

## **Marijuana use impacts midlife cardiovascular events in HIV-infected men**

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**40-word summary:** Long-term heavy marijuana use was associated with increased cardiovascular events in HIV-infected men ages 40-60 independent of tobacco smoking, viral load, and other risk factors, while there was no significant association with HIV disease markers, progression to AIDS, or mortality.

**Running title:** Marijuana use and cardiovascular events in HIV

## **ABSTRACT**

**Background.** Marijuana use is prevalent among persons infected with HIV, but its long-term effects on HIV disease progression and comorbidities are unknown.

**Methods.** A prospective study of 558 HIV-infected men enrolled in the Multicenter AIDS Cohort Study between 1990-2010: 182 HIV seroconverters and 376 with viral suppression on combination antiretroviral therapy (ART). Associations between heavy marijuana use and HIV disease markers or white blood cell (WBC) count were examined using mixed-effects and linear regression models. Effects of marijuana use on cardiovascular (CV) events and other endpoints were estimated by Kaplan-Meier and logistic regression analyses.

**Results.** The median baseline age of participants was 41, 66% were white, 79% had education > 12 years, and 20% reported heavy marijuana use at  $\geq 50\%$  of biannual visits during follow-up. Long-term heavy marijuana use showed no significant associations with viral load, CD4 counts, AIDS, cancer, or mortality in both cohorts, but was independently associated with increased CV events between ages 40-60 after adjusting for age, tobacco smoking, viral load, and traditional risk factors (odds ratio [OR], 2.5; 95% confidence interval [CI] 1.3, 5.1). Marijuana and tobacco use were each independently associated with higher WBC counts in adjusted models ( $P < 0.01$ ); in turn, the highest quartile of WBC counts ( $\geq 6500$  cells/ $\mu\text{L}$ ) was associated with increased CV events (OR 4.3; 95% CI, 1.5, 12.9).

**Conclusions.** Heavy marijuana use is a risk factor for CV disease in HIV-infected men ages 40-60, independent of tobacco smoking and traditional risk factors.

Key words: HIV, AIDS, marijuana, cardiovascular disease

## INTRODUCTION

Marijuana use for recreational and medicinal purposes is prevalent among individuals infected with human immunodeficiency virus type 1 (HIV): recent estimates in cohorts of HIV-infected (HIV+) men who have sex with men (MSM) range from 24% to 62% [1-3]. Despite this high prevalence, few studies have investigated health effects of long-term heavy marijuana use among HIV+ individuals [4]. Observational studies reported contradictory findings regarding associations between marijuana use and HIV viral load [5-9] or CD4 cell counts [1, 3, 5, 6, 10]. Animal model studies also report inconsistent effects of tetrahydrocannabinol administration on viral replication and disease progression [11, 12]. Thus, the effects of marijuana use on HIV disease and associated comorbidities remain unclear.

Cardiovascular (CV) disease is a comorbidity of particular concern in HIV+ populations [13], given that the estimated incidence is up to two-fold higher than that of age-matched uninfected controls [14, 15]. The high prevalence of tobacco smoking and other traditional CV risk factors, combination antiretroviral therapy (ART)-associated dyslipidemia, and health care disparities explain only a portion of this elevated risk [16-21]. Thus, identifying non-traditional risk factors that explain elevated CV risk in HIV-infected populations is a priority for improving health outcomes [13-15]. Marijuana smoking has been associated with myocardial infarction and other adverse cardiovascular events in a few studies of HIV-uninfected individuals [22-25], yet the evidence regarding its effects on cardiovascular disease remains unclear [4]. To our knowledge, its effects on CV disease in HIV+ individuals have been evaluated in only one study [20].

The aims of this longitudinal study were to examine the effect of long-term heavy marijuana use on HIV disease markers in men with acute and chronic HIV infection, and evaluate differences in cardiovascular events and other health outcomes during long-term follow-up.

## **METHODS**

### **Study Cohort**

The Multicenter AIDS Cohort Study (MACS) is an ongoing prospective study of HIV-infected and -uninfected MSM [26, 27]. Participants undergo standardized interviews detailing behavioral characteristics, medical treatments or conditions, physical examinations, and collection of biological specimens at biannual study visits. All subjects provided written informed consent, and each site's institutional review board approved the study protocols.

This study examined two independent cohorts from the MACS public data release P23 using visits from 1990 through 2010 (**Figure 1A**): HIV-negative (HIV-) participants who seroconverted after MACS enrollment (HIV seroconverters), and HIV+ participants with baseline viral suppression on combination ART (chronic HIV). Inclusion criteria for HIV seroconverters were first HIV antibody positive visit after 1990; baseline was anchored to midpoint between last documented HIV- (1989 or later) and first HIV+ visit. Inclusion criteria for chronic HIV subjects were a baseline visit in 1996 or later with at least 1 year of prior combination ART use, and baseline viral load <400 HIV RNA copies/mL and CD4 cell count  $\geq 300$  cells/ $\mu$ L. HCV antibody positive subjects and those reporting 1 year or more of other heavy drug use (daily nitrite inhalants (poppers) or uppers use, daily or weekly cocaine or heroin use) were excluded from the study.

### **Exposure variables**

Marijuana was the primary exposure of interest. Subjects were classified based on self-reported frequency of marijuana use as heavy users (defined as daily or weekly use at  $\geq 50\%$  of biannual visits), non-users (less than monthly or no use at all visits), and occasional users (remaining subjects). Subjects reporting smoking an average of 0.25 cigarette packs/day or more were classified as moderate/heavy tobacco smokers.

## **Outcome and covariate definitions**

HIV disease markers included plasma viral load, CD4+ and CD8+ T lymphocyte cell counts, and CD4:CD8 ratio [20]. White blood cell (WBC) counts were examined given prior association with cigarette smoking and marijuana use [28, 29]. Ascertainment of health outcomes in the MACS, including AIDS, cancer, and cardiovascular (CV) diagnoses has been described [19, 27, 30, 31]. Study endpoints for each subject were defined as: 1) first incident event after age 30 for AIDS diagnoses, cancer, and mortality; 2) first incident event after age 40 for CV events; 3) loss to follow-up, last visit in 2010, or age 60. These age ranges were selected based on the known association of age  $\geq 40$  with CV risk and limited follow-up or events before age 30 and after age 60. AIDS diagnoses included subjects with CD4 counts  $< 200$  cells/ $\mu\text{L}$  during follow-up. Cancer diagnoses were classified using International Classification of Diseases for Oncology, third edition (ICD-O-3) codes [31]. A CV composite endpoint was comprised of the following International Classification of Diseases (ICD-9) codes [16]: myocardial infarction (410), ischemic heart disease (411, 414, 429.2), angina pectoris (413), cerebrovascular disease (433, 434, 435), atherosclerosis (440), and heart failure (428). Traditional CV risk factors (diabetes mellitus, hypertension, and hypercholesterolemia) were defined based on reported use of medications for these conditions for one year or more during 10 years prior to endpoint, or mean laboratory test values at two most recent visits prior to endpoint within five years: diabetes, hemoglobin A1C (H<sub>g</sub>A<sub>1c</sub>)  $\geq 6.5\%$ ; hypertension, systolic blood pressure  $> 140$  or diastolic blood pressure  $> 90$ ; and hypercholesterolemia, total cholesterol  $> 240$ .

## **Statistical methods**

Mixed-effects models were fit for each time-varying HIV disease marker as a continuous dependent variable. Marijuana use, smoking, age at baseline, race, and education were time-invariant categorical covariates. Duration in study was a time-varying covariate. Additional models for time-varying WBC and neutrophil count were fit for the merged HIV+ cohort with follow-up between ages 40-60 included viral load and total cholesterol as time-varying categorical covariates. All models included a random intercept and slope.

Stratified Kaplan-Meier curves were used to assess differences in mortality and health outcomes in the merged HIV+ cohort by marijuana use or smoking, with age as the time axis and allowing staggered entry. Subjects with events prior to entry age were excluded from analyses; log-rank test was used to evaluate statistical significance of differences between groups.

Case-control data at endpoint was used to assess associations between marijuana use, CV events, and WBC count in the merged HIV+ cohort with follow-up between ages 40-60. Logistic and linear regression models were fit with CV event and time-updated log<sub>2</sub>-transformed WBC count, respectively, as dependent variables. Predictors included time-updated marijuana use, tobacco smoking, age, race, HIV viral load, and traditional risk factors; linear regression models also included total cholesterol. All analyses were performed using R version 3.2.

## RESULTS

### Clinical Characteristics

A total of 558 subjects met eligibility requirements for the HIV seroconverter (n=182) and chronic HIV (n=376) cohorts, respectively; heavy marijuana users comprised approximately 20% of both cohorts (**Figure 1A**). Subjects were predominantly white, with > 12 years of education, median age 40.8 years at baseline, and median duration between last HIV- and first HIV+ visit 1.0 years for HIV seroconverters (**Table 1**). Heavy marijuana use was associated with cigarette smoking in both cohorts ( $P=.035$  and  $< .001$ ). There were no significant associations between heavy marijuana use and other demographic and baseline characteristics, including age, race, education, alcohol use, CES-D depression scores, viral load, and CD4 count. Self-reported ART use, adherence, and regimens were similar between marijuana users and non-users (**Table 1** and **Supplementary Table 1**). The patterns of marijuana and tobacco use both remained generally consistent over time within individual subjects (**Figure 1B**). An all-by-all comparison of baseline and demographic characteristics stratified by marijuana use (**Supplementary Figure 1**) indicated more cigarette smoking among heavy marijuana or alcohol users,

yet no other associations were observed. CES-D depression scores were lower with increasing age, yet these trends did not vary by marijuana use.

### **Marijuana use and HIV-related outcomes**

Marijuana use showed no significant association with mean trajectories of HIV disease markers, including no dose-response relationship when stratifying by daily vs. weekly use (**Figure 2A**). In mixed-effects models adjusted for age, smoking, race, and education, daily marijuana use was associated with lower CD4:CD8 ratio at baseline in the chronic HIV cohort ( $P = .009$ ; **Supplementary Table 2**), but this effect was not reproduced in HIV seroconverters ( $P = .457$ ). There was no significant association between daily or weekly use and other HIV disease markers.

Next, we evaluated associations between marijuana use and HIV-related outcomes during long-term follow-up. There were no significant associations between heavy marijuana use and progression to AIDS, cancer diagnoses, or mortality in either cohort (**Table 1**). AIDS-related causes of death comprised 68.1% of all mortalities; the AIDS-defining malignancies (ADMs) Kaposi sarcoma (32.8%) and Non-Hodgkin lymphoma (20.7%) comprised the majority of cancer diagnoses. By contrast, a higher proportion of heavy marijuana users had cardiovascular events between ages 40-60 compared with non- and occasional users in both cohorts ( $P = .311$  and  $P < 0.001$ ; **Table 1**). Consistent with these findings, Kaplan-Meier curves for the merged HIV+ cohort between ages 40-60 showed elevated rates of incident cardiovascular events among heavy marijuana users ( $P < 0.001$ ) (**Figure 2B**), while there was no significant difference in rates of AIDS diagnoses, cancer, or mortality (**Figure 2B**).

### **Marijuana use, increased WBC count, and cardiovascular events**

Previous studies demonstrated significant associations between tobacco smoking, elevated WBC count, and coronary heart disease [28, 29, 32], but these associations have not been explored in the context of marijuana use during HIV infection. We therefore evaluated mean trajectories of WBC counts stratified by marijuana and tobacco use over the first 6 years of follow-up in both cohorts. Mean WBC

counts were elevated in cigarette smokers vs. non-smokers; the highest mean WBC counts were observed in marijuana/tobacco co-users in both cohorts (**Supplementary Figure 2**). In mixed-effects models adjusted for age, race, education, viral load, and total cholesterol, cigarette smoking was associated with higher WBC count (estimate =.153;  $P < 0.0001$ ), and heavy marijuana use was associated with increasing WBC count in the merged HIV+ cohort between ages 40-60 (marijuana \* age interaction: estimate =.020;  $P < 0.001$ ; **Figure 3A** and **Supplementary Table 3**). Comparable estimates were observed for neutrophil (**Figure 3A** and **Supplementary Table 3**), but not monocyte or lymphocyte counts, in models adjusted for the same covariates. Thus, neutrophils are the main leukocyte population explaining higher WBC counts. Using case-control data at endpoint, heavy marijuana use (estimate =1.11;  $P=0.01$ ) and cigarette smoking (estimate =1.15;  $P < 0.0001$ ) were each independently associated with increased WBC count in linear regression models (**Table 2**). The interaction between marijuana use and smoking was not significant in mixed-effects and linear regression models, providing further evidence for independent effects of these factors.

Given the high prevalence of cigarette smoking among marijuana users, we evaluated associations between marijuana use, cigarette smoking, and CV event rates in the merged HIV+ cohort between ages 40-60. CV events occurred in 19.7% of heavy marijuana users, compared with 8.7% of occasional and non-users ( $P = .012$ ), while demographics and HIV disease characteristics were similar at endpoint (**Supplementary Table 4**). Kaplan-Meier curves showed increased CV and non-AIDS defining malignancy rates among cigarette smokers ( $P < 0.001$  and  $P < 0.05$ ; **Figure 3B**, left panel, and **Supplementary Figure 3**) while there was no significant effect on progression to AIDS, mortality, or ADM diagnoses (**Supplementary Figure 3**). CV event rates were also increased among marijuana users who did not smoke cigarettes compared to non-smokers, and highest among marijuana/tobacco co-users ( $P < 0.01$  and  $P < 0.001$ ; **Figure 3B**, right panel). Among cigarette smokers, average cigarette packs/day was similar between marijuana users vs. non-users (mean 0.88 vs. 0.96 packs/day, respectively;  $P > 0.3$ ,  $t$ -test), indicating Kaplan-Meier estimates were not influenced by more cigarette smoking among marijuana/tobacco co-users. In logistic regression models adjusted for age, smoking, viral load, and 2-3 vs.



0-1 traditional CV risk factors, time-updated heavy marijuana use was associated with 2.5-fold increased odds of CV events (95% CI, 1.2-5.3;  $P=0.016$ ). Effects of marijuana/tobacco co-use on increased odds of CV events were additive in this model (odds ratio 4.8 [95% CI, 1.8-12.7];  $P<0.01$ ) (**Table 3**). The association between heavy marijuana use and CV risk was not modified by detectable HIV viral load (estimate for interaction=0.740;  $P=0.358$ ). Other risk factors were not significantly associated with increased odds of CV events, except for diabetes and elevated platelet count (**Supplementary Table 5**), which were not significant in adjusted models. Similar to results in logistic regression models, time-varying heavy marijuana use was associated with increased incident CV events in Cox proportional hazard models adjusted for time-varying cigarette smoking, viral load, and traditional risk factors (hazard ratio 2.16; 95% CI, 1.04-4.51;  $p=0.039$ )

In a separate multivariate logistic regression model, higher WBC count was associated with increased odds of CV events (**Table 3** and **Figure 3C**). Time-updated WBC count in the highest quartile ( $\geq 6500$  cells/ $\mu\text{L}$ ) was associated with 4.3-fold increased odds of a CV event compared with the lowest quartile ( $< 4500$  cells/ $\mu\text{L}$ ) (95% CI, 1.45-12.89;  $P = 0.009$ ). When modeled as a continuous covariate, each 1000 cells/ $\mu\text{L}$  WBC increment was associated with 25% increased odds of a CV event independent of marijuana use, cigarette smoking, age, viral load, and traditional risk factors (95% CI, 1.05-1.49;  $P = 0.012$ ); including WBC count as a covariate significantly improved model fit ( $P = 0.013$ , likelihood-ratio test).

## **DISCUSSION**

In this longitudinal nested study of HIV+ men on ART, heavy marijuana use was associated with increased rates of CV events in men ages 40-60 independent of cigarette smoking and other known risk factors. Heavy marijuana use was also associated with elevated WBC count within the normal physiological range, an indicator of systemic inflammation that has been previously associated with increased cardiovascular disease in the general population [32, 33]. Furthermore, this elevation in WBC counts was explained by increased neutrophil counts. Elevated rates of CV events were observed among

HIV+ heavy marijuana users beginning at relatively young ages (< 50 years), and were further increased in marijuana/tobacco co-users compared to users of only one substance. Further studies are needed to determine whether the effect of long-term heavy marijuana use on CV events is related to pro-inflammatory effects of non-tobacco smoke exposure, non-inflammatory effects (e.g., transient tachycardia and/or hypertension, elevated carboxyhemoglobin, hemodynamic effects, effects on platelets) [22-24], or a combination of these effects.

In healthy individuals, relatively few physical health outcomes have been clearly linked to marijuana use [4, 34-36]. However, adverse cardiovascular and cerebrovascular effects of recent marijuana use have been reported in some studies of the general population [4, 22-25]. The association between long-term heavy marijuana use and increased CV event rates has not been previously reported in HIV+ individuals. A study examining associations between substance use and coronary plaque measures in the MACS reported no strong effect of marijuana [20]. However, the study design was different from ours in several respects including the narrower definition of CV disease, and less stringent criteria for defining marijuana exposure. Furthermore, we excluded HCV+ subjects and other heavy illicit drug users from our study cohort. The association between marijuana use and CV events we detected was weaker than that of other known CV risk factors including age, cigarette smoking, and viral load > 400 copies/mL [14, 15, 17-19, 37]. In contrast to some prior studies [14, 15, 18, 19], we found weak or no significant associations between CV events and hypertension or cholesterol risk factors, which may reflect the younger age and high smoking prevalence in our study cohort.

The association we found between heavy marijuana use and elevated WBC has been reported in healthy young men [28], but to our knowledge has not previously been reported in HIV+ individuals. In line with previous studies of HIV-uninfected populations [28, 29, 32, 33], cigarette smoking was associated with elevated WBC count in our cohort of mostly middle-aged HIV+ men. The mechanism by which marijuana exposure increases WBC count remains poorly defined, but may be related to toxic or pro-inflammatory effects of smoke combustion products [36, 38].

The lack of association between marijuana use and HIV disease markers, progression to AIDS, mortality, or cancer reported here is consistent with prior observational [1, 3, 6, 8, 39] and case-control [5] studies. However, other studies reported associations between marijuana use and higher CD4 counts and/or lower viral load [9], higher CD4 counts [10], and slightly lower viral loads during the first year post-seroconversion [7]. While differences between these studies likely reflect differences in study populations, selection criteria, adjustment for confounders, and length of follow-up, the results reported here are consistent with other studies in MSM cohorts [1, 3, 6].

Strengths of this study include more selective inclusion and exclusion criteria compared to most previous studies: the chronic HIV cohort had controlled disease characteristics at baseline (CD4 count > 300 cells/ $\mu$ L, viral load  $\leq$  400 copies/mL), and subjects with heavy use of other illicit drugs and HCV co-infection were excluded. These factors have substantial effects on HIV disease markers and other outcomes, and therefore could confound efforts to detect effects of marijuana exposure. A further strength was the classification of long-term heavy marijuana use over years of follow-up, and strict criteria for classifying heavy and non-using subjects over this time span.

Limitations of this study include those inherent to studies of longitudinal cohorts, including the possibility that results may be specific to the MSM population recruited for the MACS, and non-random ascertainment and dropout biases. Available measures of marijuana exposure were self-reported frequency of use with no information regarding route of administration, source, quantity and potency. Based on available knowledge, the predominant mode of exposure was likely via marijuana smoking. These concerns are mitigated in part by the evaluation of two independent cohorts selected from differing calendar periods, which nonetheless show a consistent lack of association between marijuana use and HIV disease markers. Furthermore, we assessed cardiovascular events using time-updated data for marijuana and tobacco exposure during 10 years prior to endpoint.

In summary, we found no significant association between marijuana use and HIV disease progression or mortality. However, long-term heavy marijuana use was associated with increased midlife CV events in HIV+ men, independent of cigarette smoking and traditional risk factors. Furthermore, the

increase in CV events was additive in marijuana/tobacco co-users. These findings suggest that clinicians should consider heavy marijuana smoking as a modifiable risk factor when optimizing preventive care, particularly for individuals already at increased risk. In recent years, the prevalence of cigarette smoking has declined, while the prevalence of heavy marijuana use has remained high or increased in HIV-infected populations [3, 10, 40]. Given that traditional factors are not sufficient to explain elevated cardiovascular risk among HIV+ individuals [13, 15, 22], the identification of heavy marijuana smoking as a non-traditional risk factor helps to explain a portion of this elevated risk and warrants further investigation in other HIV-infected and uninfected populations.

**Funding:** This work was supported by NIH grants to D.G. (DP1 DA028994 and R01 DA30985). A.D. was supported in part by NIH T32-AI007386. S.S.M was supported in part by Harvard Catalyst Master's Program in Clinical and Translational Investigation funded by the NIH Clinical and Translational Science Award Program (1UL1-TR001102), Biostatistical consultation was provided by the Harvard Center for AIDS Research (P30 AI060354). The MACS is funded by the National Institute of Allergy and Infectious Diseases (NIAID) [U01-AI35039, U01-AI35040; U01-AI35041; U01-AI35042; and UM1-AI35043], with additional co-funding from the National Cancer Institute (NCI), National Institute on Drug Abuse (NIDA), and National Institute of Mental Health (NIMH) at the National Institutes of Health (NIH). MACS data collection is also supported by UL1-TR000424 (JHU CTSA).

**Conflicts of Interest:** The authors report no conflicts of interest.

**Acknowledgments:** The data for this manuscript were obtained by the Multi-center AIDS Cohort Study (MACS) with centers at Baltimore (U01- AI35042): The Johns Hopkins University Bloomberg School of Public Health: Joseph B. Margolick (principal investigator [PI]), Jay Bream, Todd Brown, Barbara Crain, Adrian Dobs, Richard Elion, Richard Elion, Michelle Estrella, Lisette Johnson-Hill, Sean Leng, Anne Monroe, Cynthia Munro, Michael W. Plankey, Wendy Post, Ned Sacktor, Jennifer Schrack, Chloe Thio; Chicago (U01-AI35039): Feinberg School of Medicine, North-western University, and Cook County Bureau of Health Services: Steven M. Wolinsky (PI), John P. Phair, Sheila Badri, Dana Gabuzda, David Ostrow, Frank J. Palella, Jr., Sudhir Penugonda, Susheel Reddy, Matthew Stephens, Linda Teplin; Los Angeles (U01-AI35040): University of California, UCLA Schools of Public Health and Medicine: Roger Detels (PI), Otoniel Martínez-Maza (Co-PI), Aaron Aronow, Peter Anton, Robert Bolan, Elizabeth Breen, Anthony Butch, Shehnaz Hussain, Beth Jamieson, Eric N. Miller, John Oishi, Harry Vinters, Dorothy Wiley, Mallory Witt, Otto Yang, Stephen Young, Zuo Feng Zhang; Pittsburgh (U01-AI35041): University of Pittsburgh, Graduate School of Public Health: Charles R. Rinaldo (PI), Lawrence A. Kingsley (Co-PI), James T. Becker, Phalguni Gupta, Kenneth Ho, Susan Koletar, Jeremy J. Martinson, John W. Mellors, Anthony J. Silvestre, Ronald D. Stall; Data Coordinating Center (UM1- AI35043): The Johns Hopkins University Bloomberg School of Public Health: Lisa P. Jacobson (PI), Gypsyamber D'Souza (Co-PI), Alison, Abraham, Keri Althoff, Jennifer Deal, Priya Duggal, Sabina Haberlen, Alvaro Muoz, Derek Ng, Janet Schollenberger, Eric C. Seaberg, Sol Su, Pamela Surkan.

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## FIGURE LEGENDS

### **Figure 1. Cohort selection and longitudinal patterns of marijuana and smoking exposures.** (A)

Cohort selection flowchart. (B) Lasagna plots illustrating self-reported patterns of marijuana use (top panel) and cigarette smoking (bottom panel) among subjects in the chronic HIV cohort. Each row depicts visit-level exposure data for an individual subject in the study interval used for group classification.

Abbreviations: HCV, hepatitis C virus; HIV, Human Immunodeficiency Virus; cART, combination antiretroviral therapy.

### **Figure 2. Association between marijuana use, HIV disease marker trajectories, and health**

**outcomes.** (A) Mean values of HIV disease markers by marijuana use. (B) Kaplan-Meier curves of AIDS diagnoses, mortality, cancer diagnoses, and cardiovascular events for the merged HIV+ cohort stratified by marijuana use. *P* values denote pair-wise log-rank test vs. control group. Abbreviations: HIV, Human Immunodeficiency Virus.

### **Figure 3. Heavy marijuana use and WBC count are independently associated with increased odds of cardiovascular events.** (A) Estimated mean trajectories for WBC (top panels) and neutrophil counts

(bottom panels) by cigarette smoking (left panel) or by marijuana use and cigarette smoking (right panel), for the merged HIV+ cohort with follow-up between ages 40-60 from mixed effects models adjusted for viral load, race, age at entry, education, and total cholesterol (Supplementary Table 3). (B) Kaplan-Meier

curves of first cardiovascular event stratified by smoking only (left panel) or by marijuana use and cigarette smoking (right panel), for the merged HIV+ cohort with follow-up between ages 40-60. Mj+ denotes heavy marijuana users, Mj-, occasional or non-users, Sm+, moderate or heavy smokers ( $\geq 0.25$

packs/day, average), Sm-, light or non-smokers, *P* values, pair-wise log-rank test vs. control group. (C) Forest plot showing estimated odds of adverse cardiovascular events for the indicated covariates from logistic regression Model 2 (Table 3) with WBC values by quartiles. Abbreviations: CI, confidence

interval; HIV, Human Immunodeficiency Virus; Mj, marijuana; Sm, smoking; WBC, white blood cells.

**Table 1. Baseline and Updated Cohort Characteristics.**

	HIV Seroconverters				Chronic HIV			
	Non-users (n=102)	Heavy Marijuana (n=36)	Occasional Marijuana (n=44)	P Value <sup>a</sup>	Non-users (n=244)	Heavy Marijuana (n=65)	Occasional Marijuana (n=67)	P Value <sup>a</sup>
Length of follow-up, mean (SD), years	7.7 (2.6)	7.5 (2.3)	7.9 (2.1)	0.715	5.5 (2.1)	5.1 (2.1)	5.82 (1.9)	0.157
Seroconversion window, median (IQR), years <sup>b</sup>	1.0 (0.0-1.0)	1.0 (0.0-1.0)	1.0 (0.0-1.0)	0.500	--	--	--	--
Age, median (IQR), years	40.5 (33-47)	33.3 (28-42)	37.8 (32-41)	0.015	42 (37-46)	41 (35-49)	41.5 (36-47)	0.771
Racial/ethnic group				0.161				0.411
White	83 (81.4)	23 (63.9)	36 (81.8)		146 (59.8)	33 (50.8)	42 (62.7)	
Black	12 (11.8)	9 (25.0)	7 (15.9)		55 (22.5)	21 (32.3)	17 (25.4)	
Other	7 (6.9)	4 (11.1)	1 (2.3)		43 (17.6)	11 (16.9)	8 (11.9)	
Education > 12 years	86 (84.3)	32 (88.9)	38 (86.4)	0.789	187 (76.6)	49 (75.4)	50 (74.6)	0.934
Heavy or binge alcohol use <sup>c</sup>	26 (25.5)	13 (36.1)	12 (27.3)	0.471	54 (22.1)	17 (26.2)	22 (32.8)	0.19
Moderate/heavy cigarette smoking <sup>d</sup>	27 (26.5)	18 (50.0)	15 (34.1)	0.035	60 (24.6)	35 (53.8)	27 (40.3)	< 0.001
CES-D score, median (IQR)	9 (4-16)	9 (3-16)	13 (2-20)	0.981	8 (3-16)	7.5 (5-16)	13 (7-20)	0.023
CES-D score ≥ 16	26 (26.3)	11 (30.6)	14 (34.1)	0.628	63 (26.2)	18 (28.1)	26 (40.6)	0.078
CD4 count (cells/μL), median (IQR)	629 (455-855)	667 (507-834)	680 (544-828)	0.552	559 (445-741)	548 (432-620)	553 (435-790)	0.312
HIV viral load ≥ 400 copies/mL	78 (89.7)	30 (93.8)	30 (88.2)	0.728	0 (0.0)	0 (0.0)	0 (0.0)	--
Any ART use in study period <sup>e</sup>	82 (80.4)	29 (80.6)	33 (75)	0.742	244 (100)	65 (100)	67 (100)	--
ART adherence at ≥ 95% of visits <sup>e</sup>	75 (91.5)	27 (93.1)	25 (75.8)	0.04	167 (68.4)	43 (66.15)	41 (61.2)	0.533
AIDS diagnoses <sup>ff</sup>	32 (31.4)	8 (22.2)	17 (38.6)	0.344	16 (6.6)	6 (9.2)	4 (6.0)	0.690
Mortalities <sup>ff</sup>	20 (19.6)	6 (16.6)	10 (22.7)	0.794	9 (3.7)	3 (4.6)	3 (4.5)	0.794
One or more cancer diagnoses <sup>ff</sup>	14 (13.7)	3 (8.3)	7 (15.9)	0.634	13 (5.3)	4 (6.0)	3 (4.5)	0.891
One or more cardiovascular events <sup>ff</sup>	8 (11.1)	3 (14.3)	1 (2.9)	0.311	13 (6.7)	14 (29.2)	5 (10.9)	< 0.001

Data are n (%) at baseline visit (index date of the analysis) unless otherwise specified. Study period for seroconverter and chronic HIV cohorts: -4 years to + 7 years relative to baseline and baseline to +7 years, respectively.

Abbreviations: SD, standard deviation; IQR, interquartile range; ART, antiretroviral therapy; cART, combination antiretroviral therapy; CES-D, Center for Epidemiological Studies Depression Scale; HIV, human immunodeficiency virus.

a - Chi-Squared or Fisher's Exact test, ANOVA, or Kruskal-Wallis Rank Sum Test, for categorical, normally-distributed continuous, and non-normally distributed continuous variables, respectively.

b - Duration between last HIV- and first HIV+ visits.

c - Heaviest reported usage at ≥ 2 visits in study period: Heavy, > 14 drinks/week; Binge, 5 or more drinks/occasion at least monthly.

d - Average ≥ 0.25 packs/day during study period.

e - Self-reported current ART medication use.

f - Incident events during post-baseline follow-up, restricted to ages 40 - 60 for cardiovascular events.

**Table 2. Factors Associated with Increased White Blood Cell Count.**

Predictor	Unadjusted Models		Adjusted Model	
	Estimate (95% CI)	P Value	Estimate (95% CI)	P Value
Heavy marijuana use <sup>a</sup>	1.09 (1.01, 1.18)	0.023	1.10 (1.02, 1.19)	0.012
Moderate/heavy cigarette smoking <sup>b</sup>	1.12 (1.05, 1.19)	< 0.0001	1.15 (1.08, 1.22)	< 0.0001
Age 45-60 vs. 40-44	1.05 (0.98, 1.13)	0.152	1.05 (0.98, 1.13)	0.172
Race				
White	1.00 (Ref.)	--	1.00 (Ref.)	--
Black	0.86 (0.80, 0.92)	< 0.0001	0.87 (0.81, 0.94)	< 0.001
Other	0.96 (0.88, 1.05)	0.331	0.97 (0.89, 1.05)	0.425
Diabetes risk factor <sup>c</sup>	1.03 (0.91, 1.16)	0.677	--	--
Hypertension risk factor <sup>d</sup>	1.04 (0.98, 1.11)	0.179	--	--
Total cholesterol (mg/dL)				
< 180	1.00 (Ref.)	--	1.00 (Ref.)	--
180-229	1.13 (1.06, 1.21)	< 0.001	1.11 (1.04, 1.18)	0.002
≥ 230	1.11 (1.01, 1.21)	0.023	1.10 (1.01, 1.20)	0.022
HIV viral load ≥ 400 copies/mL	0.88 (0.82, 0.96)	0.002	0.90 (0.83, 0.97)	0.006

Models were fit using linear regression with time-updated log<sub>2</sub>-transformed WBC count (cells/μL) as the dependent variable, from the merged HIV+ cohort at first incident cardiovascular event after age 40, loss to follow-up, last visit in 2010, or age 60. All laboratory test values were the mean of two most recent visits prior to endpoint.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; WBC, white blood cell.

a - Daily or Weekly marijuana use at ≥ 50% of visits in period 10 years prior to endpoint.

b - Average ≥ 0.25 packs/day in period 10 years prior to endpoint.

c - HgA1c ≥ 6.5%, or use of diabetes medications for at least one year in the 10 years prior to endpoint.

d - Systolic blood pressure > 140 or diastolic blood pressure > 90, or use of antihypertensive medications for at least one year in the 10 years prior to endpoint.

**Table 3. Association between Marijuana Use, White Blood Cell Count, and Cardiovascular Events.**

Predictor	Cardiovascular Events		Unadjusted Models		Adjusted Model 1		Adjusted Model 2	
	Controls (n=370) n (%)	Cases (n=44) n (%)	OR (95% CI)	<i>P</i> Value	OR (95% CI)	<i>P</i> Value	OR (95% CI)	<i>P</i> Value
Marijuana use								
None/occasional	313 (84.6)	30 (68.2)	1.00 (Ref.)	--	1.00 (Ref.)	--	1.00 (Ref.)	--
Heavy <sup>a</sup>	57 (15.4)	14 (31.8)	2.56 (1.28, 5.13)	0.008	2.51 (1.18, 5.31)	0.016	2.31 (1.08, 4.93)	0.031
Cigarette smoking								
None/light	261 (70.5)	21 (47.7)	1.00 (Ref.)	--	1.00 (Ref.)	--	1.00 (Ref.)	--
Moderate/heavy <sup>b</sup>	109 (29.5)	23 (52.3)	2.62 (1.39, 4.94)	0.007	2.55 (1.29, 5.04)	0.007	2.06 (1.01, 4.17)	0.045
Age								
40-44	86 (23.2)	5 (11.4)	1.00 (Ref.)	--	1.00 (Ref.)	--	1.00 (Ref.)	--
45-60	284 (76.8)	39 (88.6)	2.36 (0.9, 6.18)	0.080	3.35 (1.18, 9.57)	0.024	3.10 (1.06, 9.05)	0.039
Traditional risk factors <sup>c</sup>								
0-1	295 (79.7)	29 (65.9)	1.00 (Ref.)	--	1.00 (Ref.)	--	1.00 (Ref.)	--
2-3	75 (20.3)	15 (34.1)	2.03 (1.04, 3.99)	0.039	2.59 (1.23, 5.43)	0.012	2.44 (1.15, 5.18)	0.020
HIV viral load (copies/mL)								
< 400	311 (84.1)	27 (61.4)	1.00 (Ref.)	--	1.00 (Ref.)	--	1.00 (Ref.)	--
≥ 400	59 (16.0)	17 (38.6)	3.32 (1.70, 6.47)	< 0.001	3.73 (1.80, 7.72)	< 0.001	4.83 (2.23, 10.48)	< 0.001
WBC count - quartiles (cells/μL)								
< 4500	93 (25.1)	5 (11.4)	1.00 (Ref.)	--	-- --	--	1.00 (Ref.)	--
4500-5499	108 (29.2)	9 (20.4)	1.55 (0.5, 4.79)	0.445	-- --	--	1.69 (0.51, 5.66)	0.390
5500-6499	73 (19.7)	8 (18.2)	2.04 (0.64, 6.49)	0.228	-- --	--	2.67 (0.77, 9.20)	0.120
≥ 6500	96 (26.0)	22 (50.0)	4.26 (1.55, 11.73)	0.005	-- --	--	4.32 (1.45, 12.89)	0.009

Time-updated values from the merged HIV+ cohort at first incident cardiovascular event after age 40, loss to follow-up, last visit in 2010, or age 60. Models were fit using logistic regression.

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; OR, odds ratio; WBC, white blood cell.

- a - Daily or weekly marijuana use at  $\geq 50\%$  of visits in period 10 years prior to endpoint.
- b - Average  $\geq 0.25$  packs/day in period 10 years prior to endpoint.
- c - Number of cholesterol, diabetes, or hypertensive risk factors (see Materials and Methods).

Figure 1.

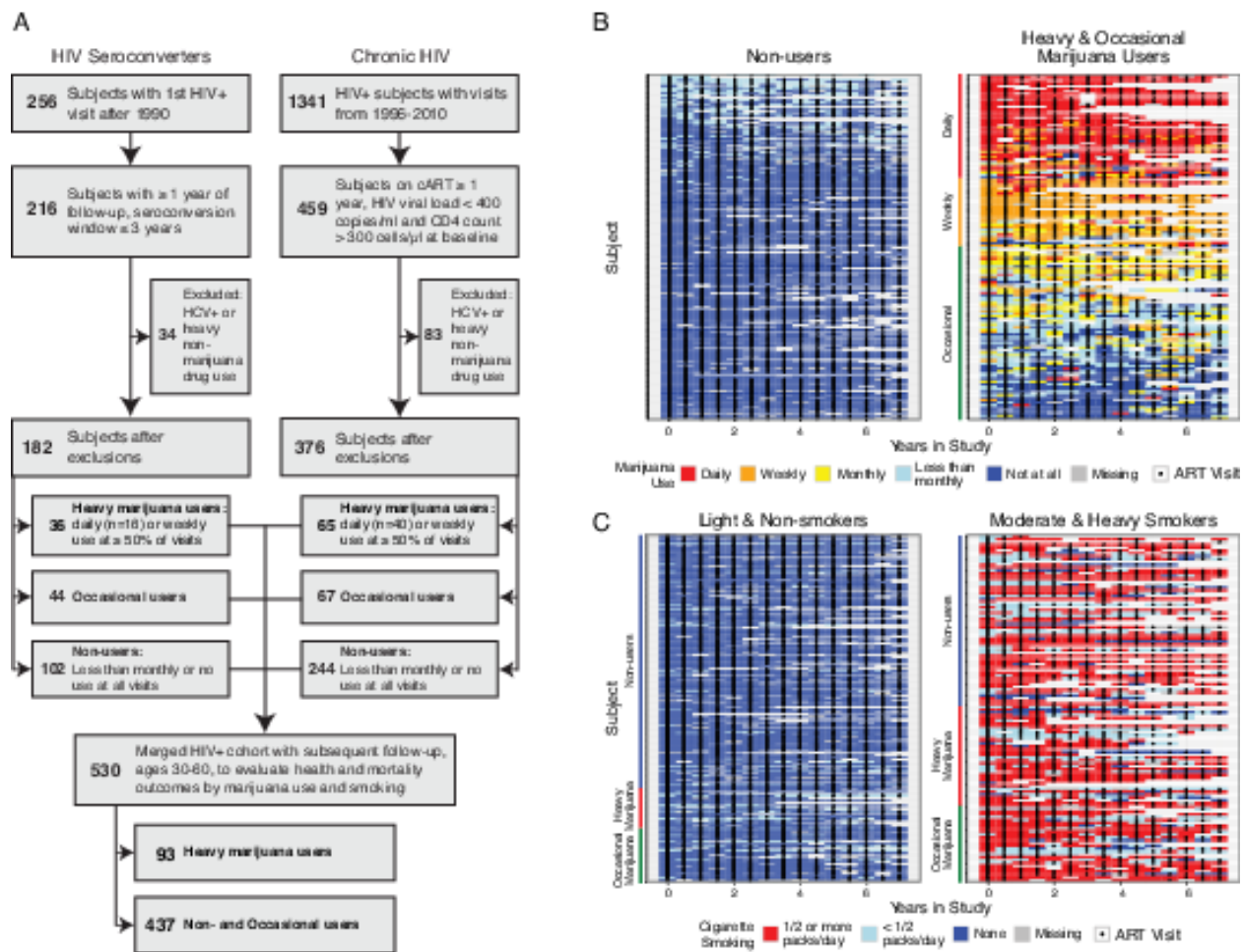
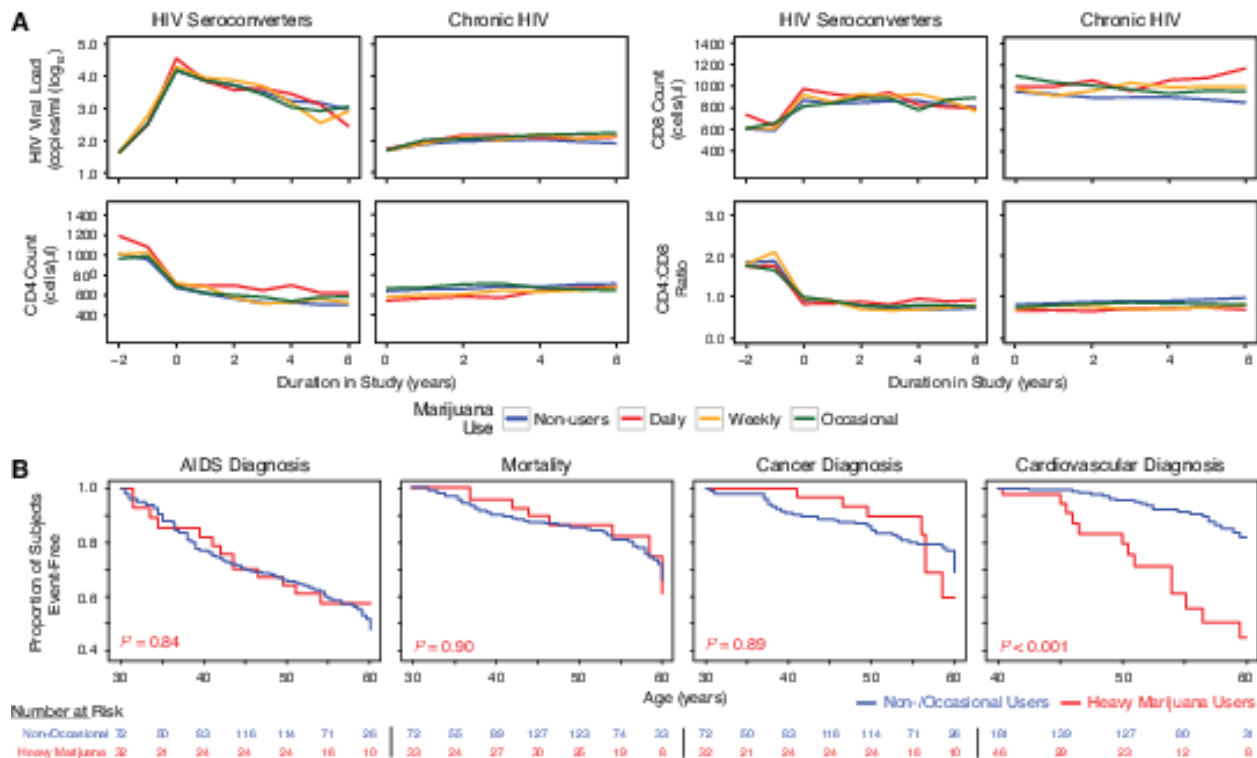


Figure 2.



**Figure 3.**

