HIV-1 DNA Decay Dynamics in Blood During More Than a Decade of Suppressive Antiretroviral Therapy

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Background. Human immunodeficiency virus type 1 (HIV-1) DNA dynamics during long-term antiretroviral therapy (ART) are not defined.

Methods. Blood mononuclear cells obtained during 7–12 years of effective ART were assayed for total HIV-1 DNA and 2-long terminal repeat (LTR) circles by quantitative polymerase chain reaction (qPCR). Slopes of HIV-1 DNA were estimated by participant-specific linear regressions. Plasma was assayed for residual viremia (HIV-1 RNA) by qPCR.

Results. Thirty participants were studied. HIV-1 DNA decreased significantly from years 0–1 and 1–4 of ART with median decay slopes of −0.86 (interquartile range, −1.05, −0.59) and −0.11 (−0.17, −0.06) $\log_{10}$(copies/10^6 CD4+ T-cells)/year, respectively ($P < .001$). Decay was not significant for years 4–7 (−0.02 [−0.06, 0.02]; $P = .09$) or after year 7 of ART (−0.006 [−0.030, 0.015]; $P = .17$). All participants had detectable HIV-1 DNA after 10 years (median 439 copies/10^6 CD4+ T-cells; range: 7–2074). Pre-ART HIV-1 DNA levels were positively associated with pre-ART HIV-1 RNA levels (Spearman = 0.71, $P < .001$) and with HIV-1 DNA at years 4, 7, and 10 on ART (Spearman ≥ 0.75, $P < .001$). No associations were found ($P ≥ .25$) between HIV-1 DNA slopes or levels and % activated CD8+ T-cells (average during years 1–4) or residual viremia (n = 18). 2-LTR circles were detected pre-ART in 20/29 and in 8/30 participants at last follow-up.

Conclusions. Decay of HIV-1 DNA in blood is rapid in the first year after ART initiation (86% decline), slows during years 1–4 (23% decline/year), and subsequently plateaus. HIV-1 DNA decay is not associated with the levels of CD8+ T-cell activation or persistent viremia. The determinants of stable HIV-1 DNA persistence require further elucidation.

Clinical Trials Registration. NCT00001137.

Keywords. HIV-1 persistence; antiretroviral therapy; HIV-1 DNA decay; immune activation.

Combination antiretroviral therapy (ART) suppresses but does not cure human immunodeficiency virus type 1 (HIV-1) infection because of the persistence of viremia and latent HIV-1 reservoirs [1, 2]. Although the decay kinetics of plasma HIV-1 RNA after ART initiation are well described [3, 4], long-term changes in total and episomal HIV-1 DNA in blood are not [5, 6]. We therefore conducted a longitudinal study of HIV-1 DNA changes in participants on suppressive ART for 10 or more years.

Several studies reported that the proviral HIV-1 DNA copy number on ART is directly related to the level of persistent immune activation [7, 8], although contradictory results showing no relationship with immune activation have recently been published [9]. Hence, we also investigated the relationship between HIV-1 DNA decay and immune activation.

MATERIALS AND METHODS

Study Participants

Study participants had been enrolled in the ACTG A5001 cohort (AIDS Clinical Trials Group longitudinal linked randomized trials—ALLRT [10]) and followed to evaluate clinical, virological, and immunological outcomes. For the current study, selection criteria included the following: ART-naive; plasma HIV-1 RNA suppressed (<50 copies/mL) from week 32 of initial therapy for 10 or more years; at least 1 pre-ART peripheral blood mononuclear cell (PBMC) sample (≥5 million cells) and plasma sample (≥1.0 mL); at least 1 PBMC sample (≥5 million cells) at year 1 and at least 1 PBMC sample (≥5 million cells) and plasma sample (≥3.0 mL) at year 10. Seven of 30 participants had, at or after week 48, either transient HIV-1 RNA blips >50 copies/mL (n = 3, 81–538 copies/mL), ART interruption ≥14 consecutive days (n = 1), or both (n = 2, blip of 62 copies/mL 72 weeks prior to ART interruption; ART interruption 72 weeks prior to blip of 64 copies/mL) or treatment failure followed by resuppression after a change of regimen (n = 1).

Samples

Cryopreserved PBMCs were obtained pre-ART, 1 year post-ART, and at years 4, 7, and 10. For a subset of 10 participants, an additional sample was obtained at 12 years post-ART. All samples from each participant were assayed in batch for total HIV-1 DNA and 2-long terminal repeat (LTR) circles. Stored plasma samples at approximately years 4, 7, 10, and 12 (if available) post-ART from a subset of participants (n = 18) were tested for persistent viremia by single-copy HIV-1 RNA assay (SCA).

Quantitative Polymerase Chain Reaction Assays for Total HIV-1 DNA and 2 LTR Circles

Extraction of Nucleic Acid

Cryopreserved PBMCs were extracted following a previously described protocol [11] with sonication during the lysis step and after resuspension of nucleic acids (Branson high-intensity cup horn, sonication cycle ID#01, 60% amplitude for 10 seconds). Following estimation of total nucleic acid by NanoDrop 1000 (Thermo Scientific), samples were diluted to a final concentration of <170 ng/μL to prevent inhibition of quantitative polymerase chain reaction (qPCR).

qPCR for Total HIV-1 DNA and 2-LTR Circles

Each sample was assayed in triplicate for total HIV-1 DNA and 2-LTR circles using published qPCR methods with normalization for cellular input [11–13]. The 95% limits of detection (LOD) for the HIV-1 DNA and 2-LTR assays were 5 7.5 copies/10^6 and 7.5 copies/10^6 PBMC, respectively. Results were further normalized by the percentage of all blood lymphocytes that

Table 1. Participant Characteristics

Characteristic	Total (N = 30)
Pre-ART age (years)	
Median (Q1, Q3)	37 (31, 42)
Min, Max	24, 57
Sex	
Male	23 (77%)
Race/ethnicity	
White non-Hispanic	16 (53%)
Black non-Hispanic	5 (17%)
Hispanic (regardless of race)	7 (23%)
Asian, Pacific Islander	2 (7%)
Pre-ART human immunodeficiency virus type 1 RNA (log_{10}copies/mL)	
Median (Q1, Q3)	4.7 (4.3, 5.4)
Min, Max	2.3, 6.5
Pre-ART CD4+ T-cell count (cells/mm^3)	
Median (Q1, Q3)	193 (111, 360)
Min, Max	9, 881
Pre-ART CD4+ T-cells percent	
Median (Q1, Q3)	15 (10, 24)
Min, Max	2, 35
Initial ARV regimen	
EFV + 2/3 NRTI	16 (53%)
EFV + NFV + 2 NRTI	4 (13%)
NFV + 2 NRTI	2 (7%)
ABC/AZT/3TC	8 (27%)
ARV regimen at final time point	
ATV/r + 2 NRTI	3 (10%)
EFV + 2/3 NRTI	21 (70%)
LPV/r + 2 NRTI	1 (3%)
NVP + 2/3 NRTI	3 (10%)
NFV + 2 NRTI	1 (3%)
ABC 3TC EFV RAL	1 (3%)

Abbreviations: ABC, abacavir; ART, antiretroviral therapy; ARV, antiretroviral, ATV, atazanavir; AZT, azidothymidine; EFV, efavirenz; LPV, lopinavir; NFV, Nelfinavir; NRTI, nucleoside reverse transcriptase inhibitor; NVP, nevirapine; RAL, raltegravir; 3TC, lamivudine.

were CD4+ T-cells by flow cytometry and expressed as copies per 10^6 CD4+ T-cells.

Single-Copy HIV-1 RNA Assay for Residual Viremia

HIV-1 RNA in plasma was measured by SCA as previously described [3, 4]. Three milliliters of plasma were assayed, resulting in an LOD of 0.6 copies/mL.

Flow Cytometry

Flow cytometric analysis was performed on fresh cells [14]. Naive T cells were defined as those that stained positive for CD45RA and CD62L. Activated T cells were defined as CD3+ lymphocytes that stained positive for CD38 and HLA-DR. CD8+ T-cell activation was measured as the percentage of

Table 2. Summary of Total Human Immunodeficiency Virus Type 1 (HIV-1) DNA Levels, 2-LTR Circles, CD4+ T-cell Count, and Plasma HIV-1 RNA

Characteristic	Years on Antiretroviral Therapy					
	0	1	4	7	10	12
CD4+ T-cell count (cells/mm^3)						
Median (Q1–Q3)	193 (111–360)	347 (257–484)	549 (379–768)	666 (520–899)	770 (566–924)	697 (472–1105)
Min-Max	9–881	78–1039	174–1528	306–1493	155–2397	306–1398
Total HIV-1 DNA (copies/10^6 CD4+ T-cells)						
N	30	30	30	29	29	10
Median (Q1–Q3)	7319 (1920–16 469)	1054 (455–2193)	446 (185–1224)	434 (128–701)	439 (180–1030)	417 (105–1260)
Min-Max	315–144 331	113–9033	43–2664	49–2086	7–2074	77–1356
2-LTR circles (copies/10^6 CD4+ T-cells)						
Positive	20/29 (69%)	17/30 (57%)	8/30 (27%)	6/29 (21%)	8/29 (28%)	4/10 (40%)
Median (Q1–Q3)[a]	146 (48–297)	66 (29–89)	10 (3–24)	14 (10–23)	1 (1–7)	5 (1–11)
Min-Max[a]	17–4851	1–155	2–27	4–25	0–28	1–15
HIV-1 RNA by single-copy HIV-1 RNA assay						
Undetectable (<1 copy/mL)	Not applicable	Not done	8 (44%)	10 (63%)	13 (76%)	5 (83%)
Detectable (≥1 copy/mL)	Not applicable	Not done	10 (56%)	6 (38%)	4 (24%)	1 (17%)

Abbreviations: HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat.
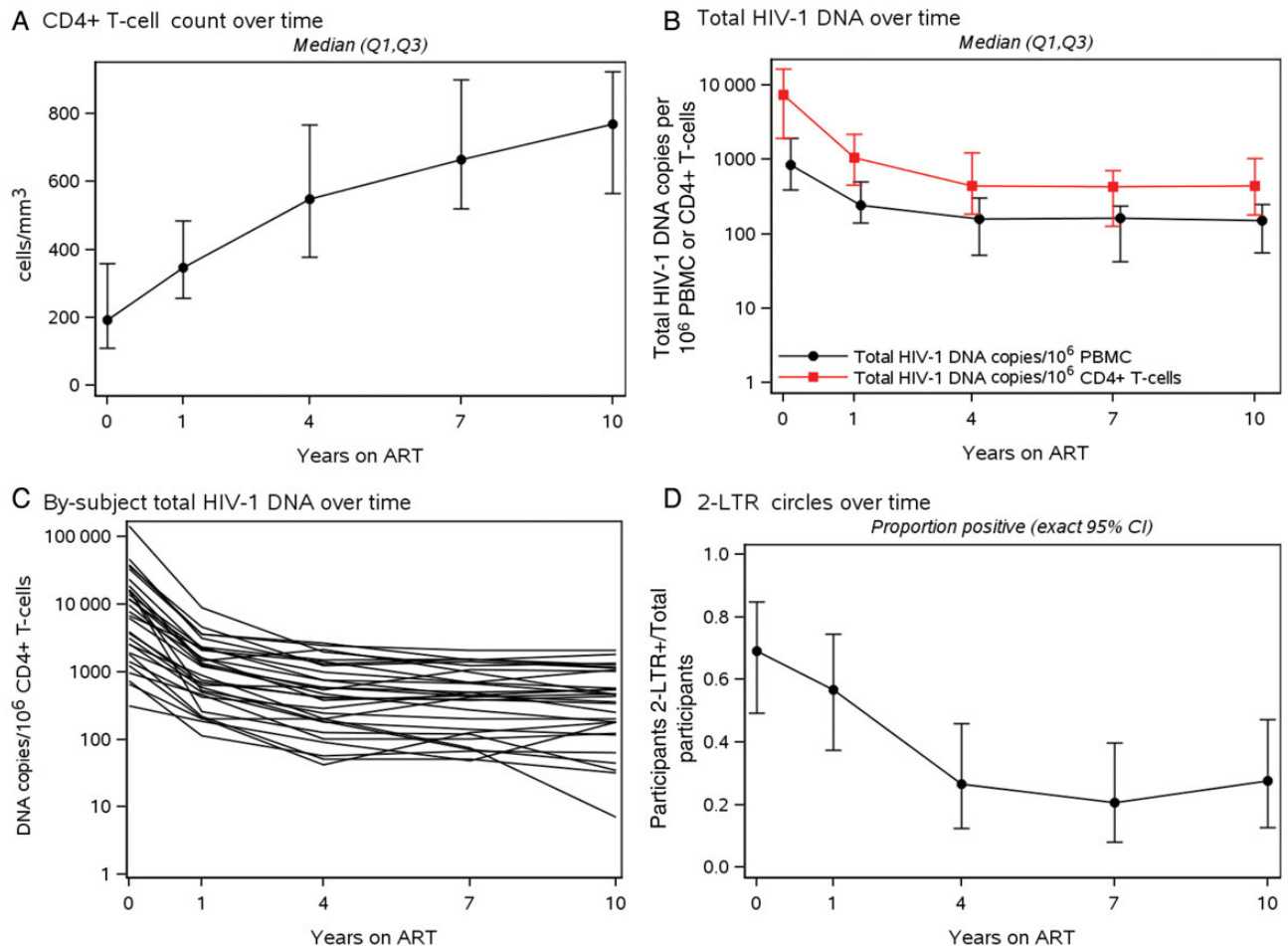
[a] Among 2-LTR–positive results.

Figure 1. Longitudinal measures of CD4+ T-cell count, total human immunodeficiency virus type 1 (HIV-1) DNA, and 2-LTR circle detection. *A*, CD4+ T-cell count over time for all participant pre-antiretroviral therapy (ART) and at years 1, 4, 7, and 10. *B*, Total HIV-1 DNA levels per million peripheral blood mononuclear cells (black circle) and per million CD4+ T-cells (red square) over time for all participants pre-ART at years 1, 4, 7 and 10. *C*, Participant-specific decay patterns of total HIV-1 DNA per million CD4+ T-cells. *D*, The proportion of participants with positive (detectable) 2-LTR circles over time pre-ART and at years 1, 4, 7, and 10. Abbreviations: CI, confidence interval; LTR, long terminal repeat; PBMC, peripheral blood mononuclear cells.

CD38+/HLA-DR+ cells. At least 1 measure of immunological parameters was available for years 1–4 post-ART in 28 participants.

Statistical Analysis

Within-participant total HIV-1 DNA log_{10}copies/10^6 CD4+ T-cell slopes were estimated by fitting participant-specific linear regressions. These slopes were compared between time periods and also against the null hypothesis of no change using the Wilcoxon signed rank test. Associations with total HIV-1 DNA levels and slopes were determined using Spearman rank-based correlations (ie, with pre-ART characteristics and measures of immune recovery and immune activation) or repeated measures analyses using generalized estimating equations (ie, with HIV-1 RNA by SCA).

RESULTS

Participant Characteristics

Table 1 shows baseline characteristics of the 30 study participants. Median pre-ART age was 37 years; 77% were male; and 53% were white non-Hispanic. Median plasma HIV-1 RNA pre-ART was 4.7 log_{10}copies/mL (interquartile range [IQR], 4.3–5.4) and median CD4+ T-cell count was 193 (IQR, 111–360) cells/mm^3. The median total duration of ART with HIV-1 DNA measurements was 10.2 years.

HIV-1 DNA Decay Kinetics
Total HIV-1 DNA

All participants had detectable HIV-1 DNA at each time point. The median HIV-1 DNA level at baseline (pre-ART)

Table 3. Summary of Total Human Immunodeficiency Virus Type 1 DNA Slopes Over Time

log$_{10}$(Total HIV-1 DNA cps/10^6 CD4+ T-cells) Slope/Year	Pre-ART to Year 1 on ART[a]	Year 1 to Year 4 on ART[a]	Year 4 to Year 7 on ART[a]	Year 7 Through Follow-up on ART[a,b]
N	30	30	29	29
Median (Q1, Q3)	−0.856 (−1.048, −0.590)	−0.111 (−0.169, −0.062)	−0.017 (−0.061, 0.020)	−0.006 (−0.030, 0.015)
Min, Max	−1.965, −0.246	−0.241, 0.057	−0.195, 0.166	−0.415, 0.252
Negative slope				
Yes	30 (100%)	29 (97%)	20 (69%)	19 (66%)
No		1 (3%)	9 (31%)	10 (34%)

Abbreviations: ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1.

[a] Wilcoxon signed rank P = <.001, <.001, .09, and .17, respectively.

[b] Last year of ART follow-up with available total HIV-1 DNA: median 10 (range, 7–12; interquartile range, 10–12).

was 7319 copies/10^6 CD4+ T-cells (IQR, 1920–16 469), declining to a median of 1054 copies/10^6 CD4+ T-cells at year 1 of ART (IQR, 455–2193), then to a median of 446 copies/10^6 CD4+ T-cells after 4 years of ART (IQR, 185–1224; Table 2). Subsequent changes in the HIV-1 DNA levels were small over the next 6–8 years, reaching a median of 417 copies/10^6 CD4+ T-cells at year 12 (IQR, 105–1260; n = 10). Figures 1B and 1C illustrate the decline of total HIV-1 DNA through year 10 while on ART. The most rapid decay (7.2-fold, 86% decline) occurred within the first year, followed by a slower decline (2.2-fold) from years 1 to 4, and then a plateau during which no significant decline occurred. Specifically, HIV-1 DNA decreased from years 0–1 and 1–4 on ART, with median decay slopes of −0.86 (IQR, −1.05, −0.59) and −0.11 (IQR, −0.17, −0.06) log$_{10}$(copies/10^6 CD4+ T-cells)/year, respectively (comparing yearly decay years 0–1 with years 1–4; each P < .001 vs null of no change; Table 3). During these time intervals, 100% and 97%, respectively, had a negative HIV-1 DNA slope. HIV-1 decay slope/year was not significant from years 4 to 7 of ART (median [IQR], −0.02 [−0.06, 0.02]; P = .09) or after year 7 (−0.006 [−0.030, 0.015]; P = .17), although 69% and 66% of participants, respectively, had a negative HIV-1 DNA slope. A by-participant listing of all slopes is available as Supplementary Table 1.

Figure 2 shows individual longitudinal plots of total HIV-1 DNA, 2-LTR circles, and plasma HIV-1 RNA from 6 representative participants (remaining participants' plots are available in Supplementary Figure 1). The kinetics of HIV-1 DNA decay are similar, with normalization per million PBMC or CD4+ T-cells confirming that CD4+ T-cells are the predominant HIV-infected cells in blood (Figure 1B and Figure 2). The levels of residual plasma HIV-1 RNA show no obvious relation to total HIV-1 DNA levels or 2-LTR detection (Figure 2).

2-LTR Circles

Figure 1D shows that 2-LTR circles were detected in 20/29 (69%) participants at pre-ART (median 146 copies/10^6 CD4+ T-cells) and remained detectable in 8/30 (27%) participants at the last time point (median 3 copies/10^6 CD4+ T-cells). The median fraction of 2-LTR circle HIV-1 DNA relative to total HIV-1 DNA (among 2-LTR–positive participants) was 2.5% pre-ART (range, 0.04%–42%) and declined to <0.6% after a decade of ART.

Associations With Total HIV-1 DNA Decay

Age, Pre-ART Plasma HIV-1 DNA, and RNA

Higher pre-ART HIV-1 RNA was associated with higher pre-ART HIV-1 DNA levels (Spearman = 0.71, P < .001) and larger declines in HIV-1 DNA from pre-ART to year 1 of ART (Spearman = −0.42, P = .022; Table 4, Figure 3A). After adjusting for pre-ART CD4+ T-cells, there was no longer evidence of an association between pre-ART HIV-1 RNA and HIV-1 DNA slope during year 1 of ART (Spearman = −0.09, P = .64). Strong, positive correlations were evident between HIV-1 DNA levels pre-ART and after 4, 7, and 10 years of ART (Spearman ≥ 0.75, P < .001; Figure 3B). Older pre-ART age was marginally associated with a smaller decline in total HIV-1 DNA from year 1 to year 4 of ART (Spearman = 0.38, P = .039; Table 4).

CD4+ T-Cells and Immune Activation

There was a strong, negative correlation between pre-ART CD4+ T-cell count and pre-ART HIV-1 DNA (Spearman = −0.70, P < .001; Table 4, Figure 3C). In addition, higher pre-ART CD4+ T-cell count was associated with smaller decline in HIV-1 DNA from pre-ART to year 1 of ART (Spearman = 0.55, P = .002; Table 4, Figure 3D). A similar correlation was seen after adjusting for pre-ART HIV-1 RNA (Spearman = 0.40, P = .033). No association was observed between CD4+ T-cell count slopes and HIV-1 DNA slopes during either year 1 (Spearman = 0.19, P = .32) or years 1–4 (Spearman = 0.06, P = .77) of ART.

Twenty-eight of 30 participants had advanced flow data between year 1 and year 4. There was no evidence of association
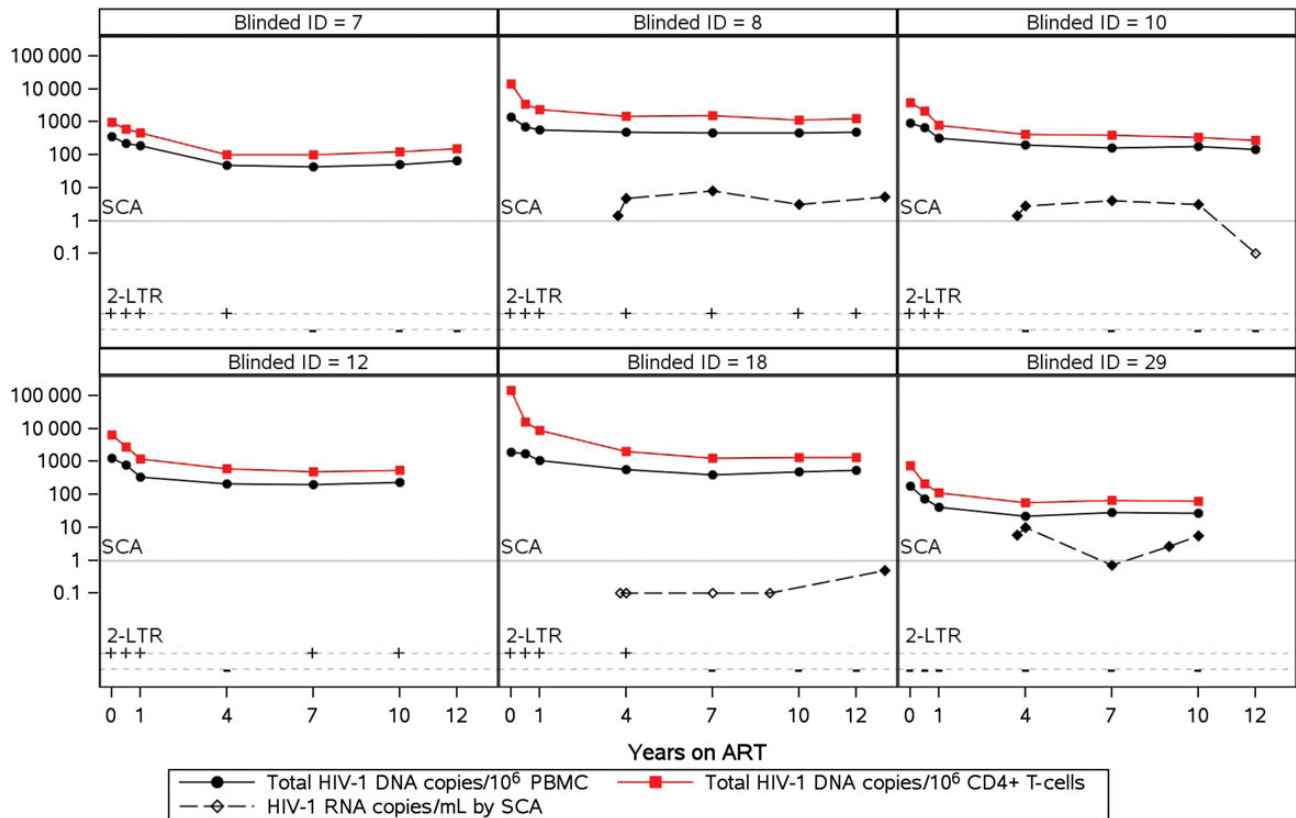
Figure 2. Longitudinal plots for a subset of representative participants (6 of 30) of total human immunodeficiency virus type 1 (HIV-1) DNA levels (black circle, per million peripheral blood mononuclear cells; red square, per million CD4+ T-cells), 2-LTR circles positivity (+/−), and HIV RNA levels over time (black diamond, copies/milliliter detected; open symbol, undetected values; gray line, limit of quantification). Longitudinal plots of the remaining 24 patients are displayed in Supplementary Figure 1. Abbreviations: ART, antiretroviral therapy; ID, participant identification; LTR, long terminal repeat; PBMC, peripheral blood mononuclear cells; SCA, single-copy HIV-1 RNA assay.

between average percent activated CD8+ cells (HLA-DR+/ CD38+) during years 1–4 and HIV-1 DNA $\log_{10}$copies/10^6 CD4+ T-cell slopes from year 1 to year 4 and from year 4 through follow-up (Spearman $P \geq .25$; Table 4), or from cross-sectional correlations at year 1 or year 4 between percent activated CD8+ cells and HIV-1 DNA levels ($r = 0.08$, $P = .7$, n = 28, and $r = 0.06$; $P = .9$, n = 11, respectively).

Residual Plasma Viremia (HIV-1 RNA by SCA)

At year 4 of ART, 17/18 participants with plasma HIV-1 RNA data had HIV-1 DNA data from the same specimen date. At year 4, there was no evidence of an association between HIV-1 DNA $\log_{10}$copies/10^6 CD4+ T-cells and HIV-1 RNA when treating HIV-1 RNA values continuously (Spearman = −0.13, $P = .61$) or discretely (< vs ≥1 copy/mL: Spearman = −0.073, $P = .78$). From year 4 through follow-up on ART, there was also no evidence of an association between total HIV-1 DNA $\log_{10}$copies/10^6 CD4+ T-cells and HIV-1 RNA ≥1 copy/mL ($P = .34$, repeated measures analysis). Finally, there was no evidence that total HIV-1 DNA at year 4 modified the HIV-1 RNA

slope/year or the probability of HIV-1 RNA being ≥1 copy/mL. Four of the 7 participants with transient blips or ART interruption were included in these analyses. Exclusion of these participants from the analyses did not change the results; there was no evidence at year 4 of an association between HIV-1 DNA $\log_{10}$ copies/10^6 CD4+ T-cells and HIV-1 RNA when treating HIV-1 RNA values continuously (Spearman $r = 0.02$, $P = .96$) or discretely (< vs ≥1 copy/mL: Spearman $r = 0.00$, $P = 1.0$).

DISCUSSION

This study reveals that total HIV-1 DNA levels in blood are remarkably stable after the fourth year of suppressive ART. Although biphasic decline of HIV-1 DNA is observed during the first 4 years of ART, little to no decay occurs thereafter. This pattern of HIV-1 DNA decay was observed consistently across the participants studied. The level of HIV-1 DNA during the plateau phase was directly correlated with the level of HIV-1 DNA before ART initiation and inversely correlated with the pretherapy CD4+ T-cell count, indicating that the number of

Table 4. Correlations With Human Immunodeficiency Virus Type 1 DNA Slopes

| | | log$_{10}$(Total HIV-1 DNA copies/10^6 CD4+ T-cells) Slope/Year | | | | | |
| | | Pre-ART to Year 1 on ART | | Year 1 to Year 4 on ART | | Year 4 Through Follow-up on ART | |
Characteristic	N	Spearman	P Value	Spearman	P Value	Spearman	P Value
Pre-ART age (years)	30	−0.20	.29	0.38	.039	0.17	.38
Pre-ART CD4+ T-cell count (cells/mm^3)[a]	30	0.55	.002	0.15	.42	0.15	.43
Pre-ART HIV-1 RNA (log$_{10}$copies/mL)[b]	30	−0.42	.022	−0.03	.88	−0.28	.13
Average activated CD8% (HLA-DR+/CD38+) during years 1–4	28	Not applicable		−0.22	.25	−0.11	.56

Abbreviations: ART, antiretroviral therapy; HIV-1, human immunodeficiency virus type 1.

[a] Correlation with pre-ART total HIV-1 DNA (log$_{10}$copies/10^6 CD4+ T-cells): Spearman = −0.70, $P < .001$.

[b] Correlation with pre-ART total HIV-1 DNA (log$_{10}$copies/10^6 CD4+ T-cells): Spearman = 0.71, $P < .001$.

HIV-1–infected cells and the extent of immunodeficiency at the time of ART initiation are important determinants of the number of HIV-1 DNA-containing cells that persist after long-term ART. These findings are consistent with those reported by Hocqueloux et al [15] and von Wyl et al [16]. However, the current study extends the duration of observation and provides long-term longitudinal data on episomal HIV-1 DNA, which decays in parallel with total HIV-1 DNA during the first year of ART but stays above the limit of detection in approximately one-third of participants, raising the possibility of a longer half-life for episomal HIV-1 DNA than previously appreciated [17]. Lastly, our analyses did not reveal an association between CD8+ T-cell activation and either HIV-1 DNA levels or decay kinetics, which is contrary to recent reports [8, 18] but consistent with others [9, 19].

The largest decay of HIV-1 DNA occurred within the first year of initiating ART (86% decline). Others have reported similar findings [5, 6] that are likely due to the death of a large pool of productively infected cells and blockade of new cell infection by ART. This 7-fold decay of HIV-1 DNA contrasts with the 10 000- to 100 000-fold reduction in plasma HIV-1 RNA achieved during the first year of ART. The difference between decay of HIV-1 DNA and RNA in blood reveals that a substantial fraction of HIV-1–infected cells persist on ART but contribute little, if anything, to plasma viremia. A slower second phase of HIV-1 DNA decay occurs over the next 3 years of ART and averages a 2.2-fold decrease, likely from death of a smaller pool of infected cells with a longer half-life. Finally, a plateau phase is reached after 4 years, after which there is no significant decay for a median of 10.2 years after ART initiation.

Three mechanisms could explain the indefinite plateau phase of HIV-1 DNA. First, it could be maintained by the survival, without proliferation, of long-lived HIV-infected cells. The persistence of the same infected CD4+ T-cells over more than a decade seems unlikely given that the half-life of most immune

cell subsets is short [20], although this possibility has not been excluded. Second, ongoing, complete cycles of HIV-1 replication could explain the stability of proviral HIV-1 DNA. Whether ongoing replication occurs on ART is controversial [8, 12, 18, 21, 22]. A few studies suggest that replication occurs in participants on protease inhibitors, as evidenced by an increase in 2-LTR circles after treatment intensification with the integrase inhibitor raltegravir [18, 23] or by reduction of gut-associated HIV-1 RNA [19]. However, most studies show no effect of antiretroviral intensification on persistent viremia [12, 21, 22, 24]. In addition, studies of HIV-1 population genetics on ART have not revealed consistent evidence of HIV-1 evolution [25–27]. The third potential explanation for maintenance of a plateau phase is proliferation of cells containing HIV-1 DNA [2], with expansion of some infected-cell clones and loss of other infected cells. In this case, clonal expansion would be evident by the appearance of identical proviral sequences with the same integration sites. Indeed, Wagner et al [28] have shown that the proportion of identical sequences in blood increases with time on suppressive ART despite stable HIV-1 DNA levels and that identical sequences often have the same cellular integration site; the latter provides clear evidence of clonal expansion. In addition, Maldarelli et al [29] have shown that the distance of HIV-1 populations in plasma from the most recent common ancestor decreases over time on suppressive ART, suggesting loss of HIV-1 variants. Taken together, these observations are consistent with stable HIV-1 DNA levels that result from a dynamic equilibrium between clonal expansion and loss of HIV-1 DNA-containing cells through cell death. The relative contributions of these mechanisms to stable HIV-1 DNA levels require further investigation.

Interestingly, 2-LTR circles decayed gradually but stayed detectable in 27% of participants after 7–12 years of ART. In vitro studies have estimated the half-life of HIV-1 episomes to be 10–20 hours [17, 30, 31], leading to the conclusion that they are a
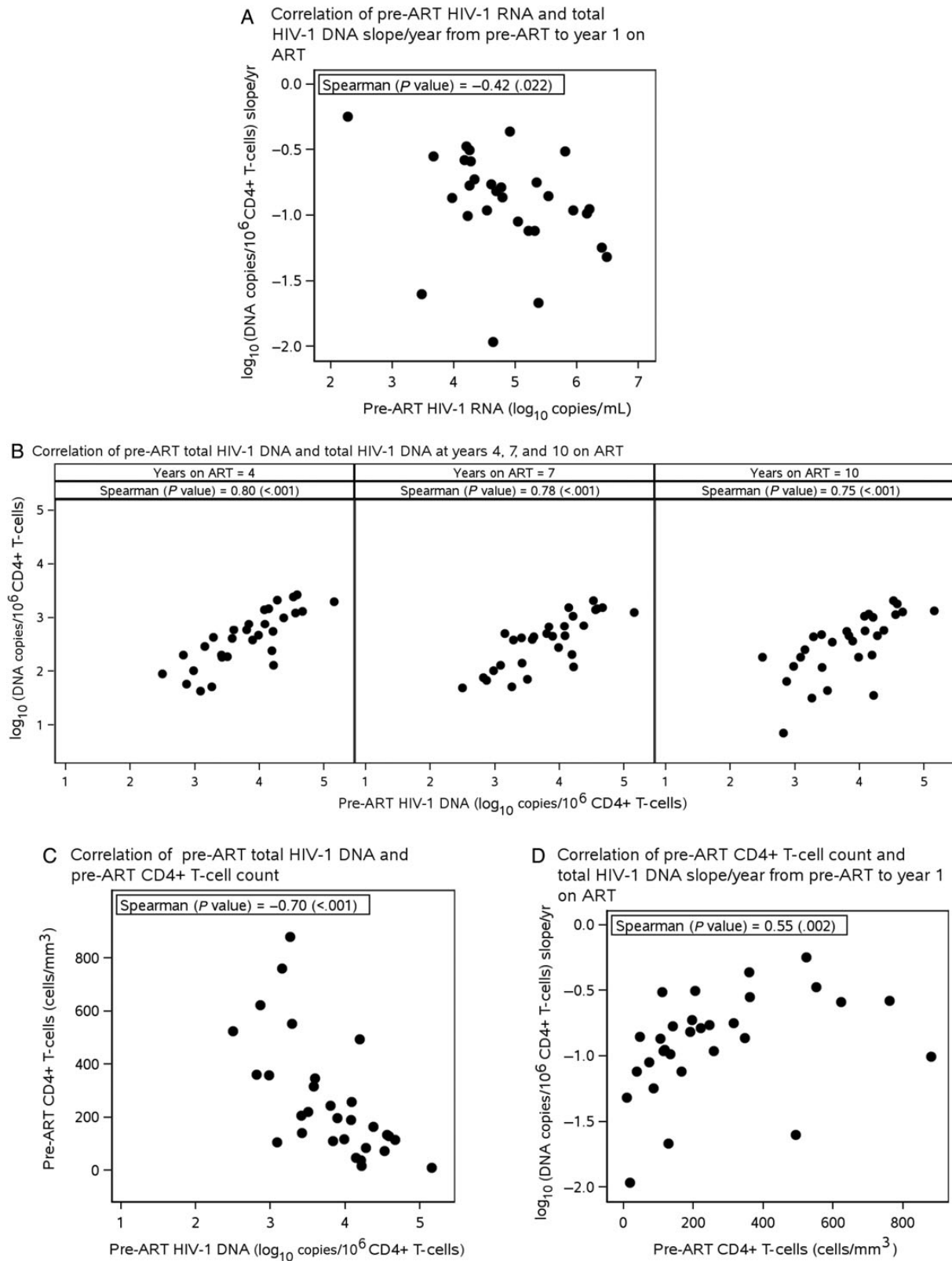
Figure 3. *A*, Correlation between pre-antiretroviral therapy (ART) plasma human immunodeficiency virus type 1 (HIV-1) RNA ($\log_{10}$copies/mL) and total HIV-1 DNA decline pre-ART to year 1 [$\log_{10}$(copies/10^6 CD4+ T-cells) slope/year]. *B*, Correlation between pre-ART total HIV-1 DNA and total HIV-1 DNA at years 4, 7, and 10 on ART. *C*, Correlation between pre-ART total HIV-1 DNA and pre-ART CD4+ T-cell count. *D*, Correlation between pre-ART CD4+ T-cell count and total HIV-1 DNA slope/year from pre-ART to year 1 on ART.

marker of recent HIV-1 replication. This may be the case for proliferating cell populations in which cell division progressively reduces the frequency of episome-containing cells. Alternatively, episomes may persist in long-lived cell populations that do not proliferate. In this regard, Pace et al [32] showed that episomes have a half-life in vitro that exceeds 30 days. In the current study, a similar decay pattern of 2-LTR circles and total HIV-1 DNA, along with the persistence of 2-LTR circles throughout follow-up in a subset of participants, suggest that 2-LTR circles may persist in long-lived cells and are not necessarily an indicator of ongoing HIV-1 replication.

We examined the correlation between HIV-1 DNA levels and immune recovery and activation. Intriguingly, higher CD4+ T-cell levels at ART initiation were associated with lower HIV-1 DNA levels and smaller declines of total HIV-1 DNA within the first year of ART. The reason for this inverse relationship is not clear but may be a result of fewer productively infected cells in participants with higher levels of CD4+ T-cells. In addition, we did not find an association between HIV-1 decay and CD8+ T-cell activation, suggesting that immune activation is not related to the persistence of HIV-1 DNA. This finding is consistent with the recent report by Poizot-Martin et al [9] that HIV-1 DNA levels and immune activation are not correlated after long-term ART. By contrast, Hatano et al [8] showed a consistent relationship between immune activation and HIV-1 persistence. The latter observations were made following short-term treatment intensification, making them difficult to compare with our study. However, the immunologic assessments in our study have limitations. Our flow data are restricted to a few time points between year 1 and year 4 of ART, and we did not examine activation of CD4+ T-cells. In addition, we only quantified total HIV-1 DNA and immune activation in blood. This may not accurately reflect other sites of HIV-1 persistence, including lymphoid tissues and gut-associated lymphoid tissue [19, 33, 34]. Also, we did not observe an association between persistent plasma viremia and HIV-1 DNA levels. This is consistent with other studies that indicated that most proviruses are defective, that is, incapable of producing infectious virions [27, 35, 36]. There are several possible mechanisms by which intact HIV proviruses in CD4+ T-cells become silenced, including nuclear retention of multispliced HIV-1 RNA [37], DNA methylation [38], Apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like-induced mutation [39], or histone deacetylation [40]. In addition, it is not known whether the residual virions in plasma are replication competent or defective. Finally, the cellular and compartmental sources of persistent viremia are undefined and may not be PBMCs [25]. Taken together, it is not surprising that the level of total HIV-1 DNA in PBMC is not predictive of residual viremia.

Importantly, long-term persistence of HIV-1 DNA is not an insurmountable obstacle to curing HIV-1 infection. Only a minority of proviruses (<1%) can be activated to produce infectious virus that could lead to relapse of viremia after cessation of ART [36, 41]. Consequently, curative strategies should focus on eliminating the small population of replication-competent proviruses. Furthermore, post-treatment controllers from the Viro-Immunological Sustained CONtrol after Treatment Interruption study have provided a model in which functional cure can be achieved without complete elimination of proviral DNA [42].

In conclusion, our study has revealed the remarkable stability of HIV-1 DNA during long-term suppressive ART. The mechanisms of this HIV-1 DNA stability require further elucidation. A better understanding of the dynamics of proviral persistence is needed to inform therapies to cure HIV-1 infection.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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