UC San Diego

UC San Diego Previously Published Works

Title

Effect of Cannabis Use on Human Immunodeficiency Virus DNA During Suppressive Antiretroviral Therapy

Permalink

https://escholarship.org/uc/item/93f2w7b7

Journal

Clinical Infectious Diseases, 70(1)

ISSN

1058-4838

Authors

Chaillon, Antoine

Nakazawa, Masato

Anderson, Christy

et al.

Publication Date

2020

DOI

10.1093/cid/ciz387

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Journal : CLINID

Article Doi : 10.1093/cid/ciz387

OXFORD
UNIVERSITY PRESS

Article Title : Effect of Cannabis Use on Human Immunodeficiency Virus DNA During Suppressive Antiretroviral Therapy

INSTRUCTIONS

1. **Permissions:** Permission to reproduce any third party material in your paper should have been obtained prior to acceptance. If your paper contains figures or text that require permission to reproduce, please confirm that you have obtained all relevant permissions and that the correct permission text has been used as required by the copyright holders. Please contact jnls.author.support@oup.com if you have any questions regarding permissions.
2. **Author groups:** Please check that all names have been spelled correctly and appear in the correct order. Please also check that all initials are present. Please check that the author surnames (family name) have been correctly identified by a pink background. If this is incorrect, please identify the full surname of the relevant authors. Occasionally, the distinction between surnames and forenames can be ambiguous, and this is to ensure that the authors' full surnames and forenames are tagged correctly, for accurate indexing online. Please also check all author affiliations.
3. **Figures:** If applicable figures have been placed as close as possible to their first citation. Please check that they are complete and that the correct figure legend is present.
4. **Missing elements:** Please check that the text is complete and that all figures, tables and their legends are included.
5. **Funding:** Please provide a Funding statement, detailing any funding received. Remember that any funding used while completing this work should be highlighted in a separate Funding section. Please ensure that you use the full official name of the funding body, and if your paper has received funding from any institution, such as NIH, please inform us of the grant number to go into the funding section. We use the institution names to tag NIH-funded articles so they are deposited at PMC. If we already have this information, we will have tagged it and it will appear as coloured text in the funding paragraph. Please check the information is correct. [red text to be used for suppliers who are tagging the funding]
6. **Conflict of interest:** All authors must make a formal statement indicating any potential conflict of interest that might constitute an embarrassment to any of the authors if it were not to be declared and were to emerge after publication. Such conflicts might include, but are not limited to, shareholding in or receipt of a grant or consultancy fee from a company whose product features in the submitted manuscript or which manufactures a competing product. The following statement has been added to your proof: 'Conflict of Interest: none declared'. If this is incorrect please supply the necessary text to identify the conflict of interest.
7. **Abbreviations:** some commonly used abbreviations have been automatically expanded for clarity to readers. By using the tracked changes proof for reference, please check the expanded abbreviations have been made correctly and mark up any corrections (if required) here or by email, listing the line and page numbers your correction refers to.

MAKING CORRECTIONS TO YOUR PROOF

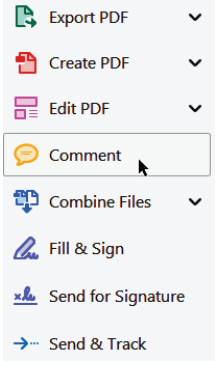
These instructions show you how to mark changes or add notes to your proofs using Adobe Acrobat Professional versions 7 and onwards, or Adobe Reader DC. To check what version you are using go to **Help** then **About**. The latest version of Adobe Reader is available for free from get.adobe.com/reader.

DO NOT OVERWRITE TEXT, USE COMMENTING TOOLS ONLY.

DISPLAYING THE TOOLBARS

Adobe Reader DC

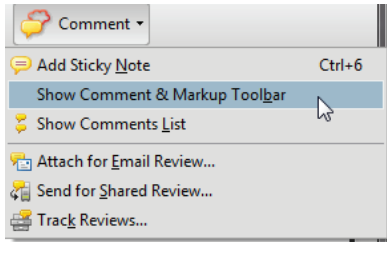
In Adobe Reader DC, the Comment toolbar can be found by clicking 'Comment' in the menu on the right-hand side of the page (shown below).



The toolbar shown below will then display along the top.

Acrobat Professional 7, 8, and 9

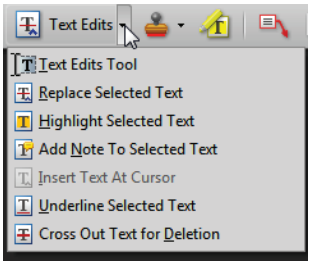
In Adobe Professional, the Comment toolbar can be found by clicking 'Comment(s)' in the top toolbar, and then clicking 'Show Comment & Markup Toolbar' (shown below).



The toolbar shown below will then be displayed along the top.

USING TEXT EDITS AND COMMENTS IN ACROBAT

This is the quickest, simplest and easiest method both to make corrections, and for your corrections to be transferred and checked.



1. Click **Text Edits**
2. Select the text to be annotated or place your cursor at the insertion point and start typing.
3. Click the **Text Edits** drop down arrow and select the required action.

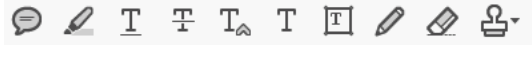
You can also right click on selected text for a range of commenting options, or add sticky notes.

SAVING COMMENTS

In order to save your comments and notes, you need to save the file (**File, Save**) when you close the document.

USING COMMENTING TOOLS IN ADOBE READER

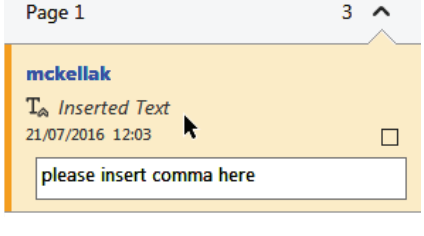
All commenting tools are displayed in the toolbar.
To edit your document, use the highlighter, sticky notes, and the variety of insert/replace text options.



POP-UP NOTES

In both Reader and Acrobat, when you insert or edit text a pop-up box will appear. In **Acrobat** it looks like this:

In **Reader** it looks like this, and will appear in the right-hand pane:



AUTHOR QUERY FORM

Journal : CLINID

Article Doi : 10.1093/cid/ciz387

Article Title : Effect of Cannabis Use on Human Immunodeficiency Virus DNA During Suppressive Antiretroviral Therapy

First Author : Antoine Chaillon

Corr. Author : Sara Gianella

AUTHOR QUERIES - TO BE ANSWERED BY THE CORRESPONDING AUTHOR

Please ensure that all queries are answered, as otherwise publication will be delayed and we will be unable to complete the next stage of the production process.

Please note that proofs will not be sent back for further editing.

The following queries have arisen during the typesetting of your manuscript. Please click on each query number and respond by indicating the change required within the text of the article. If no change is needed please add a note saying "No change."

	Please note that inclusion or deletion of names to the author group, on the title page, cannot be made directly in the manuscript at the proof stage, as certain formalities have to be completed for doing the same. Hence, kindly confirm whether you require any changes to be made in the author group, and CID Editorial will follow up.
AQ1	Please confirm all authors are in the same department or provide separate affiliations for separate departments.
AQ2	Please provide expansion of pol.
AQ3	Please provide expansion of "m" in mRNA.
AQ4	Please provide expansion for Ln in Figure 1.
AQ5	Please confirm expansion of IFN to interferon.
AQ6	Please confirm edits convey intended meaning.
AQ7	Please provide expansion for CHRP, if applicable.
AQ8	Please check use of "E" in author name of reference 1.
AQ9	Please provide publisher name and location for reference 1
AQ10	Please confirm all references with et al have 7 or more authors.
AQ11	Please provide expansion for CHARTER in reference 11.
AQ12	Please provide publisher name for reference 13.
AQ13	Please provide expansion for HNRC in reference 14.
AQ14	Please update publication details for reference 18.

Clinical Infectious Diseases

BRIEF REPORT

Effect of Cannabis Use on Human Immunodeficiency Virus DNA During Suppressive Antiretroviral Therapy

Antoine Chaillon, Masato Nakazawa, Christy Anderson, Aaron Christensen-Quick, Ronald J. Ellis, Donald Franklin, Sheldon R. Morris, and Sara Gianella

AQ1 Division of Infectious Diseases, University of California, San Diego, La Jolla

Cannabis use is frequent among people living with human immunodeficiency virus (HIV) and is associated with reduced systemic inflammation. We observed a faster HIV DNA decay during antiretroviral therapy among cannabis users, compared to those with no drug use. No cannabis effect was observed on cellular HIV RNA transcription.

Keywords. MSM; cannabis; HIV DNA; cellular transcription.

Cannabis is widely consumed in the United States [1], particularly among people living with human immunodeficiency virus (HIV) [2–4], and this has fostered an important debate regarding the impact of cannabis on virologic and inflammatory biomarkers. On one hand, a recent study reported reduced adherence to antiretroviral therapy (ART) and retention in care in cannabis users [5], and 2 other studies found no effects of cannabis on viral suppression [4, 6]. On the other hand, 1 study has demonstrated beneficial impacts of cannabis on plasma HIV RNA among recently infected people living with HIV (PLWH) [7]. More recently, Manuzak et al [8] evaluated the impact of cannabis use on inflammation and immune activation in ART-treated PLWH and reported that cannabis use was associated with (1) reduced activation of $CD4^+$ and $CD8^+$ T cells and (2) a shift in the composition of the monocyte populations in peripheral blood.

Here, we investigated the effect of cannabis use (with or without other drugs) on the decay and transcriptional activity of the HIV reservoir during suppressive ART.

METHODS

Ethics Statement

The study was approved by the University of California San Diego Human Research Protections Program. All adult participants provided written informed consent.

Received 29 January 2019; editorial decision 29 April 2019; accepted 9 May 2019; published online May 10, 2019.

Correspondence: S. Gianella, Division of Infectious Diseases, Department of Medicine, University of California, San Diego, 9500 Gilman Drive, Stein Clinical Research Building #324, La Jolla CA, 92093-0679 (gianella@ucsd.edu).

Clinical Infectious Diseases® 2019;XX(XX):1–4

© The Author(s) 2019. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

DOI: 10.1093/cid/ciz387

Study Cohort and Clinical Data

There were 48 men who have sex with men (MSM) coenrolled in the San Diego Primary Infection Resource Consortium [9, 10] and in the Translational Methamphetamine Acquired Immunodeficiency Syndrome (AIDS) Research Center cohort [11]. The estimated duration of infection (EDI) was determined using serologic and virologic parameters [12]. A median of 4 (interquartile range [IQR] 1–5) longitudinal peripheral blood mononuclear cells (PBMCs) and plasma samples per individual were collected over a median of 22 months (IQR 12–31) following ART initiation. Absolute $CD4^+$ and $CD8^+$ T cell counts, plasma HIV RNA levels (Amplicor, Roche), and self-reported ART adherence were measured.

To ascertain substance use (not including alcohol), we utilized 3 validated questionnaires, including the World Health Organization Composite International Diagnostic Interview–Substance Abuse Module 2.1 [11, 13, 14]. We were primarily interested in the effect of cannabis on cellular HIV DNA and HIV RNA. Due to the small sample size and to reduce the false discovery rate, we combined all other drugs (except cannabis). This yielded 4 groups, based on self-reported drug use: (1) no drug use; (2) cannabis use only; (3) use of drugs other than cannabis ("other drugs"); and (4) use of both cannabis and other drugs ("both drugs").

Total Cellular Human Immunodeficiency Virus DNA and RNA

DNA was extracted from 5 million PBMCs for each time point using an AllPrep DNA/RNA Mini Kit (Qiagen, CA). Total HIV DNA (gag) was quantified by droplet digital polymerase chain reaction and normalized to the housekeeping gene RPP30 [15]. Levels of all fully elongated and correctly processed HIV mRNA molecules were quantified [16] and normalized to total RNA concentration [17].

Flow Cytometry

Markers of T cell activation ($CD38^+HLA\text{-}DR^+$) and monocyte activation ($CD16^+$) were assessed by flow cytometry [18].

Statistical Analyses

We developed (generalized) linear mixed-effects regression models to investigate the relationship of cannabis and other drugs on 2 outcomes during ART: (1) changes in HIV DNA levels, and (2) cell-associated HIV RNA transcripts (detectable or undetectable). For each model, we included a random intercept for subject and the following fixed effects: (1) time from ART initiation (ie, slope); (2) drug use; and (3) time by drug use (ie, the difference in slopes). To determine which fixed effects to include in the final model, we tested whether the outcome changed over time among all participants. If it did change at

the 10% significance level, we subsequently tested whether the drug user groups differed in the rate of change and in the outcome level at ART initiation; if not, we tested whether the groups differed in the overall outcome level. To control for other sources of variability, we added relevant covariates (ie, age, time from EDI to ART, CD4 count, CD4/CD8 ratio, mean % activated [$CD38^+HLA\text{-}DR^+$] of $CD4^+$ and $CD8^+$ T cells, and mean % of $CD14^+CD16^+$ monocytes), if associated with the outcome at $P < .10$ in individual models. Our final models include all predictors and covariates that were significant at $P < .05$. Because 34% of all time points exhibited levels of cellular HIV RNA below the limits of detection, we used mixed-effects logistic regression to analyze this outcome. Data are expressed as median (IQR).

RESULTS

Study Cohort and Samples

This study evaluated 206 blood samples from 48 MSM who initiated ART within a median of 4 months (IQR 1–6) from EDI and achieved suppressed HIV RNA within a median of 5 months (IQR 3–8), without any documented blips of HIV RNA replication. Of the 48 individuals, 10 (21%) reported no drug use, 5 (11%) reported use of cannabis only, 16 (33%) reported use of drugs other than cannabis, and 17 (35%) reported use of cannabis and other drugs. Summaries of cohort characteristics and drug self-reports at ART initiation are provided in Supplementary Tables 1 and 2.

Predictors of Human Immunodeficiency Virus DNA Levels and Decay

The median unadjusted HIV DNA level at ART initiation was 2.4 $\log_{10}$ copies/10^6 cells (IQR 1.0–3.1) with a significant decrease of HIV DNA over time among all participants ($P < .01$; Supplementary Table 3). Cannabis-only users had significantly greater HIV DNA levels at the time of ART initiation ($P = .04$), but subsequently experienced a faster HIV DNA decay, compared to participants with no drug use ($P = .04$; Figure 1). Participants who used other drugs (with or without cannabis) had similar levels of HIV DNA at ART initiation ($P = .41$ and $P = .66$, respectively) and during ART ($P = .43$ and $P = .76$, respectively), compared to those who used no drugs. The 4 groups did not differ in baseline CD4 counts ($P = .80$) or EDIs ($P = .46$).

Regarding markers of cellular activation, study participants taking other drugs had a persistent, higher proportion of activated ($CD38^+HLA\text{-}DR^+$) $CD8^+$ T cells, as compared to those who used no drugs, even after controlling for significant covariates. There were no differences by groups related to $CD38^+HLA\text{-}DR^+CD4^+$ T cells or $CD14^+CD16^+$ monocytes. Overall, the mean percentage of $CD38^+HLA\text{-}DR^+CD8^+$ T cells was positively associated with HIV DNA levels in the individual model ($P < .02$), but this was no longer significant when drug groups were included ($P = .08$).

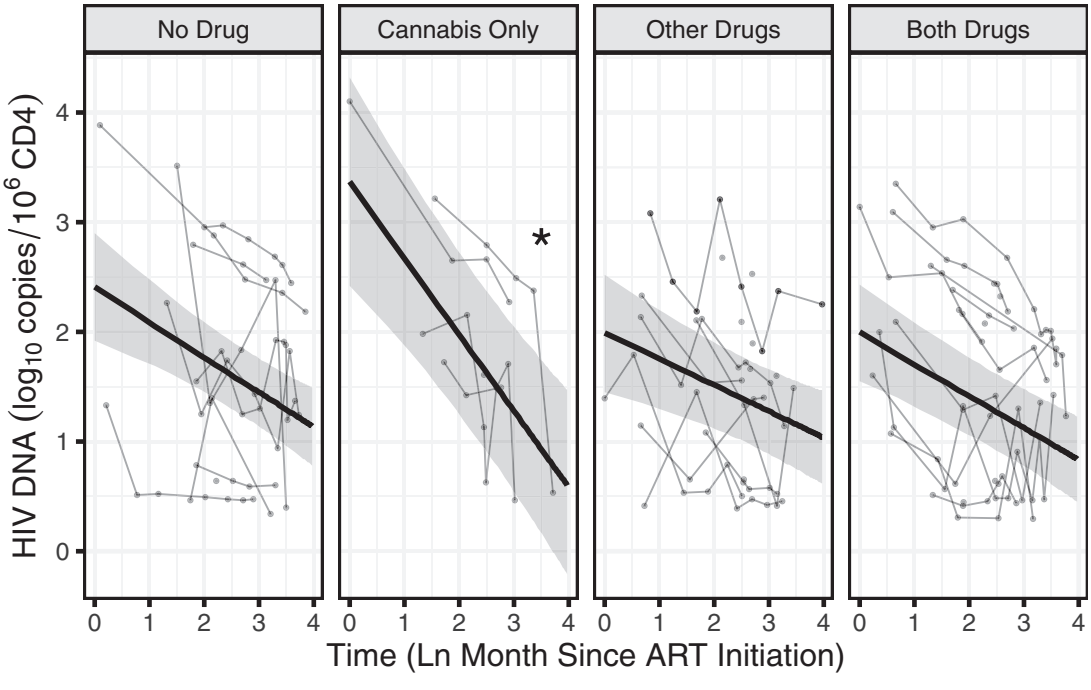


Figure 1. Observed HIV DNA ($\log_{10}$ copies/10^6 CD4+) in cohort during ART as a function of time (Ln months since ART Initiation) and drug use. The thin lines and dots indicate individual data points. Thick lines and shaded areas indicate model-estimated values and their 95% confidence intervals. *$P < .05$: the mean HIV DNA level at ART initiation and slope differences compared to the no-drug group. Abbreviations: ART, antiretroviral therapy; HIV, human immunodeficiency virus.

Cellular Human Immunodeficiency Virus RNA Transcription

The median levels of cellular HIV RNA at baseline were 4.9 $\log_{10}$ copies/ml (IQR 3.8–5.7; Supplementary Table 1), and all of the participants had detectable levels at ART initiation. Participants who reported use of cannabis (with or without other drugs), did not exhibit significantly different proportions of detectable cellular HIV RNA ($P = .59$ and $P = .28$, respectively). However, participants who used other drugs were significantly more likely to have detectable cellular HIV RNA levels during ART ($P = .01$). Similarly, participants with higher peak HIV RNA levels (before ART initiation) and higher mean percentages of activated $CD8^+$ T cells had more detectable cellular HIV RNA levels during ART ($P < .05$). Participants with higher CD4/CD8 ratios had less detectable cellular HIV RNA levels ($P < .10$). Interestingly, only the other drug effect remained significant for cellular HIV RNA when included in a final multivariate model ($P = .01$; other effects, $P > .4$; Supplementary Table 4).

DISCUSSION

In this longitudinal study of PLWH with well-characterized histories of substance use, we found that exclusive use of cannabis was associated with a faster decay of HIV DNA, but had no impact on cell-associated HIV RNA transcription or cellular activation during suppressive ART. However, the consumption of other drugs was associated with increased $CD8^+$ T cell activation and with increased cellular HIV RNA transcription during ART. Our results are consistent with a previous study reporting that cannabis use was associated with lower plasma HIV RNA levels among recently infected PLWH [7], and with reports of reduced HIV replication and cellular infection rates in the presence of cannabinoids in vitro [19, 20]. Further, cannabis use has been associated with decreased frequencies of interleukin 23 and tumor necrosis factor α [8] and, more recently, with the reduction of interferon-γ inducible protein 10 levels in plasma [21]. Altogether, these findings point to a potential anti-inflammatory effect of cannabis that, in turn, might positively impact HIV replication and persistence [22], although another study has suggested that tetrahydrocannabinol may enhance HIV replication by suppressing immune functions in mice [23], and this possibility should be evaluated in future, larger clinical studies. In our study, the effect of cannabis on HIV DNA was not evident when cannabis was used in combination with any other drugs, highlighting the complex interplay between drug use and viral infections.

The small sample size and the multiple potential confounding factors among participants with a complex history of drug use are major limitations to this report. Furthermore, cannabis use was not confirmed by the direct measurement of cannabis metabolites, and only relied on self-report. While inaccurate self-reporting could confound our observations, studies have demonstrated the reliability of self-reported drug use [24].

The mechanisms by which cannabis impacts the HIV reservoir—whether directly or indirectly by limiting immune activation, the infection of $CD4^+$ T cells, and macrophages and inflammation—remain uncertain. Further work is necessary to explore these mechanisms and the interactions with other drugs, which have been associated with contradictory effects on the immune system. These findings may have implications for and reaffirm the need for regular screening for substance use in HIV care settings and for a holistic approach to HIV cures from healthcare providers, physicians, epidemiologists, and researchers.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Financial support. This work was supported by the National Institute on Drug Abuse (grant number P50 DA026306).

Potential conflicts of interest. A. C. has received funding from the National Institutes of Health (grant number R21 AI131971-01) and the University of San Diego Center for Acquired Immunodeficiency Syndrome Research, a National Institutes of Health–funded program (grant number P30 AI036214). S. G. has received funding from the National Institutes of Health (grant numbers HD094646, AI027763, AI134295, and AI68636). R. J. E. has received funding from the National Institutes of Health (grant numbers R01 AG048650, P30 MH62512, and R01 MH107345). S. R. M. has received grants from Gilead Sciences and CHRP and other support from Bristol Myers Squibb, Forty Seven Inc., and Impact Biomedicines (now Celgene), outside the submitted work. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. National Academies of Sciences E, and Medicine Health and Medicine Division; Board on Population Health and Public Health Practice. Cannabis: prevalence of use, regulation, and current policy landscape. **2017**.
2. Harris GE, Dupuis L, Mugford GJ, et al. Patterns and correlates of cannabis use among individuals with HIV/AIDS in maritime Canada. Can J Infect Dis Med Microbiol **2014**; 25:e1–7.
3. Mimiaga MJ, Reisner SL, Grasso C, et al. Substance use among HIV-infected patients engaged in primary care in the United States: findings from the Centers for AIDS Research Network of Integrated Clinical Systems cohort. Am J Public Health **2013**; 103:1457–67.
4. Okafor CN, Zhou Z, Burrell LE 2nd, et al. Marijuana use and viral suppression in persons receiving medical care for HIV-infection. Am J Drug Alcohol Abuse **2017**; 43:103–10.
5. Kipp AM, Rebeiro PF, Shepherd BE, et al. Daily marijuana use is associated with missed clinic appointments among HIV-infected persons engaged in HIV care. AIDS Behav **2017**; 21:1996–2004.
6. Lake S, Kerr T, Capler R, Shoveller J, Montaner J, Milloy MJ. High-intensity cannabis use and HIV clinical outcomes among HIV-positive people who use illicit drugs in Vancouver, Canada. Int J Drug Policy **2017**; 42:63–70.
7. Milloy MJ, Marshall B, Kerr T, et al. High-intensity cannabis use associated with lower plasma human immunodeficiency virus-1 RNA viral load among recently infected people who use injection drugs. Drug Alcohol Rev **2015**; 34:135–40.
8. Manuzak JA, Gott TM, Kirkwood JS, et al. Heavy cannabis use associated with reduction in activated and inflammatory immune cell frequencies in antiretroviral therapy-treated human immunodeficiency virus-infected individuals. Clin Infect Dis **2018**; 66:1872–82.
9. ~~Quinn GP, Murphy D, Pratt C, et al. Altruism in terminal cancer patients and rapid tissue donation program: does the theory apply? Med Health Care Philos **2013**; 16:857–64.~~
10. ~~Lintz KC, Penson RT, Chabner BA, Mack S, Lynch TJ. Schwartz center rounds. A staff dialogue on Phase I trials: psychosocial issues faced by patients, their families, and caregivers. Oncologist **1998**; 3:357–64.~~

11. Byrd DA, Fellows RP, Morgello S, et al; CHARTER Group. Neurocognitive impact of substance use in HIV infection. J Acquir Immune Defic Syndr **2011**; 58:154–62.
12. Le T, Wright EJ, Smith DM, et al. Enhanced CD4+ T-cell recovery with earlier HIV-1 antiretroviral therapy. N Engl J Med **2013**; 368:218–30.
13. World Health Organization W. Composite international diagnostic interview. Version 2.1. Geneva, Switzerland: **1998**.
14. Rippeth JD, Heaton RK, Carey CL, et al; HNRC Group. Methamphetamine dependence increases risk of neuropsychological impairment in HIV infected persons. J Int Neuropsychol Soc **2004**; 10:1–14.
15. Strain MC, Lada SM, Luong T, et al. Highly precise measurement of HIV DNA by droplet digital PCR. PLOS One **2013**; 8:e55943.
16. Shan L, Rabi SA, Laird GM, et al. A novel PCR assay for quantification of HIV-1 RNA. J Virol **2013**; 87:6521–5.
17. Gianella S, Massanella M, Richman DD, et al; California Collaborative Treatment Group 592 Team. Cytomegalovirus replication in semen is associated with higher levels of proviral HIV DNA and CD4+ T cell activation during antiretroviral treatment. J Virol **2014**; 88:7818–27.
18. Christensen-Quick A, Massanella Luna M, Frick A, et al. Subclinical CMV DNA is associated with CD4 T cell activation and impaired CD8 T Cell CD107A expression despite early antiretroviral therapy. J Virol **2019**; accepted for publication.
19. Costantino CM, Gupta [illegible] wdall AW, Dale BM, Devi LA, Chen BK. Cannabinoid receptor 2-mediated attenuation of CXCR4-tropic HIV infection in primary CD4+ T cells. PLOS One **2012**; 7:e33961.
20. Ramirez SH, Reichenbach NL, Fan S, et al. Attenuation of HIV-1 replication in macrophages by cannabinoid receptor 2 agonists. J Leukoc Biol **2013**; 93:801–10.
21. Rizzo MD, Crawford RB, Henriquez JE, et al. HIV-infected cannabis users have lower circulating CD16+ monocytes and IFN-γ-inducible protein 10 levels compared with nonusing HIV patients. AIDS **2018**; 32:419–29.
22. Eisenstein TK, Meissler JJ. Effects of cannabinoids on T-cell function and resistance to infection. J Neuroimmune Pharmacol **2015**; 10:204–16.
23. Roth MD, Tashkin DP, Whittaker KM, Choi R, Baldwin GC. Tetrahydrocannabinol suppresses immune function and enhances HIV replication in the huPBL-SCID mouse. Life Sci **2005**; 77:1711–22.
24. Napper LE, Fisher DG, Johnson ME, Wood MM. The reliability and validity of drug users' self reports of amphetamine use among primarily heroin and cocaine users. Addict Behav **2010**; 35:350–4.