Non-structured treatment interruptions are associated with higher HIV reservoir size measured by intact proviral DNA assay in people who inject drugs

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Summary: We quantitated the latent reservoir for HIV- in persons who inject drugs. Reservoir size was not dramatically different than in other cohorts. A history of frequent treatment interruptions, but not active drug use, was associated with larger reservoir size.

Footnotes

Conflict of Interests Statement. Aspects of the IPDA are the subject of patent application PCT/US16/28822 filed by JHU with RFS as an inventor and licensed to AccelevirDx . RFS holds no equity interest in AccelevirDx. RFS consults for Merck and Abbvie on HIV cure related issues. KDR is an employee of AccelevirDx. GML is an employee of and equity holder in AccelevirDx. All other authors have no conflicts of interest to declare.

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Abstract

The latent reservoir for HIV-1 in $CD4^+$ T cells is a major barrier to cure. HIV-1-infected persons who inject drugs (PWID) often struggle to maintain suppression of viremia and experience non-structured treatment interruptions (NTIs). The effects of injecting drugs or NTIs on the reservoir are unclear. Using the intact proviral DNA assay (IPDA), we found no apparent effect of heroin or cocaine use on reservoir size. However, we found significantly larger reservoirs in those with frequent NTIs or a shorter interval from last detectable HIV RNA measurement. These results have important implications for inclusion of PWID in HIV-1 cure studies.

Keywords: HIV-1 latent reservoir; HIV-1 viral suppression; people who inject drugs (PWID); anti-retroviral treatment (ART): non-structured treatment interruption (NTI)

Background

The latent reservoir for HIV-1 in resting $CD4^+$ T cells is a major barrier to cure [1-8]. It is comprised of latently infected resting $CD4^+$ T cells carrying integrated, replication-competent viral genomes that are not expressed unless cells are activated. The reservoir has not been extensively studied in persons who inject drugs (PWID). In mixed cohorts with various modes of acquisition, the frequency of latently infected cells as detected by the quantitative viral outgrowth assay (QVOA) varies over a 2 log range centered around 1 infectious unit per million (IUPM) $CD4^+$ T cells [3, 5, 7-9]. The size of the reservoir in PWID likely falls in this range, but whether there are major differences relative to other populations of infected individuals is unclear. As cure interventions move into clinical trials [10-12], it is important to understand how injection drug use affects the latent reservoir and the potential for cure.

Drugs such as heroin and cocaine may affect the reservoir directly and indirectly. Increased intensity of active injection drug use is directly associated with markers of inflammation, independent of the effects of HIV-1 or HCV infections [13], and inflammatory mediators could affect both the establishment and maintenance of the reservoir [14]. In addition, a major effect of drug use on the latent reservoir may operate indirectly through drug-use associated problems with adherence to ART. Many PWID have a history of non-structured treatment interruptions (NTI), with one-third having multiple interruptions, cycling on and off ART almost annually [15].

An additional problem is that the reservoir cannot be accurately measured unless active viral replication is suppressed for >6 months. Following ART initiation, the frequency of resting $CD4^+$ T cells harboring inducible, replication-competent HIV-1 genomes as detected by the QVOA falls down to a steady state level of ~1 IUPM over 6-9 months [16]. Prior to that, the small pool of cells that constitute the stable latent reservoir is masked by a much larger pool of recently infected cells that carry replication–competent HIV-1 genomes but that do not

enter the stable reservoir. For this reason, the reservoir is generally measured only after >6 months of suppressive ART. This complicates reservoir analysis in individuals with frequent NTIs.

The QVOA is the gold standard assay for the latent reservoir, but it requires large blood volumes which can be problematic in PWID. In addition, it requires an *in vitro* stimulation to reverse latency and allow viral outgrowth [3, 5, 7-9].[3, 4] Recent studies have shown that not all replication-competent proviruses are induced by a single round of *in vitro* T cell activation [17-19]. Thus the QVOA may underestimate reservoir size. Simpler approaches to reservoir measurement involving subgenomic PCR assays to detect proviral DNA are also problematic because a large fraction of proviruses are defective as result of large deletions and/or APOBEC3G-mediated hypermutation [9, 17, 20-24]. Hence standard DNA PCR assays dramatically overestimate reservoir size by including defective proviruses that cannot contribute to viral rebound. The recently described intact proviral DNA assay (IPDA) separately quantitates intact and defective proviruses [21, 25, 26], and has been used to show that these two populations of proviruses have different *in vivo* decay rates [21, 27, 28]. The IPDA may therefore provide the best way to assess the reservoir in PWID.

To evaluate the effects of heroin and cocaine use on the latent reservoir, we used the IPDA to analyze samples from the ALIVE cohort of PWID [29]. The large size and high retention rates of this cohort coupled with an extensive longitudinal database linked to a biorepository allowed us to identify participants who had suppression of viremia on ART to below the limit of detection in 2 consecutive study visits 6 months apart [30]. We analyzed the size of the reservoir in 120 samples from 99 study participants who met these criteria and had four different patterns of drug use reported over the prior 6 months: no current drug use, heroin use, cocaine use, or use of both drugs. The results provide new insights into factors affecting reservoir size and the potential for cure in PWID.

Methods

Study participants and samples. ALIVE participants have study visits every 6 months for collection of drug use data and cryopreservation of PBMC samples. Because stable suppression of viremia for ≥ 6 months is required for accurate reservoir analysis [16], we identified participants who had plasma HIV-1 RNA levels below the limit of detection on two consecutive study visits and analyzed samples from the second of these visits (Fig. 1A). From participants meeting these criteria, we selected four balanced sets of 30 samples based on self-reported patterns of drug use at the sampled visit: no active illicit drug use, active heroin use only, active cocaine use only, and active use of both heroin and cocaine. For some participants, these criteria were met for more than one pair of study visits. An attempt was made to balance each group with respect to one additional parameter, the number of previous non-structured treatment interruptions (NTIs) as defined by switches between detectable and undetectable HIV-1 RNA values on consecutive study visits (Fig. 1A). Within each drug use group, participants with more frequent NTI's (determined to be viral switch at ≥18% of study visits) were selected in a 2:1 ratio to those with less frequent switches. The mean time participants were followed within the ALIVE cohort before sampling was 10.6 years. Some of earlier samples used in this study were obtained when clinical assays for HIV-1 plasma RNA had a limit of detection of 400 copies/ml. For consistency, we have used this value in the analysis of NTIs. This choice lessens the chance of misclassifying clinically insignificant blips as an indication of treatment interruption [31]. Study participants were mostly positive for hepatitis C infection by serology (93.5%) but negative for hepatitis B surface antigen (98.1%). The Johns Hopkins Bloomberg School of Public Health IRB reviewed and provided continuous approval for study procedures, and all participants provided written informed consent.

IPDA. De-identified, previously collected samples blinded as to participant characteristics and drug use history were provided to Accelevir Diagnostics as cryopreserved PBMCs for IPDA analysis. In-depth descriptions of the IPDA rationale and procedure have been previously published [21, 25, 27].

Results

Study participants. We analyzed a total of 120 samples from 99 unique individuals (Table 1). The study population was predominantly male (72.6%) and African American (95.6%) with a median age at sampling of 52.8 years (IQR = 49.1-56.8). Most participants were unemployed (91.0%), but few had experienced incarceration (7.0%) or homelessness (5.5%) during the previous 6 months. The study population had a median CD4 count of 448 cell/µl at the time of sampling and a median CD4 nadir of 173 cells/µl. Relative to the sample time point, the median time since first ART was 7.8 years. However, median time since the last detectable HIV-1 RNA was estimated at 3.4 years, and 67.0% of the cohort had HIV-1 RNA switches at ≥18% of study visits. These parameters were well balanced between the 4 drug use groups (Table 1).

IPDA analysis. The IPDA directly quantities intact proviruses, defined here as proviruses lacking the two most common types of fatal defects: large deletions and APOBEC3G-mediated hypermutation [21]. DNA from purified $CD4^+$ T cells is dispersed into thousands of nanoliter-sized droplets so that individual proviruses can be interrogated at two informative positions using duplex PCR reactions occurring within the droplets (Fig. 1A). Amplicon positions are chosen so the vast majority of defective proviruses will fail to give amplification at both positions, and thus intact proviruses can be directly counted as double positive droplets [21]. A separate digital droplet PCR (ddPCR) measures input cellular DNA and allows correction for DNA shearing [21].

IPDA analysis was carried out on $CD4^+$ T cells purified from cryopreserved PBMC from the sampling time point as shown in Fig. 1A. For 5 of 99 participant samples (5.1%), there was signal failure for one of the IPDA amplicons. As previously described [25, 27], sequence polymorphism can cause amplification issues, precluding the measurement of intact proviruses without alternative primers or probes. All samples (n=7) from these participants

were excluded from the analysis as were 5 samples for which instrument malfunction precluded measurement. For the remaining samples (N=108), the median frequency of intact proviruses was 175/10^6 $CD4^+$ T cells (IQR 29-345). Notably, these values are similar to those recently reported for a distinct cohort of HIV-1-infected adults in whom injection drug use was rare (151/10^6 $CD4^+$ T cells, IQR 40–398, reference [27]). For 13 of 108 samples (12%), no intact proviruses were detected in the cells analyzed, suggesting a low reservoir size (Fig. 1B). Both 3' and 5' defective proviruses were detected in all of these samples, excluding amplicon failure as a cause of the absence of intact proviruses. As with most reservoir measurements [8, 9, 25, 32], intact provirus values varied over a 2-3 log range in different individuals (Fig. 1B). Consistent with previous studies [21, 25], the frequency of cells with intact proviruses was significantly lower than the frequency of cells with defective proviruses (median values of 576 and 357 per 10^6 $CD4^+$ T cells for 3′ defective and 5′ defective proviruses, respectively, Fig. 1B). Based on median values, intact proviruses represent only 13% of total detected proviruses (Fig. 1B), highlighting the importance of selective measurement of intact proviruses. Overall, these results suggest that the HIV-1 reservoir is not substantially larger in PWID than in other infected individuals.

Effect of current drug use. To assess the effects of ongoing use of heroin and/or cocaine on reservoir size, we compared the frequency of $CD4^+$ T cells with intact proviruses in four groups with different patterns of self-reported drug use (Fig. 2A). In participants denying active use of heroin or cocaine but with a history of injection drug use, the median frequency of cells with intact proviruses was 90/10^6 $CD4^+$ T cells (IQR 12-306). Intact proviral levels for study participants in the other drug use groups were also in this range (heroin, median 199, IQR 31-307; cocaine, median 277, IQR 42-502; both, median 105, IQR 23-316). The differences in intact provirus levels between the groups were not significant (Fig. 2A). In addition, levels of proviruses with defects at the 3’ or 5’ end of the genome were also similar among the drug use groups (Fig. S1). Together these results show that neither a history of

injection drug use nor self-reported active use of heroin and/or cocaine has a major effect on the size of the latent reservoir in PWID on suppressive ART.

Effect of NTIs. Drug addiction is associated with problems with adherence and engagement in care, with some PWID experiencing repeated NTIs [15, 30]. To determine whether NTIs affect reservoir size, we determined the number of switches in plasma HIV-1 RNA from undetectable to detectable (or detectable to undetectable) on consecutive study visits for each participant over the course of follow-up (median 10.6 years). We then compared intact provirus values in participants who had relatively stable suppression of viremia on ART ("Low switches") to values in participants with frequent switches ("High switches", defined as switches on >18% of biannual study visits). As shown in Fig. 2B, high switch participants had significantly higher levels of intact proviruses than low switch participants (median 249 vs 43 per 10^6 $CD4^+$ T cells, respectively; p = 0.0004).

Effect of time since last detectable HIV-1 RNA level. As part of the study design, all participants had HIV-1 RNA levels below the limit of detection at the time of sampling and at the study visit 6 months previously (Fig 1A). However, some participants had maintained suppression of viremia on ART for many years, while for other participants, particularly those with frequent NTIs, the period of sustained virologic suppression was much shorter. The median time since last detectable viral load for the overall study population was 3.4 years (IQR, 1.6 – 6.1). As shown in Fig. 2C, levels of intact provirus declined with increasing time since last detectable HIV-1 RNA level. After exploring multiple cutpoints, we dichotomized years since last detectable HIV-1 RNA at three years for further analysis. Participants with <3 years compared to those with ≥3 years since last detectable viral load had significantly higher levels of intact proviruses (median 282 vs 80 per 10^6 $CD4^+$ T cells, respectively; p = 0.0004).

Correlates of intact provirus levels. To understand the relationship between NTIs, duration of viral suppression, and size of the latent reservoir, we analyzed correlates of intact provirus levels in univariate and multivariable regression models. In univariate analysis (Table 2), we found no association with pattern of self-reported drug use. However, age ≥50 years and female gender were both associated with lower intact provirus levels. In addition to these demographic variables, both higher switch frequency and shorter time since last detectable HIV-1 viral load were significantly associated with higher intact provirus levels (Table 2). These two variables are related (illustrated in Fig. 2C); a higher proportion of viral switches was strongly correlated with shorter time since last detectable HIV-1 viral load (higher switches: median of 2.1 years, IQR, 1.5 - 4.1; lower switches: 5.0 years, IQR, 4.3 - 8.7). In separate multivariable models adjusting for demographics and drug use groups, higher switching (p=0.0032) and <3 years from the last detectable HIV-1 viral load (p=0.0087) were each significantly and independently correlated with higher intact provirus levels (Table 2; Models A and B, respectively). Intact and total proviruses levels are highly correlated (Spearman's ρ = 0.76; P < 0.0001, reference 21). Thus associations with higher switching and <3 years from the last detectable HIV-1 viral load were also seen using the log of total proviruses as the dependent variable (p = 0.0009 and p = 0.0098, respectively). In a final model adjusting for demographics and drug use groups (Table 2, Model C), we incorporated an interaction term representing joint exposure to both of these variables (high switching alone; <3 years from last detectable viral load alone, and both high switching and <3 years from last detectable viral load. These were compared to low switching and ≥3 years from last detectable viral load as the reference group. In this multivariable model, we observed a dose-response association of intact provirus levels increasing with joint exposure. Of note, although the point estimate suggested substantial increase in intact provirus levels, there were only 4 samples from participants with a low proportion of switching and a short interval since last detectable viral load. As a result, this comparison was not statistically significant. Overall, these results suggest that frequent NTIs and a short time of sustained suppression of viremia on ART can both contribute to higher levels of intact proviruses.

Effect of reservoir decay. Given that NTIs and shorter time since the last detectable viral load were both associated with higher intact provirus levels, we hypothesized that frequent NTIs might simply shorten the period of sustained viral suppression during which reservoir decay could occur. Most of the high switch participants had shorter periods of sustained suppression prior to sampling (Fig. 2C). The slope of the regression line for the correlation between the log of the intact provirus levels and time since last positive viral load (m = -0.090) was similar to the expected decay slope of the latent reservoir ($t_{½}$ = 3.67 yrs, m = -0.078, references [7, 8]). To determine whether the apparent effect of NTIs on intact provirus levels was due solely to shorter time for reservoir decay, we calculated the expected intact provirus level at the time stable suppression of viremia was first achieved assuming uniform exponential reservoir decay (Fig. 2D). After adjustment for reservoir decay, a history of NTIs was still associated with higher estimated initial intact provirus levels (p = 0.0447). Comparison of the slopes of the regression lines for high and low switch participants suggested slower decay for high switch participants (Fig. 2C), and thus the higher levels of intact proviruses seen in participants with frequent NTIs might reflect increased deposition of virus in the reservoir during NTIs and decreased reservoir decay as well as shorter times of sustained suppression during which decay can occur. For participants sampled longitudinally with more than a year and no NTIs between sampling (n=10 for high switch participants, 5 for low switch participants), we also found a slower mean decay rate for high switch participants (0.0691 ± 0.237 yr^{-1} vs. 0.0997 ± 0.0957 yr^{-1} for low switch participants). These rates are equivalent to half-lives of 10.03 years vs. 6.95 yrs, for high and low switch participants, respectively. These rates are slower than the mean decay rate observed in cohorts not selected for a history of injection drug use (0.189 $yr^{-1,}$ $t_{½}$ = 3.67 yr, references 7,8). Further study is need to understand the mechanisms underlying the observed effect of NTIs and injection drug use on reservoir size.

Discussion

We examined latent reservoir size among PWID using a novel assay that selectively detects the intact proviruses that cause rebound upon ART interruption. In PWID with current suppression of viremia on ART, we found no apparent effect of active heroin and/or cocaine use on reservoir size as measured by IPDA and no marked difference in reservoir size relative to other cohorts of infected people. However, we found notably larger reservoirs in PWID with past or recent periods of viremia due to NTIs compared to PWID with more stable suppression of viremia. Further, our data suggest that NTIs contribute to reservoir size directly and do not simply reflect shorter times for reservoir decay.

Our data have important implications for the field. First, they support the inclusion of PWID with stable suppression of viremia on ART in cure studies. Second, they demonstrate that a history of viremia due to NTIs may have lasting effects on the size of the reservoir, and as such, virologic history should be considered when designing or analyzing HIV-1 cure studies. We and others have clearly documented how illicit drug use, particularly the intensity of injecting drugs, is directly associated with the inability of PWID to maintain durable HIV-1 viral suppression [30, 33, 34]. Our data indicate that injecting drugs is more likely to impact latent reservoir size through behavioral mechanisms resulting in repeated loss of viral suppression in contrast to biological effects of the illicit drugs themselves.

Both the accumulation of episodes of virologic failure through NTIs and a shorter duration of sustained viral suppression appear to directly contribute to reservoir size. Through direct analysis of multivariable models incorporating both of these variables, as well as back calculation of initial intact provirus levels at time of initial viral suppression, we show that the effect of NTIs on increasing latent reservoir size does not appear to be solely related to duration of viral suppression. This finding suggests that repeated episodes of HIV-1 viral rebound may result in larger latent reservoirs, potentially making eradication more difficult in

individuals with this history. Of note, brief structured treatment interruptions done as part of carefully controlled clinical studies do not appear to increase reservoir size [35, 36]. Our results suggest that further investigation of the mechanisms by which viral rebound contributes to the latent reservoir are needed and that a history of frequent NTIs should be considered in the evaluation of participants in cure studies.

In the analysis of factors contributing to reservoir size in PWID, we did not find an association with patterns of self-reported active cocaine or heroin use. In addition to the statistically significant associations with NTIs and short duration of virologic suppression, we did find effect of gender and age. There were lower intact provirus levels in female participants and in participants over 50 years of age. Previous studies of gender differences in the HIV-1 reservoir have given conflicting results, likely influenced by the assay used and other factors [26, 37, 38].

This study had several strengths including access to a well-characterized longitudinal PWID cohort linked to biorepository samples, a novel study design which allowed concurrent assessment of both types of illicit drug use as well as viral suppression switching, and application of state-of-the-art IPDA assay for reservoir characterization. While our study was limited to characterization of drug use exposure based on self-report, ongoing work will provide biological measures to validate exposure data.

In summary, the latent reservoir size among PWID is comparable to that of other HIV-1 populations and does not appear dependent on type of illicit drug used. Cure studies should be designed to include broadly representative populations of infected individuals; exclusion of PWID with substantial duration of viral suppression is unjustified. Both the use of longer-acting ART and implementation of effective HIV care engagement interventions (e.g., substance use treatment, peer navigation) hold promise for prolonging the duration of non-interrupted viral suppression among PWID [39, 40], and thereby increase eligibility for HIV-1 cure interventions.

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Table 1. Characteristics of Study Participants

Characteristic	Entire Cohort	Group 1 (none)	Group 2 (heroin)	Group 3 (cocaine)	Group 4 (both)
Samples analyzed	108[1]	28	28	23	29
General					
Unique participants	91[1]	28	28	23	29
Gender (% male)	72.6	67.9	71.4	65.2	79.3
Race (% AA[2])	95.6	92.9	92.9	100.00	96.6
Age[3] (median,IQR)	52.8 (49.1-56.8)[4]	52.6 (46.7-56.6)	53.1 (49.5-56.7)	51.2 (49.4-56.9)	56.0 (50.9-59.8)
Social					
Employed[5] (%)	9.0[4]	10.7	7.4	9.1	3.4
Incarceration[5] (>1wk, %)	7.0[4]	0.0	0.0	0.0	17.2
Homelessness[5] (any, %)	5.5[4]	0.0	7.1	8.7	6.9
Alcohol use[5] (%)	49.5[4]	14.3	67.9	47.8	58.6
Tobacco use[5] (%)	78.0[4]	78.6	75.0	82.6	86.2
Injection drug use[5] (%)	28.6[4]	0.0	28.6[6]	26.1	70.0
Clinical					
Proximal CD4[3] (median, cells/µl)	448[4]	408	464	482.0	432
CD4 nadir (cells/µl)	173	137	183.5	220	188
Time from first ART (yrs)	7.8	10.6	7.6	6.7	7.4
Time from last positive HIV vl[3] (yrs)	3.40	3.19	4.07	2.49	4.14
Proportion with high switch[3,7] (%)	67.0	64.3	64.3	73.9	62.1

[1]A total of 99 participants provided 120 samples. 20 participants were sampled at 2 time points, and 1 participant was sampled at 3 time points. Of the 120 samples, 7 samples from 5 different participants were excluded due to IPDA signal failure, and 5 samples from 5 different participants were excluded due to instrument failure, resulting in a final set of 108 samples for analysis from 91 unique donors.

[2]African-American

[3]At the time of sampling, except where indicated

[4]At the first sample time point for participants sampled more than once.

[5]During the 6 months prior to sampling.

[6]Heroin use by other than injection accounts for the low fraction of participants in the heroin use group reporting iv injection.

[7]Percentage of participants experiencing a high number of switches in HIV-1 RNA status. A switch was defined as a change in HIV-1 RNA from detectable to undetectable (or undetectable to detectable) relative to the previous study visit. Switches were considered high if a switch was seen at ≥18% of study visits (10% for the heroin only group due to lower numbers).

Table 2. Correlates of Intact Provirus Levels.

	Univariate		Model A[1]		Model B[1]		Model C[2]	
	Estimates (95% CI)	p-value	Estimates (95% CI)	p-value	Estimates (95% CI)	p-value	Estimates (95% CI)	p-value
Age >50 years	-0.58 (-0.97 – -0.19)	**0.0038**	-0.67 (-1.04 – -0.29)	**0.0006**	-0.62 (-1.00 – -0.24)	**0.0017**	-0.64 (-1.00 – -0.24)	**0.0010**
Female	-0.41 (-0.83 – -0.00)	**0.0489**	-0.47 (-0.86 -0.08)	**0.0191**	-0.49 (-0.88 – -0.09)	**0.0165**	-0.42 (-0.88 – -0.09)	**0.0384**
Black	0.67 (-0.23 – 1.56)	0.1436	0.54 (-0.28 – 1.36)	0.1927	0.47 (-0.36 – 1.30)	0.2628	0.48 (-0.36 – 1.30)	0.2524
Drug use group[3]:								
None	1.00		1.00		1.00		1.00	
Heroin only	0.29 (-0.24 – 0.82)	0.2816	0.32 (-0.15 – 0.79)	0.1845	0.37 (-0.11 – 0.85)	0.1320	0.33 (-0.11 – 0.85)	0.1707
Cocaine only	0.38 (-0.18 – 0.94)	0.1788	0.30 (-0.20 – 0.80)	0.2385	0.29 (-0.22 – 0.80)	0.2611	0.27 (-0.22 – 0.80)	0.2832
Cocaine and heroin	0.18 (-0.34 – 0.71)	0.4957	0.20 (-0.27 – 0.68)	0.4037	0.24 (-0.24 – 0.72)	0.3194	0.24 (-0.24 – 0.72)	0.3226
Injection drug use frequency[3]:			--		--		--	
None	1.00							
<1 per day	-0.39 (-0.89 – 0.12)	0.1342						
Daily	-0.10 (-0.65 – 0.46)	0.7313						
Lower switching[4]	1.00		1.00		--		--	
Higher switching	0.65 (0.27 – 1.03)	**0.0011**	0.56 (0.19 – 0.93)	**0.0032**				
Years with UVL[5]:			--				--	
≥3	1.00				1.00			
<3	0.62 (0.25 – 0.98)	**0.0011**			0.49 (0.13 – 0.85)	**0.0087**		
Low switch; UVL≥3 yrs	--		--		--		1.00	
Hi switch; UVL≥3 yrs							0.49 (0.02 – 0.96)	**0.0396**
Low switch; UVL<3 yrs							0.59 (-0.38 – 1.56)	0.2280
Hi switch; UVLD<3 yrs							0.73 (0.31 – 1.16)	**0.0009**

[1]Multivariable model adjusting for demographics and drug use groups.

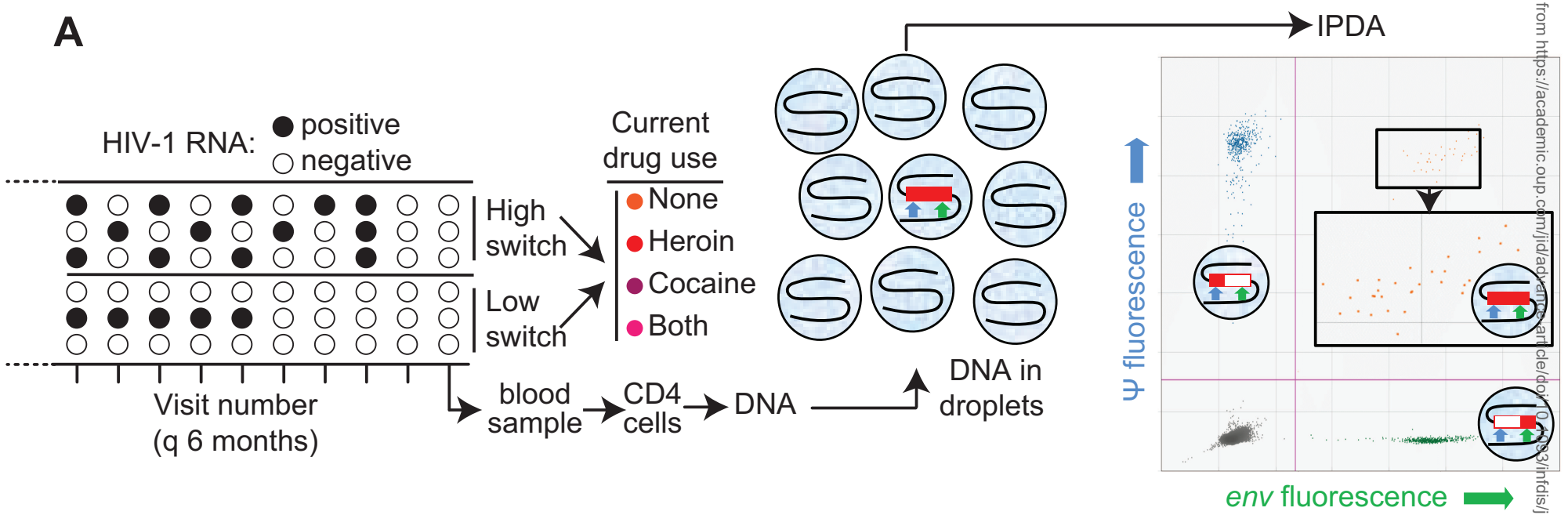
[2]Multivariable model adjusting for demographics and drug use groups and incorporating an interaction term representing joint exposure to high switching and <3 years of undetectable viral loads (UVL).

[3]Based on self-report at sample time point.

[4]Based on fraction of study visits at which there was a switch in HIV-1 RNA suppression status (e.g., from undetectable to detectable, or detectable to undetectable). Higher switching defined as a switch at >18% of study visits. Participants were followed biannually for a median of 10.6 years.

[5]Years with undetectable viral load (UVL). Calculated as years from last detectable viral load prior to sample visit.

Figure 1



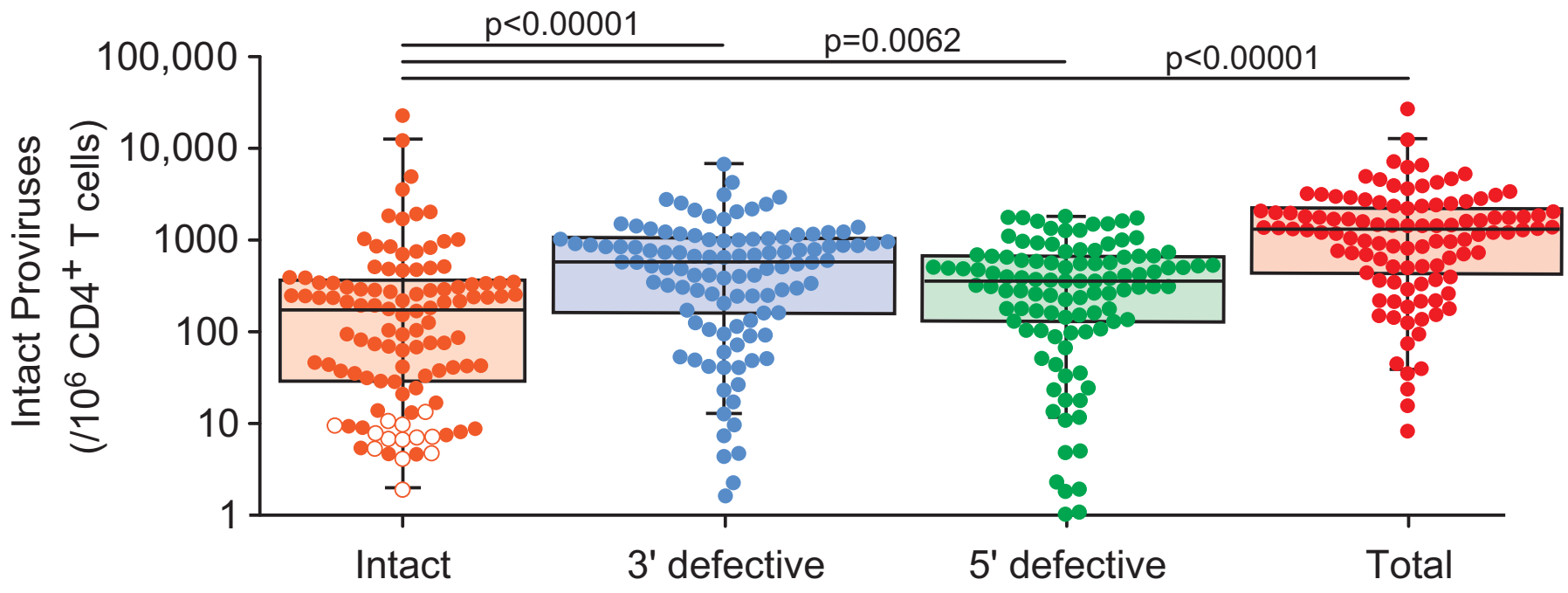


Figure 2