with a reduction in markers of inflammation, reduction in frequency of activated T cells and reduction in HIV reservoir size.

Study outcome measures

The primary objective is to evaluate the safety and tolerability of TN-TC11LM and TN-TC19LM oral capsules in PLWHIV on effective ART. The primary between group comparison is the percentage of participants without any signs of significant toxicity; percentage of participants who are able to complete the study and scores on the WHOQOLHIV-BREF Scale, EQ-D5 and POMS questionnaires from week 0 to week 12 are secondary outcomes that will also be compared between groups. The secondary objective is to determine the effect of TN-TC11LM and TN-TC19LM oral capsules on frequency of activated T-cells and markers of inflammation association with HIV disease progression. Exploratory objectives are to determine the effect of TN-TC11LM and TN-TC19LM oral capsules on 1) the size of the peripheral HIV reservoir 2) the composition of the gastrointestinal bacterial and fungal microbiome.

Safety and tolerability

Safety will be assessed at regular intervals (Table 3) by vital signs and adverse effects (AE) monitoring, as reported by the participant and actively sought at each study visit by the coordinator or physician. Biological safety will be evaluated by hematology, biochemistry and other clinical, laboratory or other diagnostic tests done on participants during the course of the study. Lab results for all participants for assessed safety variables will be reviewed by the trial

investigator. The Data safety monitor will also review safety information. Toxicity of TN-TC11LM and TN-TC19LM will be assessed using the World Health Organization (WHO) toxicity scale. All AEs, regardless of the grade, will be documented and it will be noted whether or not these symptoms were already present at baseline. Any AEs that occur during the study will be evaluated by the trial investigators and grade 3 and 4 AEs will be recorded on the CRFs. If required, blood specimens will be collected for hematology and biochemistry tests. Participants having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the trial investigator. The trial investigator will report ongoing AEs at the completion of the clinical study to the primary treating physician at the CVIS who will determine the need for and provide standard medical care. The trial investigator will ensure that the event is satisfactorily resolved or that no additional follow-up is needed. Any participant who discontinues the study for an unresolved clinically significant AE will be followed until satisfactory clinical resolution is achieved and the AE recorded on the case report form (CRF), regardless of severity grading. AEs that may be related to TN-TC11LM and TN-TC19LM will be managed by dose reduction of TN-TC11LM and TN-TC19LM. TN-TC11LM and TN-TC19LM will be discontinued permanently in the event of any life-threatening AEs.

T-cell subsets and Immune activation

The frequency of different CD4+ and CD8+ T cell subsets will be defined using multi-parameter flow cytometry (BD Fortessa X-20) in peripheral blood. The expressions of CD3, CD4 and/or CD8, CD45RA, CCR7 and CD27 will be used to measure the frequency of naïve (CD45RA$^+$CCR7$^+$CD27$^+$), central memory (CD45RA$^-$CCR7$^+$CD27$^+$), transitional memory (CD45RA$^-$CCR7$^-$CD27$^+$), effector memory (CD45RA$^-$CCR7$^-$CD27$^-$) and terminally differentiated (CD45RA$^+$CCR7$^-$CD27$^-$) cells. Regulatory T (Treg) cells will be defined as CD3$^+$CD4$^+$CD25^{high}FoxP3^{high}CD127^{low} cells. Expression of the CD39 and CD73 ectoenzymes involved in Treg-mediated immunosuppression and HIV disease progression (via the adenosine pathway) will be also assessed [62] [63]. Various subsets of Th cells (T helper) will be defined as Th1 (CD45RA$^-$CCR6$^-$CCR4$^-$CXCR3$^+$), Th2 (CD45RA$^-$CCR6$^-$CCR4$^+$CXCR3$^-$), Th17 (CD45RA$^-$CCR6$^+$CCR4$^+$CXCR3$^-$) and Th1/Th17 (CD45RA$^-$CCR6$^+$CCR4$^-$CXCR3$^+$). Levels of CD8+ and CD4+ T cell immune activation (CD38/HLA-DR co-expression) and senescence (CD28$^-$CD57$^+$) will be also assessed on all T cell subsets. These markers will be assessed at week 0, 1, 2, 6, 12 and 14.

Inflammatory markers assessments

Plasma levels of various inflammatory markers including interferon-α, interleukin (IL)-1β, IL-6, IL-10, IL-17, TGF- β, interferon-gamma-induced protein (IP)-10, will be assessed via Luminex (Millipore) and levels of d-dimer, C-reactive protein, and markers of microbial translocation lipopolysaccharide and sCD14 will be assessed by ELISA in batch from blood drawn at weeks 0, 1, 2, 6, 12 and 14.

Peripheral blood HIV reservoir size

Blood for HIV reservoir assessment will be collected at 2 time points prior to cannabis initiation (1 week prior to cannabinoid capsule initiation and immediately prior to cannabinoid capsule initiation) to account for normal fluctuations in baseline levels of HIV persistence markers. Subsequently, virological measures will be done at weeks 1, 2, 6, 8, 12, 14. CD4+ T cells isolated form PBMCs by magnetic negative selection. To capture all infected cells, the frequency of cells harbouring total and integrated HIV DNA will be measured using well-established assays on a total of 500,000 cells (sensitivity of 1 copy/reaction)[17 64]. As most of the HIV genomes are defective, the recently developed "tat/rev induced limiting dilution assay" (TILDA), which provides a more functional measurement of the HIV reservoir[65], will be

employed. To assess if residual levels of viral replication may occur, we will measure 2-LTR circles, which are proposed to be a surrogate marker for ongoing HIV replication during ART[64] [66] [67]. Specifically, this combination of assays will indicate if cannabis has an impact on the size of the total reservoir (DNA), the functional reservoir (TILDA) and ongoing viral replication (2-LTR circles). Measurements will be performed in batch.

Gastrointestinal Microbiome Composition

A stool sample without preservative will be collected from each participant at the beginning of the study prior to consuming the capsules and during the final week (week 12) of capsule consumption. Specimens will be stored at -80°C until analyzed in batch, as previously described[68]. Bacterial DNA will be extracted with PCR amplified targeting of the 16S rRNA gene using universal primers which flank the V3-V4 region of the 16S gene modified with the addition of TruSeq Illumina adapters, also as previously outlined[69]. Internal Transcribed Spacer for Fungal DNA extraction will be used for fungi. PCR amplification, PCR amplicon quantification and sequencing will be performed as previously described[68] [69].

Sample size and statistical analyses

In this proof-of-concept study, we are exploring a phenomenon with little *in vivo* data and with a limited study budget. For this reason we have chosen a convenience sample of 26 participants, 13 per arm, without formal power calculations. This number of individuals will enable us to assess feasibility (willingness of patients to participate, attend study visits and complete questionnaires, numbers of drop-out participants) as well as safety and tolerability. The data obtained will help to guide future sample size calculations for future studies. Although the small number of participants may result in wide confidence intervals for adverse events, this number of participants should give us an idea about the degree of variability for continuous outcomes we are measuring.

For the primary endpoint, the proportions of participants without any signs of significant toxicity (Grades 0-2 scores on the WHO toxicity scale), proportions of participants who complete the study and scores on the WHOQOLHIV-BREF, EQ-5D and POMS questionnaires will be examined using descriptive statistics. We will also compare these proportions for Group 1 vs. Group 2 using a Fisher's exact test. For Quality of Life and mood measures, we will use analysis of covariance with 12 week score as outcome and baseline score as covariate and treatment as independent variable. With regards to the POMS questionnaire, we will consider only overall scores (and not sub-scores) due to the small sample size which would make comparisons of the sub-scale inappropriate.

Immune activation levels for Groups 1 vs. Group 2 at week 12 will be compared using analysis of co-variance with adjustment for the week 0 activation levels. The mean change and associated 95% confidence interval will be reported for each of the secondary endpoints described above. Furthermore, the study drug treatment period will be compared to the baseline period with each arm. If the treatment effect is similar within the two arms, then an analysis of the treatment effect will be pooled over the two arms, using analysis of covariance. The change is immune activation levels following discontinuation of study drug (i.e., from week 12 to week 14) will be reported as a mean with corresponding 95% confidence interval. A 50% reduction will be considered significant[70].

Analogous analyses will be conduction for reservoir assessments other outcome measures listed above as endpoints. A signed rank (non-parametric) test will be used to compare number of copies of total and integrated DNA at baseline 2 vs. at week 12. Group differences in the change of HIV reservoir size from baseline to 12 weeks will be assessed by the Mann-Whitney U test. Wilcoxon signed-rank test will be used to compare the HIV reservoir and inflammatory markers in blood samples of the same patient from baseline to 12 weeks. At least a 2-fold decrease in frequency of infected cells in both groups from baseline to 12 weeks of treatment will be considered significant [71]. Microbiome composition will be described with regards to the

frequencies of microorganisms families for the groups at baseline and then at 12 weeks of

treatment. Due to the exploratory nature of this objective, no formal statistics will be applied.

Patient and Public Involvement

The CTN Community Advisory Committee (CAC) was involved in the peer review process of

this study proposal, deemed that the research questions addressed were of very high priority to

people living with HIV and voted for the funding of this study. The CAC's critiques of the

initial proposal were taken into account in the revised proposal. Two members of the CAC (SM

and EM) were involved in finalizing the study design, inclusion/exclusion criteria, outcome

measures and monitoring plans and are formal study investigators and co-authors. Preliminary

and final results of the study will be shared with community members, patient participant and the

public at bi-annual CTN meetings, through the CTN newsletter and on the CTN website in

addition to the annual Canadian Association of HIV Research meeting.

Data management

All clinical data and electronic files will be stored in the secure environment of the CVIS

of the MUHC. All data published will be anonymized. Only researchers affiliated with the

study will have access to participant data. Study progress and safety will be evaluated in an

ongoing fashion by the principal and co-investigators. The study will be monitored for safety

and ongoing progress by a standing Data and Safety Monitoring Committee (DSMC) of

clinicians and methodologists established by the Canadian HIV Trials Network. The committee

meets every six months or as needed.

Storage of biological specimens

. Within 1 hour of being drawn, blood from the CVIS will be transferred to the laboratory at the

RI-MUHC (in the adjacent, connected building) and the plasma will be separated from peripheral

blood mononuclear cells (PBMCs) by Ficoll density centrifugation by an experienced laboratory

personnel. PBMCs and plasma will be stored in liquid Nitrogen tanks at the RI-MUHC

laboratory until time for analysis. Patients will be contacted the day before their clinic visit to

remind them when a stool specimen is due. They will be instructed to record the time of the

provision of the sample on the paper bag containing the sterile container and store it in a

refrigerator (-4C) until brought to the clinic. Once at the CVIS, the stool specimen will be placed

in a large fridge designated specifically for the storage of stool specimens.

Ethics and dissemination

Written informed consent will be obtained from all study participants. The study protocol and informed consent have been approved by the Research Ethics Board of the McGill University Health Centre (MUHC-2018-4336) and is in the process of being reviewed by Health Canada's Therapeutic Product Directorate. The study will be conducted in accordance with the application Health Canada regulations, International Conference on Harmonization guidelines on current Good Clinical Practice and the Declaration of Helsinki. Patient enrollment for this trial is anticipated to begin August 2018.

Regardless of outcome, trial results will be disseminated through scientific peer review publication, international and national conferences and the CTN according the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) and CONSORT (Consolidated Standards of Reporting Trials) guidelines for transparent reporting of trials[72][73]. CTC will be responsible for initially drafting the manuscripts and professional writers will not be used for any of the publications. Authorship will be determined based on criteria defined by the International Journal of Medical Editors[74]. We aim to write the manuscript of the final results within 6 months of completing the study. Participants who have been involved in the trial will be given the option of having a summary of results sent to them.

Discussion

Since the advent of ART, people living with HIV now have a longevity which approaches that of their HIV-uninfected counterparts but have a higher burden of non-communicable comorbidities including cardiovascular, pulmonary, renal and hepatic diseases[1][2]. Heightened inflammation in people living with HIV despite ART is believed to be the driving before behind the increased rates of non-infectious comorbidities. Similarly, chronic immune activation fosters HIV persistence[10-15]. As cannabinoids possesses both anti-inflammatory and anti-fibrotic properties[19], cannabinoids may represent a feasible method to reduce immune activation and enhance immune profile. This, in turn, may hasten the progression of non-opportunistic complications associated with HIV. Although some studies have examined whether there are beneficial effects on inflammation resulting from treatment with integrase inhibitors compared to protease inhibitors (PIs), between PIs and non-nucleoside reverse transcriptase inihibitors (NNRTIs), between specific nucleoside reverse transcriptase inhibitors, or with maraviroc in ART-naïve patients, to date insufficient to conclude that any class of antiretrovirals is superior to other classes of antiretrovirals with regards to effects on inflammation[75]. Furthermore, cannabis may induce cytochrome P450 (CYP) 1A2 via activation of the aromatic hydrocarbon receptor[76]. CYP3A4 inducers and inhibitors alter the pharmacokinetics of Δ9-THC and CBD when administered as Δ9-THC/CBD oromucosal spray. To date only one study has

ever examined the effects of cannabinoids on the pharmacokinetics of antiretrovirals. Kosel et al. studied the pharmacokinetics of smoked marijuana and dronabinol in people living with HIV receiving either indinavir and nelfinavir (2 protease inhibitors no longer used due to their toxicity and adverse effect profiles)[77]. Individuals on stable regimens of indinavir 800 mg every 8 hours or nelfinavir 750 mg three times daily were randomized to one of three treatment arms: 1) 3.95% THC marijuana cigarettes 2) dronabinol 2.5 mg capsules or 3) placebo capsules given three times daily. Although there were statistically significant decreases in maximum concentration (Cmax) of indinavir in the smoked marijuana arm, the size of the changes in the pharmacokinetic parameters of both indivnavir and nelfinavir were sufficiently small not to impose any short-term clinical consequence[77]. Furthermore, the investigators concluded that use of marijuana or dronabinol is unlikely to impact antiretroviral therapy[77]. For these reasons and based on clinical experience at our clinic, we have not precluded individuals on any particular antiretroviral regimens from participating in this study.

In this pilot study, our primary objective is to assess the safety and tolerability of TN-TC11LM and TN-TC91LM taken by people living with HIV on suppressive ART. We hypothesize that these agents will be safe and well tolerated in people living with HIV, given that similar products are safe and well-tolerated in other populations. Sativex® is currently licensed as an adjunctive treatment for symptomatic relived of spasticity in adult patients with Multiple

Sclerosis (MS) who have not responded adequately to other therapy[78-80]. It is an oromucosal spray containing CBD and THC in1:1 ratio. Marinol®, which is a synththetic THC-containing capsule, is currently used for the treatment of anorexia associated with weight loss in persons with Acquired Immunodeficiency Syndrome (AIDS) and nausea and vomiting associated with cancer chemotherapy in patients with insufficient response to conventional antiemetics[81]. It has been estimated that 5,472 patients have been exposed to Sativex® and there have been no safety concerns identified and the product remains well-tolerated.   The primary safety concerns of both Sativex® and Marinol® are consistent with the known pharmacological activity of cannabinoids. The primary safety concerns associated with Sativex® included abuse potential, cardiovascular effects and central nervous system adverse effects[82]. Although there is some evidence to suggest that individuals can develop "cannabis use disorder", individuals do not develop the same extremes of behavior as observed with other drugs of abuse[83]. In two clinical trials, nabiximols such as Sativex® have been used in two clinical trials whereby treatment was abruptly ceased to study whether withdrawal symptoms would develop[84 85] In both studies, no withdrawal syndromes were observed[86]. Cannabinoids have cardiovascular effects that include tachycardia and fluctuations in blood pressure, including episodes of postural hypotension. Therefore these agents should not be used in patients with serious cardiovascular disease, such as ischemic heart disease, arrhythmias, poorly controlled hypertension or severe heart failure. THC has complex

effects on the CNS and should not be used in patients with a personal or strong family history of psychosis. Examples of such conditions include schizophrenia and affective psychosis since symptoms of these disorders may be aggravated by cannabinoids. In multiple sclerosis (MS) patients receiving Sativex® in clinical studies, psychiatric-related adverse effects included disorientation, depression including depressed mood, dissociation, euphoric mood, hallucination, hallucinations (auditory and visual), illusions, paranoia, suicidal ideation and delusional perception[87].   Interestingly, there is some evidence to demonstrated that CBD may actually improve psychotic symptoms in persons suffering from schizophrenia[88].

For Marinol®, which contains only THC, the most frequency reported adverse effects experienced by patients with AIDS during placebo controlled clinical trials involved CNS and were reported in 33% of patients receiving Marinol®. About 25% reported a CNS adverse event during the first 2 weeks and about 4% reported such an event each week for the next 6 weeks thereafter[89]. By combining CBD with THC, we anticipate that tolerability will be greatly enhanced. When combined with THC, CBD reduces the risk for many adverse effects [88]. Furthermore, individuals will be instructed to titrate up the dose based on their own tolerability and reduce the dose if they experience any undesirable effects.   Furthermore, due to the extremely low levels of CB1 receptors in the brainstem[90], death due to overdosing on cannabis or cannabinoids alone has never been described.

The study medications and doses were chosen after a lengthy review of the existing literature and discussion with experts in the field of pain management. A high degree of inter-individual variability in metabolism following administration of cannabinoids is observed due to polymorphisms in cytochrome isoenzymes [61]. Given that the therapeutic doses of cannabinoids are highly variable between individuals, a dose titration schedules are usually recommended. When used to treat specific conditions, persons may be told to increase the dose until they achieve adequate symptom relief without adverse effects. This method was observed to work well when used in the first cohort study on the long-term safety of medicinal cannabis for non-cancer chronic pain in seven Canadian clinics[34].

Given this is a pilot study and given our budgetary restrictions, a convenience sample of 26 participants was selected without formal power calculation. If this study demonstrated that TN-TC11LM and/or TN-TC91LM are safe and tolerable in people living with HIV and can reduce systemic inflammation, future studies will be performed to address the potential of these agents to ameliorate specific conditions in people living with HIV. Should future studies be conducted, data generated from this trial will assist with power calculations. Similar to a study conducted by members of our group on the ability of Niaspan® (extended-release niacin) to reduce immune activation, as determined by percentage of activated CD8+ CD38+ HLA-DR+ T-cells, we decided that a 50% reduction in activated CD8+ CD38+ HLA-DR+ T-cells would be

considered significant[70]. This is based on previous reports indicating that a 10-fold difference exists between uninfected healthy controls and treated aviremic HIV-infected individuals in level of activated CD8+ CD38+ HLA-DR+ T-cells[91] [92].   All of the inflammatory mediators we selected for this study are known to drive immune activation[1 6 93].

In addition, we decided to make our objective examining the ability of TN-TC11LM and TN-TC91LM capsules to reduce the HIV reservoir, through the reduction of systemic inflammation, an exploratory objective. It is unclear if 3 months of treatment will be long enough to produce any meaningful reduction in the size of the HIV reservoir. Furthermore, it is unclear what reduction in reservoir size is required to have a meaningful effect on clinical outcomes. As mentioned earlier, we will consider a 50% decrease in the number of HIV-infected cells at baseline vs. week 12 to be a significant reduction in the reservoir, based on a study by *Hill et al* [71]. To our knowledge, there is no other randomized clinical trial examining the effect of cannabinoids on inflammation and HIV reservoir size in people living with HIV.

Our study is unique in being the first randomized trial in the world to examine the association between ingestion of precise quantities of cannabinoids and effect on inflammation and peripheral HIV reservoir size. It is also noteworthy that we chose not to have a placebo arm as the effects of psychoactive effects of THC would be difficult to camouflage. Furthermore, we

chose to use oral formulations of cannabinoids so that we could precisely control the dose

ingested by participants. The active pharmaceutical ingredients are extracted from the cannabis

plant and purified according to pharmaceutical standards (>98%). This level of purity will enable

investigators to know that the dosing provided is accurate and quantifiable, and will also enable

us to draw conclusions about the efficacy of the active ingredients being studying. When

cannabis is smoked or vaped, there is variability in the methods and duration of inhalation used

by participants which can influence dosage of cannabis ingested. The oral administration option

also removes undesirable pulmonary effects such as symptoms wheezing or breathlessness in

addition to inhalation of toxic chemicals [94]. Of special interest to our group is the recent

discovery that administration of oral cannabis with lipids leads to high levels of cannabinoids in

the intestinal lymphatic system and prominent immunomodulation, as demontrated by *Zgair et

al*.[20] This finding is especially important given the prominent role of the mesenteric lymph nodes

and gut to HIV persistence[7]. If oral cannabinoids can modify gut microbiome and the enteric

immune system favorably, larger clinical trials could be conducted to examine this phenomena in

further detail. As gut microbiota differs by sexual orientation, we are attempting to enrol

approximately equal numbers of MSM as well as heterosexual individuals[95] and will describe the

demographics of the individuals enrolled in our study in detail.   Similarly, we will report

participants' current and nadir CD4 T counts as in some studies the enteric bacterial microbiome

of patients with lower CD4 T counts exhibited reduced phylogenetic diversity and richness[96]. There were increases in Enterobacteriaceae, which have been associated with inflammation. Therefore, immunodeficiency in progressive HIV infection is associated with alterations in the enteric virome and bacterial microbiome[96].

The Canadian government has declared that cannabis' regulatory status will change from being an illegal substance to that of a legal substance in October 2018. Cannabis' change in regulatory status will likely stimulate more discussion amongst patients and physicians and thus physicians need to be informed about the potential risks and benefits of cannabis use. The change in the regulatory landscape will likely also foster more research into cannabis' therapeutic potential. We hope that this study will be a stimulus towards more open discussion between patients and their physicians and that it will reduce stigma associated with cannabinoids use. We also hope that this study will be the cornerstone for future studies investigating the therapeutic benefits of cannabis in PLWHIV and its potential not only at the individual level but also at the population level in the form of harm reduction strategies.

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Authors' contributions

CTC conceived and designed the study, drafted the grant and the protocol manuscript, will organize and supervise trial implementation and will be responsible for trial management. She will also be responsible for trial managements, staff training and supervision. MAJ contributed to study design and participated in grant writing. ZS, BL, JPR, JC, J Szabo, MJB and MK will participate in study implementation. MJB provided input on questionnaires while CTC and MAJ provided immunological expertise and NC provided expertise related to the HIV reservoir. SM and EM contributed to study design. CTC, MAJ and NC designed the experiments. J Singer contributed to the statistical analysis plan. CTC, MAJ and NC designed the experiments. All authors participated in refinement of the study methods, critical reviewed the manuscript drafts and approved the final manuscript. The CTN provides regulatory support. CTC and BL are Fonds de recherche du Québec-Santé (FRQ-S) chercheur-boursier-clinicien Junior 1. BL holds a Strategy for Patient-Oriented Research (SPOR) Mentorship Chair in Innovative Clinical Trials. JPR holds the Louis Lowenstein Chair in Hematology and Oncology at McGill University. MAJ is holder of a Tier 2 Canada Research Chair in immunovirology.

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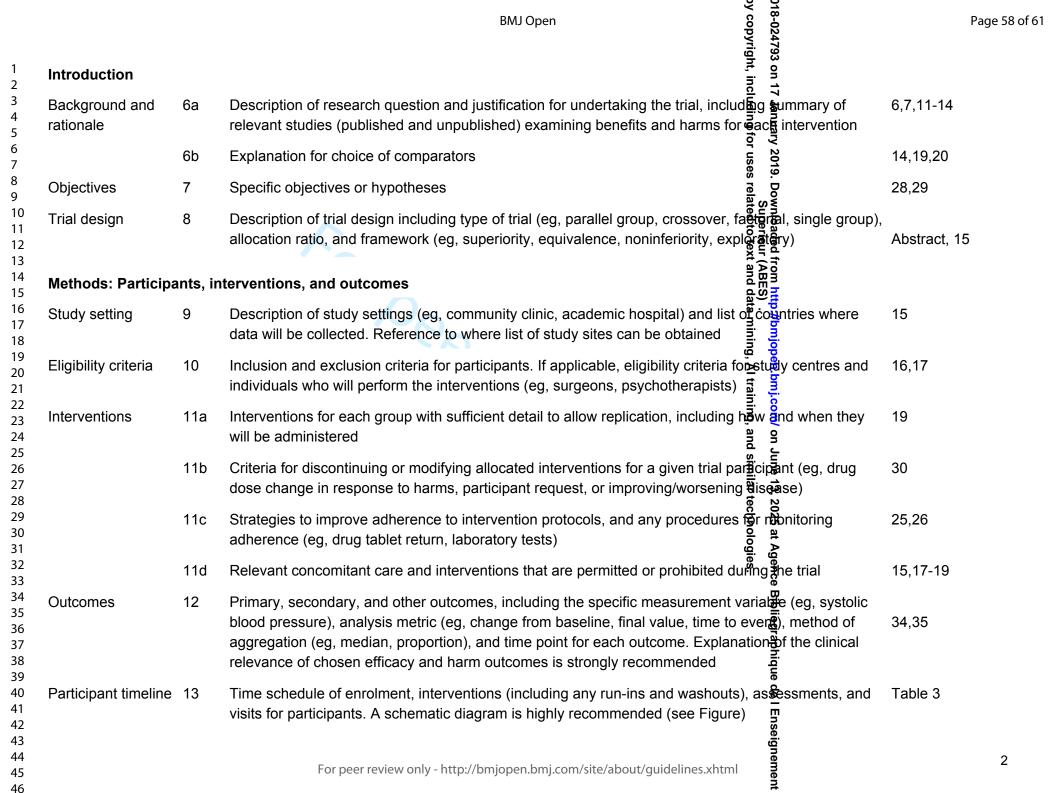
Competing interests: Tilray Inc. is supplying the study medications free of charge. All elements of the study are being undertaken independently of Tilray Inc. The authors declare there are no conflicts of interests.

Data sharing: There are no data yet to share.

SPIRIT ✓

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description	Addressed on 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	Abstract, 4
	2b	All items from the World Health Organization Trial Registration Data Set	3,4
Protocol version	3	Date and version identifier	Abstract /cover letter
Funding	4	Sources and types of financial, material, and other support	54,55
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 54,55
	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	38
	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)	30,36,37,cover letter

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	6,7,11-14
	6b	Explanation for choice of comparators	14,19,20
Objectives	7	Specific objectives or hypotheses	28,29
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)	Abstract, 15

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	15
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)	16,17
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	19
	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)	30
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	25,26
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	15,17-19
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	34,35
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)	Table 3

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		34,35
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size		Cover letter, already advertised by Canadian HIV Trials Network newsletter and website, meetings involving community; 16

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	22
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	22
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	22
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	22 (non-blind)
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	22 (non-blind)

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	22-24
	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	22-24
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	36,37
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	34,35
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	34,35
	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)	N/A

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	Cover letter, 29,30,36,37
	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	Health Canada, Canadian HIV Trials Network;29, 30, 36
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	Health Canada, CTN; 29, 30, 36
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	36

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	38
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)	38
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	38
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A at this time
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	36,37
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	57
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	35,36
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	30
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	38
	31b	Authorship eligibility guidelines and any intended use of professional writers	38
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	Not determined at this time

Appendices

5

| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Available upon request |
| 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 | 37 |

*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.