PEER REVIEW HISTORY

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

(This paper received three reviews from its previous journal but only two reviewers agreed to published their review.)

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Costiniuk, C; Saneei, Zahra; Routy, JP; Margolese, Shari; Mandarino, Enrico; Singer, Joel; Lebouché, Bertrand; Cox, Joseph; Szabo, Jason; Brouillette, Marie-Josée; Klein, Marina; Chomont, Nicolas; Jenabian, Mohammad-Ali

VERSION 1 – REVIEW

REVIEWER	Dr Myron R Szewczuk Queen's University, Kingston, Ontario, K7L3N6 Canada
REVIEW RETURNED	12-Aug-2018

GENERAL COMMENTS	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. Critique #1: Hileman and Funderburg (Curr HIV/AIDS Rep. 2017 June ; 14(3): 93–100. doi:10.1007/s11904-017-0356-x.) have reported on Inflammation, Immune Activation, and Antiretroviral Therapy in HIV. Their review focuses on the differential effects of contemporary antiretrovirals on systemic inflammation as heightened immune activation is linked to important co-morbidities and mortality with HIV infection. It is unclear whether there are beneficial effects on inflammation resulting from treatment with integrase inhibitors compared to PIs, between PIs and NNRTIs, between specific nucleoside reverse transcriptase inhibitors, or with maraviroc in ART-naïve patients. In ART switch studies, changing to an integrase inhibitor from a PI-, NNRTI-, or enfuvirtide-containing regimen has resulted in improvement in several markers of inflammation. In the present protocol application, the patients will be on ART treatment; however, there is little description on how ART and THC:CBD oils consumed orally – as TN-TC11LM and TN-TC19LM oral capsules may be contraindication or beneficial. It is important to discuss this issue in the protocol. In this pilot study, the primary objective is to assess the safety and tolerability of TNTC11LM 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. Critique #2:It is very important to describe how the patient's samples such as blood and stools, etc will be handled, timing taken and analyzed and stored in order to avoid sample biased errors. There are reports in the literature of errors in non-reproducible results. As described, 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. Critique # 3: Full Spectrum Cannabis Extract: Tilray's full spectrum products contain extract cannabis oil with active ingredients refined to 80% purity along with other natural plant compounds. Quantitative data on the cannabis oils would be appropriate for comparison future studies. Tilray's website has indicated lot differences, and this needs to be addressed in this pilot study. An API for quality control is needed. Critique #4: J Neuroimmune Pharmacol (2015) 10:204–216 DOI 10.1007/s11481-015-9603-3 is a review stating that there is evidence supporting the conclusion that the immune modulating capacity of marijuana, of Δ9-THC extracted from the marijuana plant, and synthetic cannabinoids can sensitize to some infections through their immunosuppressive activities, but not to others. In another paper (https://doi.org/10.1016/j.ebiom.2018.03.024) they have discussed Fecal Microbiota Composition Drives Immune Activation in HIV-infected Individuals. The enteric microbiota of untreated HIV-infected subjects induce significantly higher levels of activated monocytes and T cells compared to seronegative subjects. FBCs from anti-retroviral therapy (ART)-treated HIV-infected individuals induced intermediate T cell activation, indicating an only partial correction of adaptive immune cell activation capacity of the microbiome with ART. In another study (http://dx.doi.org/10.1016/j.ebiom.2016.01.032), men who have sex with men (MSM) predominantly belonged to the Prevotella-rich enterotype whereas most non-SM subjects were enriched in Bacteroides, independently of HIV-1 status, and with only a limited contribution of diet effects. So, the inclusion of subjects may consider this issue. The gut microbiota differs by sexual orientation will be needed. Such a study would be challenging, given the need to avoid or minimize confounding by demographics, diet, physical activity, HIV and other infections, and medications particularly antibiotics. In the meantime, all studies of HIV-microbiota relationships should carefully investigate possible confounding or effect modification by sexual orientation, injection drug use, and demographics. Also, http://dx.doi.org/10.1016/j.chom.2016.02.011 has reported that the enteric bacterial microbiome of patients with lower CD4 T counts exhibited reduced phylogenetic diversity and richness with specific bacteria showing differential abundance, including increases in Enterobacteriaceae, which have been associated with inflammation. Thus, immunodeficiency in progressive HIV infection is associated with alterations in the enteric virome and bacterial microbiome, which may contribute to AIDS associated enteropathy and disease progression. Despite the chronic gut inflammation characteristic of HIV infection, the associated microbiota showed limited similarity with other inflammatory states

	and instead showed increased, rather than decreased, diversity (http://dx.doi.org/10.1016/j.chom.2013.08.006).

REVIEWER	Nancy Sohler City College of New York, Medical School USA
REVIEW RETURNED	31-Aug-2018

GENERAL COMMENTS	This is a well designed and described preliminary study to explore the safety and tolerability of cannabis oils containing THC and CBD in people living with HIV. Given the rationale, this is an important study that could lead to treatment protocol that will improve care for people living with HIV. My only reservation is the lack of formal sample size calculations for any of the study outcomes.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Dr Myron R Szewczuk

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.

Critique #1: Hileman and Funderburg (Curr HIV/AIDS Rep. 2017 June ; 14(3): 93–100. doi:10.1007/s11904-017-0356-x.) have reported on Inflammation, Immune Activation, and Antiretroviral Therapy in HIV. Their review focuses on the differential effects of contemporary antiretrovirals on systemic inflammation as heightened immune activation is linked to important co-morbidities and mortality with HIV infection. It is unclear whether there are beneficial effects on inflammation resulting from treatment with integrase inhibitors compared to PIs, between PIs and NNRTIs, between specific nucleoside reverse transcriptase inhibitors, or with maraviroc in ART-naïve patients. In ART switch studies, changing to an integrase inhibitor from a PI-, NNRTI-, or enfuvirtide-containing regimen has resulted in improvement in several markers of inflammation. In the present protocol application, the patients will be on ART treatment; however, there is little description on how ART and THC:CBD oils consumed orally – as TN-TC11LM and TN-TC19LM oral capsules may be contraindication or beneficial. It is important to discuss this issue in the protocol. In this pilot study, the primary objective is to assess the safety and tolerability of TNTC11LM 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.

Authors' Response: Each participant's concomitant medications list will need to be reviewed prior to study enrolment to ensure no clinically significant drug interactions with TN-CT11L and TN-CT91L oral capsules. Although theoretical HIV antiretroviral drugs 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. This finding was discussed with HIV-specialized pharmacists who are aware of the patients' recreational and prescription drug use habits. Therefore, individuals will not be precluded to participate based on their antiretroviral regimen. This information is now included on p.19.

As mentioned by the reviewer, Hileman and Funderberg published a review on this topic although the bottom line was that "Additional research is needed to conclusively state whether there are clear differences in effects of specific antiretrovirals on inflammation and immune activation in HIV." Therefore, we now say: "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 (Hileman et al.) During our study, we will record the antiretroviral regimens taken by patients and will document any changes made to ART during the course of the study" p.41-42.

Some medications may interact with TN-CT11LM and TN-CT91LM oral capsules including those metabolized through the CYP2C and CYP3A isoenzymes. Examples of drugs which may interact with TN-CT11LM and TN-CT91LM oral capsules include sedatives, drugs with sedating or psychotropic effects and hypnotics which can result in additive effects. Examples include amitriptyline, fentanyl and certain other opioids. Antimicrobials such as rifampin and ketoconazole may also interact. However, the Investigator will review potential participants' current medication lists at the screening visit prior to study enrolment. If any concomitant therapy interacts with TN-CT11L and TN-CT91L oral capsules, and if this medication cannot be substituted, that participant will not be eligible to enroll in the study. If necessary the CVIS pharmacist will be consulted for an opinion as well.

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). 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. Furthermore, the investigators concluded that use of marijuana or dronabinol is unlikely to impact antiretroviral therapy (p.41-42).

Critique #2: It is very important to describe how the patient's samples such as blood and stools, etc will be handled, timing taken and analyzed and stored in order to avoid sample biased errors. There are reports in the literature of errors in non-reproducible results. As described, 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.

Authors' Response: Within 1 hour of being drawn, blood from the CVIS will be transferred to the laboratory at the RI-MUHC (in the same building) and the plasma will be separated from peripheral blood mononuclear cells (PBMCs) by Ficoll Density Centrifugation by an experienced laboratory technician. PBMCs and plasma will be stored in liquid Nitrogen tanks in the lab at the RI-MUHC 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. This more detailed description is provided on p.38.

Critique # 3: Full Spectrum Cannabis Extract: Tilray's full spectrum products contain extract cannabis oil with active ingredients refined to 80% purity along with other natural plant compounds. Quantitative data on the cannabis oils would be appropriate for comparison future studies. Tilray's website has indicated lot differences, and this needs to be addressed in this pilot study. An API for quality control is needed.

Authors' Response: The study drugs Tilray is providing for this study are not full spectrum extracts refined to 80% purity. The active pharmaceutical ingredients (APIs) are extracted from the cannabis plant and purified according to pharmaceutical standards (>98%). This level of purity is what allows us to know that the dosing we're providing is accurate and quantifiable, and also allows us to draw conclusions about the efficacy of the active ingredients we're studying. This is now indicated under "Study Intervention" on p.20 and in the discussion on p.46. Note that if the product contained up to 20% of other active ingredients that were undefined, it would be very difficult to attribute effects to THC and CBD.

Critique #4: J Neuroimmune Pharmacol (2015) 10:204–216
DOI 10.1007/s11481-015-9603-3 is a review stating that there is evidence supporting the conclusion that the immune modulating capacity of marijuana, of Δ9-THC extracted from the marijuana plant, and synthetic cannabinoids can sensitize to some infections through their immunosuppressive activities, but not to others. In another paper (https://doi.org/10.1016/j.ebiom.2018.03.024) they have discussed Fecal Microbiota Composition Drives Immune Activation in HIV-infected Individuals. The enteric microbiota of untreated HIV-infected subjects induce significantly higher levels of activated monocytes and T cells compared to seronegative subjects. FBCs from anti-retroviral therapy (ART)-treated HIV-infected individuals induced intermediate T cell activation, indicating an only partial correction of adaptive immune cell activation capacity of the microbiome with ART. In another study (http://dx.doi.org/10.1016/j.ebiom.2016.01.032), men who have sex with men (MSM) predominantly belonged to the Prevotella-rich enterotype whereas most non-SM subjects were enriched in Bacteroides, independently of HIV-1 status, and with only a limited contribution of diet effects. So, the inclusion of subjects may consider this issue. The gut microbiota differs by sexual orientation will be needed. Such a study would be challenging, given the need to avoid or minimize confounding by demographics, diet, physical activity, HIV and other infections, and medications particularly antibiotics. In the meantime, all studies of HIV-microbiota relationships
should carefully investigate possible confounding or effect modification by sexual orientation, injection drug use, and demographics.

Authors' Response: We thank the reviewer for raising these points. The fact that synthetic cannabinoids can sensitize to some infections through their immunosuppressive activities, but not to others, is interesting but beyond the scope of discussion for this manuscript. In individuals with suppressed viral loads on ART, it is extremely unlikely that cannabinoids would induce immunosuppression to a degree whereby patients would be at increased risk of any infection. In the "Measurements" section, we now indicate that we will record whether individuals have received antimicrobials with the previous 6 months (p.20). Indeed, sexual orientation can certainly affect the gut microbiota. We will attempt to enrol equal proportions of MSM, and heterosexual individuals, as now mentioned in the inclusion criteria (p.17). Current drug abuse in already an exclusion criteria. We also now reference the paper by Noguera-Julian et al, 2016 in the discussion in regards to this issue (p.47).

Also,http://dx.doi.org/10.1016/j.chom.2016.02.011 has reported that the enteric bacterial microbiome of patients with lower CD4 T counts exhibited reduced phylogenetic diversity and richness with specific bacteria showing differential abundance, including increases in Enterobacteriaceae, which have been associated with inflammation. Thus, immunodeficiency in progressive HIV infection is associated with alterations in the enteric virome and bacterial microbiome, which may contribute to AIDS associated enteropathy and disease progression. Despite the chronic gut inflammation characteristic of HIV infection, the associated microbiota showed limited similarity with other inflammatory states and instead showed increased, rather than decreased, diversity (http://dx.doi.org/10.1016/j.chom.2013.08.006).

Authors' Response: The inclusion of these references is very helpful as they shown that the gut microbiota that microbiota diversity can be either reduced or increased, depending on the specific HIV patient population studied. This information has been incorporated into the text p.47 and the papers referenced. We also now indicate that patients with lower CD4 T counts exhibited reduced phylogenetic diversity and richness with specific bacteria showing differential abundance, including increases in Enterobacteriaceae, which have been associated with inflammation p.47 and the paper referenced

Reviewer: 2
Reviewer Name: Nancy Sohler

This is a well designed and described preliminary study to explore the safety and tolerability of cannabis oils containing THC and CBD in people living with HIV. Given the rationale, this is an important study that could lead to treatment protocol that will improve care for people living with HIV. My only reservation is the lack of formal sample size calculations for any of the study outcomes.

Authors' Response: This is a pilot study whose purpose is to assess feasibility for a larger trial. The primary outcome is safety and tolerability of cannabinoid oils. Clinical trials are very expensive to run and we were restricted to a budget of maximum $50,000 Canadian. A large portion of the cost will be for immunological and virological studies and a study coordinator. We mention that our budgetary restriction was a limiting factor in our selection of a sample size. However, as we already mention in our discussion, if this study demonstrates that TN-TC11LM and/or TN-TC91LM are safe and tolerable in people living with HIV and shows a potential reduction systemic inflammation, future studies will be designed 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.

VERSION 2 – REVIEW

REVIEWER	Prof. Myron R Szewczuk Queen's University Kingston, Ontario K7L3N6 Canada
REVIEW RETURNED	31-Oct-2018

GENERAL COMMENTS	Authors addressed my comments adequately.