Dynamics of Total, Linear Nonintegrated, and Integrated HIV-1 DNA In Vivo and In Vitro

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Background. In patients infected with human immunodeficiency virus type 1 (HIV-1), HIV-1 DNA persists during highly active antiretroviral treatment, reflecting long-lived cellular reservoirs of HIV-1. Recent studies report an association between HIV-1 DNA levels, disease progression, and treatment outcome. However, HIV-1 DNA exists as distinct molecular forms that are not distinguished by conventional assays.

Methods. We analyzed HIV-1 RNA levels in plasma, CD4 cell counts, and levels of integrated and nonintegrated HIV-1 DNA in peripheral blood mononuclear cells (PBMCs) from patients with early or chronic infection before and during antiretroviral treatment. We also studied HIV-1 DNA decay in primary CD4 T cells infected in vitro. HIV-1 DNA was analyzed using an assay that is unaffected by the location of HIV-1 integration sites.

Results. HIV-1 RNA levels and total HIV-1 DNA levels decayed rapidly in patients during receipt of suppressive antiretroviral therapy. Ratios of total HIV-1 DNA levels to integrated HIV-1 DNA levels were high before initiation of therapy but diminished during therapy. Levels of linear nonintegrated HIV-1 DNA decayed rapidly in vitro ($t_{1/2} = 1–4.8$ days).

Conclusion. Total HIV-1 DNA decays rapidly with suppression of virus replication in vivo. Clearance of HIV-1 DNA during the first 6 months of therapy reflects a disproportionate loss of nonintegrated HIV-1 DNA genomes, suggesting that levels of total HIV-1 DNA in PBMCs after prolonged virus suppression largely represent integrated HIV-1 genomes.

In HIV-1–infected patients, highly active antiretroviral treatment (HAART) can lead to a substantial decrease in virus replication and to undetectable levels of HIV-1 RNA in plasma [1, 2], but HIV-1 DNA remains detectable in cells from most HIV-1–infected patients during sustained suppressive HAART. Recent studies report a potential link between HIV-1 DNA levels and disease progression, treatment success, and degree of virologic suppression during HAART [3–7]. HIV-1 DNA exists in a linear nonintegrated form, a circular nonintegrated form, and as an integrated provirus. Integrated HIV-1 provirus is a fundamental constituent of the latent reservoir, the major barrier to viral eradication [8–11]. This latent reservoir has a very long half-life; Siliciano et al. [12] observed a half-life of $\sim 44$ months among individuals followed for up to 7 years. Studies investigating the kinetics of the latent reservoir demonstrated a biphasic decay of latently infected CD4 T cells after virus suppression, suggesting that the decay dynamics during the early phase of infection may reflect the kinetics of linear
nonintegrated HIV-1 DNA [13]; however, HIV-1 DNA was not directly measured in this study.

Circular and integrated forms of HIV-1 DNA are thought to be stable [12, 14–16], whereas linear nonintegrated forms are thought to be labile [17–19]. However, few studies have directly addressed the decay of integrated and nonintegrated HIV-1 DNA in different clinical settings.

Furthermore, the biological significance of linear nonintegrated HIV-1 DNA is unclear. One study demonstrated limited transcription of nef by nonintegrated HIV-1 DNA in quiescent T cells [20], and another study showed transient expression of early and late messages by nonintegrated HIV-1 DNA [19]. In vitro decay analysis in the latter study suggested a rapid rate of decay of linear nonintegrated DNA in metabolically active transformed and primary CD4 T cells.

Most recent studies investigating the dynamics of the latent reservoir and kinetics of HIV-1 DNA have used terminal dilution coculture assays and direct measurement of HIV-1 DNA, respectively [21, 22]. Neither method distinguishes between the different molecular forms of HIV-1 DNA or their relative contributions to latency. Some assays that do distinguish between various forms of HIV-1 DNA rely on sequence-specific primers that recognize repetitive host-genome motifs [23, 24] and may be confounded by variation in the proximity of integration sites to repetitive motifs [23, 25].

The aim of the present study was to determine the decay characteristics of total, nonintegrated, and integrated HIV-1 DNA in vivo and in vitro, using an assay that is not affected by the sites of HIV-1 integration.

SUBJECTS, MATERIALS, AND METHODS

Patients with early infection were from the San Diego primary infection cohort [26]; these patients initiated antiretroviral therapy within 6 months after seroconversion and were selected on the basis of available samples and successful suppression of viremia during treatment. Patients with chronic infection were randomly selected from among the University of California–San Diego subpopulation of AIDS Clinical Trials Group 384 who experienced successful suppression of plasma viremia during their initial treatment regimen [27]. HIV-1 RNA was measured frequently (every 1–4 months), and HIV-1 DNA was measured on or around months 0, 6, and 12. Written informed consent was obtained from all study patients in accordance with local investigational review board guidelines.

Total DNA was extracted from patients’ peripheral blood mononuclear cells (PBMCs) and in vitro–infected CD4 cells, using the Qiagen DNA extraction kit (Qiagen) according to the manufacturer’s protocol. Replicates of 500 ng of total DNA were loaded onto a 0.5% SeaKem Agarose gel (Cambrex); fractionation of DNA was achieved with electrophoresis for 3 h at 60 V. The ~20-kb high molecular weight (HMW) band was excised from the gel with a Gene-Capsule cutter (Geno Technology) to achieve uniform gel-slice sizes. DNA was extracted from the gel, using the Qiaex II gel extraction kit (Qiagen) in accordance with the manufacturers’ protocol, with the following revisions: the incubation time in QS buffer was extended to 20 min after adding silica; following incubation, the silica was washed 3 times in buffer QXI and 3 times in buffer phosphatidylethanolamine. This procedure eliminated 97% to >99% of linear 9-kb HIV-1 DNA and >99% of 2-LTR episomal DNA from the HMW fraction in validation studies (data not shown).

Total DNA was quantified using a real-time polymerase chain reaction (PCR) assay. β-actin–specific primers and probe were provided with the ABI TaqMan β-actin detection reagents (ABI). Real-time PCR was performed in 25-μL solutions containing 5 μL of DNA target, 12.5 μL of TaqMan Universal Master Mix (ABI), and 7.5 μL of primers and probe (400 nmol/L forward and reverse primers, with a 200 nmol/L probe) under the following conditions: 50°C for 2 min and 95°C for 10 min, followed by 95°C for 15 s and 60°C for 1 min (45 cycles). A standard curve ranging from 30 to 0.1 ng of genomic DNA was generated using reference human DNA (TaqMan Control Genomic DNA). Sample DNA content was calculated using the sample cycle threshold signal values correlated to the standard curve cycle threshold values.

HIV-1 DNA levels were ascertained for both total DNA and HMW DNA, using a prototype HIV-1 DNA quantitative PCR assay (Roche Molecular Diagnostics). HIV-1 DNA levels were adjusted to the amount of input DNA and expressed as copies per microgram of DNA. Levels of HIV-1 DNA in total DNA and levels of integrated HIV-1 DNA in HMW DNA were determined directly. Levels of nonintegrated HIV-1 DNA were calculated by subtracting the levels of integrated HIV-1 DNA in HMW DNA from the levels of HIV-1 DNA in total DNA (nonintegrated HIV-1 DNA level = HIV-1 level in total DNA-integrated HIV-1 DNA level). The overall coefficient of variation for the Roche DNA assay is <30% for >86% of patient samples [28]. The coefficient of variation was 3% for HMW DNA determinations and 9% for total DNA determinations in separate validation studies (data not shown).

For the in vitro studies, enriched preparations of CD4 lymphocytes were isolated from peripheral blood specimens from 2 healthy, HIV-1-seronegative donors. Briefly, a lymphocyte fraction was obtained by Ficoll–Hypaque density gradient centrifugation. CD4+ lymphocytes were purified by negative selection with magnetic cell sorting, using the CD4+ T Cell Isolation Kit (Miltenyi Biotec). Trypan blue dye exclusion revealed that the resulting cell preparations were 95% viable; they contained 94–98% CD4+ lymphocytes, as monitored by automated flow cytometry involving fluorescein isothiocyanate–conjugated monoclonal antibodies to CD4.

Infectious virus stocks of the NL4-3 clone of HIV-1 were prepared by transfecting plasmid DNA into the CEM T lymphoblas-
toid cell line with Lipofectin (Invitrogen). For infection, virus stocks were diluted in RPMI 1640 medium to achieve a multiplicity of infection of 0.5 (based on results of the p24 antigen assay; Abbott). Immediately after CD4 cell isolation, aliquots of 3 spotting 10^6 – 5 spotting 10^6 quiescent lymphocytes in 1.0 mL were incubated with virus for 16 – 18 h at 37°C.

Cells were cultured at 37°C in RPMI 1640 medium containing indinavir (0.1 spotting 10^9 262 spotting 10^9 mol/L) to inhibit viral replication and supplemented with L-glutamine (1 mmol/L), penicillin (50 U/mL), streptomycin (50 spotting 10^9 262 spotting 10^9 g/mL), and 5% human AB serum (vol/vol).

Cells used for HIV-1 DNA analysis were sorted for viability, using flow cytometry involving propidium iodide staining.

Linear regression models were used to model log_{10} transformed RNA decay over time for each patient (log_{10} RNA_{ij} = \beta_0 + \beta_i \text{ week}_j, i = 1, \ldots, 12; j = 1, \ldots, t_i, where t_i is the number of visits for patient i). Exponential regression models were used to model DNA decay over time for each patient (DNA_{ij} = \gamma_0 \exp \left[ \gamma_1 \text{ week}_j \right]; graphs are presented on the log_{10} scale). For the RNA analyses, time points were included from 4 weeks after treatment initiation to the time of first RNA suppression (<50 copies per mL of plasma). For DNA analyses, time points were included from treatment initiation through 12 months of follow-up. A Wilcoxon rank-sum test was used to compare average slopes between patients with early and those with chronic infection. Wilcoxon rank-sum tests were also used to compare differences between patients with early and those with chronic infection with respect to total, integrated, and linear nonintegrated DNA levels before HAART initiation and total, integrated, and nonintegrated DNA levels during HAART. Wilcoxon signed-rank tests were used for within-patient comparisons, namely for comparing total, integrated, and nonintegrated DNA levels before and after HAART initiation (overall and separately for patients with early infection and patients with chronic infection). Spearman rank correlation coefficients are presented to describe the associations between integrated and nonintegrated DNA separately for all patients, those with early infection, and those with chronic infection. P values of \leq 0.05 are considered statistically significant. P values of > 0.05 but \leq 0.1 are considered borderline significant. No adjustments were made for multiple comparisons. For RNA values below the detection limit (<50 copies per mL of plasma), the detection limit was used.

**RESULTS**

**Study population.** Table 1 shows plasma HIV-1 RNA levels and CD4 cell counts before HAART initiation (i.e., at baseline) and during HAART (i.e., during virus suppression). All patients reached complete and sustained virus suppression (defined as <50 HIV-1 RNA copies per mL of plasma) during HAART. The median HIV-1 plasma RNA level was higher among patients with early infection, but the difference was not statistically significantly different from that among patients with chronic infection (P = 0.2). The median CD4 T cell count before initiation of

<p>| Table 1. HIV-1 RNA loads, CD4 cell counts, and highly active antiretroviral therapy (HAART) for patients with early or chronic HIV-1 infection. |
|--------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Patient, by group</th>
<th>HIV-1 RNA level before HAART, log_{10} copies/mL plasma</th>
<th>CD4 cell count, cells/µL blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early infection</td>
<td>Before HAART During HAART</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>5.76    497 577 ZDV,3TC, NFV</td>
<td></td>
</tr>
<tr>
<td>E2</td>
<td>6.71    496 839 ABC,3TC, ZDV,APVr</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>6.18    532 500 ABC,3TC, ZDV</td>
<td></td>
</tr>
<tr>
<td>E4</td>
<td>5.45    311 325 ZDV,3TC,APVr</td>
<td></td>
</tr>
<tr>
<td>E5</td>
<td>6.30    308 842 d4T,3TC,ABC,APVr</td>
<td></td>
</tr>
<tr>
<td>Overall, median value</td>
<td>6.18 496 577</td>
<td></td>
</tr>
<tr>
<td>Chronic infection</td>
<td>Before HAART During HAART</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>6.06    27 137 ZDV,3TC, EFV</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>4.71    166 349 ZDV,3TC, EFV</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>5.68    153 425 ddi, d4T, EFV, NFV</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>6.63    7 557 ddi, d4T, EFV, NFV</td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>6.10    64 535 ddi, d4T, EFV</td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>5.20    271 415 ZDV,3TC, NFV</td>
<td></td>
</tr>
<tr>
<td>C7</td>
<td>5.20    19 272 ZDV,3TC, NFV</td>
<td></td>
</tr>
<tr>
<td>Overall, median value</td>
<td>5.68 64 415</td>
<td></td>
</tr>
<tr>
<td>All patients, median value</td>
<td>5.94 218 462</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** All patients had an HIV-1 RNA level of <50 copies per mL plasma during treatment. Values obtained during HAART were recorded 12 months after HAART initiation. ABC, abacavir; APVr, amprenavir-ritonavir; ddI, didanosine; d4T, stavudine; EFV, efavirenz; NFV, nelfinavir; ZDV, zidovudine; 3TC, lamivudine.
HAART was significantly lower among patients with chronic infection \( (P = .003) \).

**HIV-1 RNA levels.** Decay of HIV-1 RNA levels in patients with early infection and patients with chronic infection were ascertained for a follow-up period of 12 months (figure 1A and 1B). All patients achieved undetectable HIV-1 RNA levels after initiation of HAART and were selected for further HIV-1 DNA decay analysis. HIV-1 RNA decay is shown in figure 1A for all patients; figure 1B shows observed data and linear fits of log_{10} RNA decay for individual patients. One patient (E4) had an HIV-1 RNA level of <50 copies per mL of plasma at the first time point after HAART initiation, and the data could not be modeled.

**HIV-1 DNA levels.** Levels of linear nonintegrated HIV-1 DNA per μg of PBMC DNA (i.e., total DNA level-integrated DNA level) and levels of integrated HIV-1 DNA per μg of PBMC DNA were determined for each patient individually before initiation of HAART and for up to 12 months of follow-up (figure 2). Note that, in figure 2C, the fit of the model appears to be suboptimal for some patients at later observations. This is because the assumed exponential decay model requires monotonicity and focuses on the initial decrease. There were no significant differences in the total DNA level \( (P = .43) \), the integrated DNA level \( (P = .76) \), and the nonintegrated DNA level \( (P = .27) \) at baseline between patients with early infection and patients with chronic infection. However, before HAART initiation, the median total HIV-1 DNA level (431 copies per μg of PBMC DNA) was significantly higher than the integrated HIV-1 DNA level (58 copies per μg of PBMC DNA) for all patients \( (P < .01) \).
For all patients, the total HIV-1 DNA level was markedly reduced after 12 months of HAART ($P = .01$) (figure 3). The non-integrated HIV-1 DNA level in particular diminished dramatically during this period. When considered separately, patients with early infection and patients with chronic infection had substantial reductions in the total HIV-1 DNA level (median reductions, 371 copies per μg of total DNA [$P = .062$] and 351 copies per μg of total DNA [$P = .047$], respectively) and the non-integrated HIV-1 DNA level (median reductions, 314 copies per μg of total DNA [$P = .062$] and 187 copies per μg total DNA [$P = .078$], respectively) after initiation of HAART, although not all reductions were statistically significant (figure 2).
lack of statistical significance likely reflects the small sample sizes for each group of patients.

The median rates of decay of total HIV-1 DNA (−1.03 vs. −0.25; \( P = .048 \)) and nonintegrated HIV-1 DNA (−0.79 vs. −0.32; \( P = .073 \)) were faster for patients with early infection than for patients with chronic infection. The median rates of decay of integrated HIV-1 DNA were not significantly different between groups.

To determine the relationship between the levels and decay rates of total HIV-1 DNA, nonintegrated HIV-1 DNA, and integrated HIV-1 DNA, we compared levels at baseline and levels after HAART initiation. Overall, although the total HIV-1 DNA level and the integrated HIV-1 DNA level were significantly different before HAART initiation (\( P < .01 \)), they were not significantly different after HAART initiation (\( P = .68 \)) (figure 3). As with baseline comparisons, there was no significant difference in the total HIV-1 DNA, integrated HIV-1 DNA, and nonintegrated HIV-1 DNA levels after HAART initiation between patients with early infection and patients with chronic infection (\( P > .10 \) for all comparisons).

**Half-life of nonintegrated HIV-1 DNA in vitro.** Assessment of the molecular half-life of nonintegrated HIV-1 DNA was determined in vitro. The levels of HIV-1 DNA in total DNA and integrated HIV-1 DNA in HMW DNA were determined over the course of 14 days after inoculation of viable, resting CD4 T cells. No integrated HIV-1 DNA was detectable at any time point after infection. HIV-1 DNA levels peaked 5 days after infection and decayed rapidly over the remaining 9 days of the experiment. In duplicate experiments, the calculated half-life of decay was consistent (mean \( t_{1/2} \) 2.9 days) (figure 4).

**DISCUSSION**

A pool of latently infected and resting, long-lived memory cells is established in the earliest stages of HIV-1 infection [8–10, 13, 29], a major obstacle to the eradication of this virus. To better understand the nature of this obstacle and the role that these stable cellular reservoirs of infection play in viral pathogenesis, detection and characterization of the different forms of HIV-1 DNA in infected cells have been performed in patients with early [10, 30] and patients with chronic [31, 32] HIV-1 infection. Measurement of HIV-1 DNA levels during successful therapy in different clinical settings, including when treatment was started early or late during disease [30, 33, 34], during treatment intensification [35], or during treatment interruption [7, 15, 36, 37], has provided additional insights on the relationship between cellular reservoirs of HIV-1 and replication of HIV-1. The pretreatment HIV-1 DNA level in PBMCs may also be a relevant marker in predicting residual viremia for patients receiving an identical HAART regimen [3], and HIV-1 DNA levels and HIV-1 DNA decay rates correlate with levels of residual replication and virus transcription during HAART [34, 37].

Interpretation of HIV-1 DNA data from in vivo studies is complicated, because although different forms of HIV-1 DNA are present, most of these studies have only measured the total HIV-1 DNA level. Because the biological characteristics and impact of the various molecular forms of HIV-1 DNA differ, a more precise analysis of HIV-1 DNA levels in various clinical settings should involve distinguishing between these forms. It
was the aim of this study to determine the decay dynamics for total, nonintegrated, and integrated HIV-1 DNA in vivo during successful HAART, using assay reagents that have been well validated for HIV-1 in clinical samples. Characteristics of the selected study population permitted some comparison of the decay characteristics for patients with early infection and those with chronic infection. We found that, in both patient groups, nonintegrated HIV-1 DNA appeared to be cleared rapidly upon initiation of HAART.

Furthermore, we showed here for the first time that, during successful therapy (defined as suppression of the plasma HIV-1 RNA level to <50 copies per mL of plasma), the median total and integrated HIV-1 DNA levels did not differ significantly, which presumably reflects the preferential clearance of nonintegrated HIV-1 DNA. These findings are important in regard to the interpretation of HIV-1 DNA data as a marker in HIV-1 disease and therapy evaluation.

Several assays have been used to characterize integrated HIV-1 DNA, linear nonintegrated HIV-1 DNA, and circular forms of HIV-1 DNA in vitro and in vivo [15, 16, 24, 38, 39]. One key problem with PCR assays that use primers targeting repetitive genomic sequence motifs is the potential variability in assay efficiency, which is attributable to the nonrandom distribution of repetitive motifs and the variation in numbers and location of integration sites [23, 25]. These assays might lead to underestimation or overestimation of the integrated HIV-1 DNA level, depending on the nature of the reference standard used. Our approach, adapted from that of others [40, 41], involved a DNA size-separation procedure, which allows for the quantitation of integrated HIV-1 DNA levels without regard for HIV-1 integration sites. DNA gel-separation procedures can lead to cross contamination of fragmented HMW DNA in the low molecular weight (LMW) fraction, with cross-contamination rates of 10%–50%. Therefore, direct quantitation of nonintegrated LMW DNA by means of this procedure is not reliable.

However, cross-contamination rates from LMW DNA into the HMW fraction (including episomal forms) are significantly lower (typically, <1% of LMW DNA is seen in the HMW fraction [K. Koelsch, Y. Kawano, P. Li, and J. Wong, unpublished data]), which makes it possible to accurately determine the amount of HIV-1 DNA in both total DNA and HMW DNA. The separate quantitation of total HIV-1 DNA levels and integrated HIV-1 DNA levels also allowed us to quantify linear nonintegrated HIV-1 DNA levels by subtraction, with excellent reproducibility (coefficients of variation, 0.091 for total DNA and 0.032 for integrated DNA). Our results show that levels of nonintegrated HIV-1 DNA are high during active virus replication but that these forms decay promptly during suppression of virus replication after initiation of HAART.

Clearance of nonintegrated HIV-1 DNA was faster in patients with early infection than in patients with chronic infection, although the difference was not statistically significant. Because the pretherapy RNA levels in patients with early infection are higher than those in patients with chronic infection, it is possible that higher numbers of cells are targeted by replicating virus before therapy initiation and that a more substantial initial clearance of these cells occurs after initiation of HAART. It was not the aim of the study to directly compare DNA decay rates among patients receiving different antiviral regimens; therefore, no effort was made to compare these varying decay rates in detail.

Remarks about the limitations of this study are warranted. First, recent data suggest that CD4 cells serving as latent reservoirs of HIV-1 may consist of phenotypically heterogeneous cellular populations that harbor HIV-1 with discrete genotypes, which, in turn, exhibit discrete clearance kinetics [21, 42]. The present study does not distinguish HIV-1 DNA clearance rates for these separate reservoirs. Such studies would be valuable once reliable markers for these different cell types have been identified. Second, we did not specifically assay for circular episomal forms, because earlier work by others [15, 38] has shown that such forms represent a minority of total HIV-1 DNA and because our validation assays indicate that the gel separation procedure largely excludes episomal circles from the HMW band. However, because direct measurements of linear unintegrated forms were not performed on patient samples, the linear unintegrated HIV-1 DNA levels we computed are in fact a composite of both linear and episomal unintegrated forms.

Before initiation of therapy, episomal forms would be expected to be a small minority of the total unintegrated HIV-1 DNA [38]; however, during HAART, they might become a more sizable constituent. Nevertheless, because total HIV-1 DNA and integrated DNA levels were not significantly different during treatment, this distinction between episomal and linear unintegrated forms should be relatively less important for patients receiving therapy. Finally, it should be noted that the number of patients studied here was small and highly selected for those with a well-documented response to therapy. It is possible that some patients receiving combination antiretrovirals with good but incomplete suppression of viral replication might retain higher levels of detectable linear unintegrated forms [10] or circular episomes [15, 16, 43]. Furthermore, even among these highly selected patients, we cannot exclude the possibility that residual unintegrated DNA persists at low levels or continues to be generated. Indeed, in individual patients, total HIV-1 DNA levels were slightly higher than integrated HIV-1 DNA levels even when assayed 6 months after the start of suppressive therapy. Our in vitro data indicating a short molecular half-life for unintegrated HIV-1 DNA species would support the conclusion that even these low levels of residual unintegrated DNA are the result of ongoing HIV-1 replication [22, 35, 44].

Other studies have reported a correlation between baseline HIV-1 DNA levels and virus rebound after structured treatment interruption, with residual levels of plasma viremia [3, 7, 36], and in response to “standard” HAART [4, 33]. It is not possible
to resolve whether these observations of pretreatment HIV-1 DNA that are a composite of integrated provirus and labile non-integrated forms reveal effects attributable to the size of the stable cellular reservoir or, alternatively, reveal the host and viral factors that correlate with efficiency of viral entry and reverse transcription. An approach such as that taken here may permit such studies to be revisited with the aim of distinguishing these effects.

Measuring HIV-1 DNA levels during successful HAART may be an important tool in determining the success of new and future approaches to virus eradication and of long-term therapy regimens that use structured treatment interruption as a way of reducing therapy-related adverse effects and costs. Our study confirms that nonintegrated HIV-1 DNA is short-lived in vitro [40] and extends this observation to the in vivo setting. Nonintegrated HIV-1 DNA decays rapidly during virus suppression, so that, after 6 months to 1 year of suppressive therapy, total HIV-1 DNA levels approximate integrated HIV-1 DNA concentrations. As a consequence, separate measurements of the different forms of HIV-1 DNA during HAART may not be necessary for patients that successfully suppress plasma viremia. New and standardized quantitation protocols for measuring HIV-1 DNA in these patients seem feasible and desirable for longitudinal analysis of HIV-1 treatment strategies.

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References


