Impact of the Timing of Initiation of Antiretroviral Therapy During Primary HIV-1 Infection on the Decay of Cell-Associated HIV-DNA

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Background. Combined antiretroviral therapy (cART) initiation during primary human immunodeficiency virus (HIV) infection (PHI) yields a larger decrease in cell-associated HIV-DNA (CA-HIV-DNA) than initiation during the chronic phase. Our objective was to model the short and long-term decay of CA-HIV-DNA blood reservoir in patients initiating cART during PHI and to assess the impact of the timing of cART initiation on CA-HIV-DNA decay.

Methods. We included patients enrolled during PHI in the Agence Nationale de Recherche sur le Sida PRIMO cohort, treated within the month following enrollment and achieving sustained virologic response. The decay of CA-HIV-DNA over time while on successful cART was modeled with a 3-slope linear mixed-effects model according to the delay between estimated date of infection and cART initiation.

Results. Three hundred twenty-seven patients were included, accounting for 1305 CA-HIV-DNA quantifications. Median time between infection and cART initiation was 41 days (interquartile range, 33–54 days). Median follow-up under cART was 2.3 years (range, 0.4–16.6 years). The timing of cART initiation had significant impact on the first slope of decrease: The earlier cART was initiated after HIV infection, the faster CA-HIV-DNA level decreased during the first 8 months of cART: −0.171, −0.131, and −0.068 $\log_{10}$ copies/10^6 peripheral blood mononuclear cells (PBMCs) per month when cART was initiated 15 days, 1 month, and 3 months after infection, respectively ($P < .0001$). The predicted mean CA-HIV-DNA level achieved after 5 years of successful cART was 1.62 and 2.24 $\log_{10}$ copies/10^6 PBMCs when cART was initiated 15 days and 3 months after infection, respectively ($P = .0006$).

Conclusions. This study provides strong arguments in favor of cART initiation at the earliest possible time point after HIV infection.

Keywords. HIV-1 DNA reservoir; slopes of HIV-DNA decay; primary HIV infection; remission; cohort.

More and more national human immunodeficiency virus (HIV) treatment guidelines recommend initiation of combined antiretroviral therapy (cART) as soon as possible at HIV type 1 (HIV-1) diagnosis, based on the benefits of early treatment on mortality, AIDS-related morbidity, and prevention of HIV sexual transmission [1, 2].

Although cART is able to achieve sustained control of viral replication in blood plasma, it cannot, to date,

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yield viral eradication. Indeed, during the earliest phases of primary HIV infection (PHI), HIV establishes a reservoir in its target cells, particularly in the CD4 memory T-cell subsets [3]. The half-life of these latent cells can reach up to several years and supplies to the persistence of the infection. An easy and reproducible way to measure HIV-1 reservoirs is the quantification of HIV-DNA level in peripheral blood mononuclear cells (PBMCs) [4]. This total cell-associated HIV-DNA (CA-HIV-DNA) level is a predictor of disease progression, independent of CD4 cell count and HIV-RNA load [5–8]. In addition, whereas plasma HIV-RNA falls rapidly below the level of detection of commercial kits during cART, CA-HIV-DNA remains detectable, and its quantification—and decrease—might be of interest when assessing the long-term efficacy of cART on reservoirs.

Very low CA-HIV-DNA loads are observed in 2 particular populations of HIV-infected individuals: the HIV controllers (who control spontaneously viral replication [9]) and the post-treatment controllers (in whom cART was initiated early during PHI, and who were subsequently able to control viral replication for several years after cART interruption [10]). Thus, reducing the CA-HIV-DNA reservoir as much as possible can be an interesting aim, as it could be a criterion for cART reduction or interruption [11–14].

Scarce data are available on the decay of CA-HIV-DNA in patients treated during chronic HIV infection (CHI), suggesting a modest decay that was blunted after 4–5 years of uninterrupted cART [15, 16]. Other studies compared patients treated during CHI to patients treated during PHI and found a faster decay and a more important reduction in the CA-HIV-DNA level among those treated during PHI [17, 18]. Furthermore, 2 recent studies in monkeys underlined the impact of the timing of cART initiation after infection [19, 20]. Thus, acute PHI seems to be a window where the biggest impact on the reduction in CA-HIV-DNA reservoir may be achieved. Indeed, early initiation of cART during PHI has an impact on the earliest stages of HIV infection establishment, characterized by major amplification of the viral replication [21], viral dissemination [22], intense immune responses, and immune activation associated with a high cytokine production [23].

The aim of this study was to model the short- and long-term decay of the CA-HIV-DNA reservoir in patients initiating cART during PHI, and to assess the impact of the timing of cART initiation during this stage on subsequent CA-HIV-DNA level decay.

METHODS

Patients

The Agence Nationale de Recherche sur le Sida (ANRS) PRIMO CO6 is an ongoing multicenter French cohort recruiting, since 1996, patients presenting with PHI. The study protocol was approved by the Paris Cochin Ethics Committee (Paris, France) and all patients gave written informed consent. PHI was diagnosed on the basis of a negative or incomplete Western blot (WB) with detectable HIV-1 RNA or an interval of <3 months between a negative and a positive enzyme-linked immunosorbent assay (ELISA). PHI was defined as "symptomatic" if at least 1 symptom associated with the acute HIV syndrome was present (fever, enlarged lymph node, pharyngitis, skin rash, etc) [3]. The date of infection was estimated as the date of symptoms onset minus 15 days, or, in asymptomatic patients, the date of the incomplete HIV-1 WB minus 30 days or the midpoint between a negative and a positive ELISA result. The timing of treatment initiation was defined as the delay between the estimated date of infection and the date of treatment initiation. Treatment interruption was defined as the discontinuation of all antiretroviral drugs, declared by the patient or decided by the physician (changes in cART regimens were not considered as interruptions). All patients were treatment-naive at enrollment. No specific recommendations were made in France for PHI before 2002; specific guidelines are regularly published since then and are based on CD4 cell count and/or the presence of clinical symptoms [24]. Among patients who were treated within the first month following PHI diagnosis, ie, enrollment in the cohort, we selected 327 patients remaining on uninterrupted successful cART (ie, on virologic response [HIV-RNA <50 copies/mL] at month 6 following cART initiation and maintained thereafter) and for whom at least 2 frozen blood samples were available for quantification of CA-HIV-DNA at cART initiation and while on cART.

Virologic Procedures

Total CA-HIV-DNA levels were determined on frozen samples in a centralized laboratory at baseline, at month 6 and month 12, and every year thereafter as long as the patient was on cART with a suppressed plasma HIV-RNA. In brief, total cellular DNA was extracted from frozen whole blood samples. Total CA-HIV-DNA was quantified by real-time HIV-1 DNA polymerase chain reaction (PCR) (Biocentric, Bandol, France) as described elsewhere [25]. The lower level of detection was 3 copies of HIV-1-DNA per PCR. Multiple replicates were performed to reach a detection limit of 5 copies per million of leukocytes. When CA-HIV-DNA level was undetectable, half of the detection limit was used as quantification. Blood formula was taken into account to express the results in copies per million of PBMCs [25].

Statistical Analyses

The decay of CA-HIV-DNA over time while on successful cART was modeled from the time of cART initiation to either treatment interruption, or end of the virologic response, or end

of follow-up. The evolution of the CA-HIV-DNA was analyzed on the common logarithmic scale (log_{10}) by using a mixed-effects model to take into account the correlation between measurements in the same subject [26]. The mixed-effects model allows having different numbers and times of HIV-DNA measurements for each patient. As the decline in the CA-HIV-DNA was not linear, it was modeled as a piecewise linear function. Different time points of slope changes were tested and chosen by minimization of the Akaike information criterion. The model included both fixed and random effects for the intercept and the slopes, which were therefore allowed to vary between subjects (Supplementary Data).

The impact of the timing of cART initiation on CA-HIV-DNA decay was studied with a continuous variable, allowing us to use the specific cART timing of each patient. This variable was the common logarithm of the delay between the estimated date of infection and the date of treatment initiation. Several transformations of this delay were tested; the common logarithm was chosen because it was the one that best fit the data (Akaike criterion). We verified that similar results were obtained when using time (in days) as a linear variable, time squared, square root of the time, and natural logarithm instead of the common logarithm. Assessing the impact of cART timing as a continuous variable has the advantage, compared to a dummy variable, to minimize loss of information, and thus to maximize statistical power.

The model was then adjusted for sex, age at inclusion (<40 or ≥40, to take into account the weakening immune functions in older patients), and calendar effect (1996–2002 vs 2003–2013, as almost all PHI patients were treated before PHI-specific treatment guidelines were introduced in 2002 [24]).

Several sensitivity analyses were undertaken to assess the robustness of the model regarding different hypotheses. First, we excluded the CA-HIV-DNA measurements available after 60 months (ie, 5 years) of cART to assess whether the rarefaction of CA-HIV-DNA levels available after this time had an impact on the estimations. We also restricted the analysis to patients with symptomatic PHI, to have the same algorithm for estimating the date of infection (ie, date of symptoms onset minus 15 days). We restricted the third sensitivity analysis to patients for whom the baseline HIV-1 WB was performed in the central laboratory. We then used the number of HIV-1 antibodies detected on WB to estimate the timing of cART initiation [27, 28]. Several transformations of this number were tested; the cube root of the number of HIV-1 antibodies was chosen because it was the one that best fit the data (Akaike criterion). We verified that similar results were obtained when using number, number square, and square root of the number instead of the cube root.

Statistical analyses were performed with SAS version 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

Baseline Characteristics

Three hundred twenty-seven patients were included in this study between 1996 and 2010, accounting for 1305 CA-HIV-DNA quantifications. Their baseline characteristics are reported in Table 1. More than 90% were symptomatic at the time of PHI; the median delay between the estimated day of infection and the day of cART initiation was 41 days (interquartile range [IQR], 33–54 days). At inclusion, the median CA-HIV-DNA level was 3.46 log_{10} copies/10^6 PBMCs (IQR, 3.04–3.80), the median plasma HIV-RNA was 5.3 log_{10} copies/mL (IQR, 4.8–5.9), and the median CD4 count was 450 cells/μL (IQR, 329–602 cells/μL). The median number of CA-HIV-DNA quantifications per patient was 3 (IQR, 2–4; min–max, 2–18). All patients had a CA-HIV-DNA measurement at cART initiation. Six hundred thirty-two quantifications were performed between month 1 and month 35, 157 between month 36 and month 59, 130 between month 60 and month 119, and 59 between month 120 and month 196.

Main Analysis

The results of the main model are reported in Table 2 and illustrated in Figure 1. The kinetics of CA-HIV-DNA fit with a 3-slope curve over 3 periods: 0–7 months, 8–32 months, and >32 months. There was no association between the intercept—that is, the estimated CA-HIV-DNA level at cART initiation, and cART timing. All slopes were significantly negative, meaning

Table 1. Baseline Characteristics of the Study Population (N = 327)

Characteristics	Distribution
Male sex, %	82.9
Median age at inclusion, y (IQR)	36 (29–43)
Symptomatic at primary HIV infection, %	91.4
Median year of inclusion (IQR)	2002 (1999–2005)
Median delay between infection and cART initiation, d (IQR)	41 (33–54)
Median HIV-DNA level at inclusion, log_{10} copies/0^6 PBMCs (IQR)	3.46 (3.04–3.80)
Median HIV-RNA PVL at inclusion, log_{10} copies/mL (IQR)	5.3 (4.8–5.9)
Median CD4 level at inclusion, cells/μL (IQR)	450 (329–602)
Median duration of follow-up in the cohort, y (IQR)	8.5 (4.3–12.5)
First-line cART including a protease inhibitor, %	81.6
First-line cART including a boosted protease inhibitor, %	47.4
Median duration of uninterrupted cART, y (IQR)	2.3 (1.0–4.6)
Median number of HIV-DNA measurements during cART (IQR)	3 (2–4)

Abbreviations: cART, combined antiretroviral therapy; HIV, human immunodeficiency virus; IQR, interquartile range; PBMCs, peripheral mononuclear blood cells; PVL, plasma viral load.

Table 2. Estimates of the Slopes of Decay of Cell-Associated HIV DNA Level Under Uninterrupted Combination Antiretroviral Therapy (cART) With Virologic Response (<50 Copies/mL From 6 Months), According to Time From HIV Infection to cART Initiation— ANRS PRIMO Cohort (N = 327; 1305 Measurements)

Variable	Unadjusted Estimate	*P* Value[a]	Adjusted Estimate[b]	*P* Value[a]
Intercept				
Time to cART initiation = 1 mo after infection	3.36		. . .	
+1 $\log_{10}$ month of cART initiation timing	+0.039	.8203	. . .	. . .
First slope (0–7 mo)				
Time to cART initiation = 1 mo after infection	−0.131	<.0001	−0.131	<.0001
+1 $\log_{10}$ mo of cART initiation timing	+0.093	<.0001	+0.093	<.0001
Second slope (8–32 mo)				
Time to cART initiation = 1 mo after infection	−0.016	<.0001	−0.016	<.0001
+1 $\log_{10}$ month of cART initiation timing	−0.0020	.8466	−0.0020	.8532
Third slope (>32 mo)				
Time to cART initiation = 1 mo after infection	−0.0021	.0007	−0.0020	.0019
+1 $\log_{10}$ month of cART initiation timing	+0.0021	.2299	+0.0019	.3007

The *P* value associated with "+1 $\log_{10}$ month of cART initiation timing" represents the statistical significance of the impact of cART timing (effect of initiating cART 1 $\log_{10}$ month later).

Abbreviations: cART, combined antiretroviral therapy; HIV, human immunodeficiency virus.

[a] The *P* value associated with the slope "Time to cART initiation = 1 month after infection" represents the statistical significance of the comparison to 0 (no decrease) of cell-associated HIV DNA decay for patients initiating cART 1 month after HIV infection.

[b] Three-slope linear mixed-effects model adjusted for sex, age at inclusion (≤40 or >40 years), and calendar period (1996–2002 vs 2003–2013).

that there was a significant decay of CA-HIV-DNA during all 3 periods. The timing of cART initiation had a statistically significant impact on the first slope (0–8 months), but not on the second and third slopes. The earlier cART was initiated, the steeper was the CA-HIV-DNA decay during the first 8 months of cART ($P < .0001$). For example, the CA-HIV-DNA level decreased by −0.171 $\log_{10}$ copies/10^6 PBMCs/month during the first 8 months when cART was initiated 15 days after infection, by −0.131 $\log_{10}$ copies/10^6 PBMCs/month when cART was initiated 1 month after infection, and only by −0.068 $\log_{10}$ copies/10^6 PBMCs/month when it was initiated 3 months after infection.

The predicted mean CA-HIV-DNA level achieved after 5 years of uninterrupted successful cART was 1.62 $\log_{10}$ copies/10^6 PBMCs (95% confidence interval [CI], 1.39–1.85) when cART was initiated 15 days after infection, 1.86 $\log_{10}$ copies/10^6 PBMCs (95% CI, 1.74–1.97) when cART was initiated 1 month after infection, and 2.24 $\log_{10}$ copies/10^6 PBMCs (95% CI, 2.08–2.40) when cART was initiated 3 months after infection. The differences were statistically significant ($P = .0006$).

Adjusting the model for sex, age at inclusion, and calendar period changed neither the estimates nor their statistical significance (Table 2).

Sensitivity Analyses

The first analysis excluded the data points available after 60 months; 1139 data points of 327 patients were analyzed. The results were consistent with the main model; the third slope (beyond 32 months) maintained almost the same steepness (−0.0027 $\log_{10}$ copies/10^6 PBMCs/month), but it was not statistically significant ($P = .54$), probably due to the smaller number of data points after 32 months (n = 347 in the main analysis, n = 181 in this analysis).

The second analysis excluded the patients who were not symptomatic at the time of PHI. A total of 1165 data points of 299 patients were analyzed. The conclusions of this model were consistent with the main model, except for the third slope, which became weaker (−0.0009 $\log_{10}$ copies/10^6 PBMCs/month) and not significantly different from 0 ($P = .18$).

The third sensitivity analysis defined cART timing according to the number of HIV-1 antibodies on the WB performed in the central laboratory on a sample collected on the day of cART initiation or the day before. Such a WB was available for 136 patients, for whom 550 data points were analyzed. Because of the smaller number of patients and observations for this analysis, a random intercept model was performed instead of a random intercept and slopes model. In line with the previous results, the CA-HIV-DNA level decreased continuously under cART, significantly from 0 to 8 months and after 32 months. In keeping with the main analysis and other sensitivity analyses, the impact of cART timing was statistically significant on the first slope but not on the other ones: The lower the number of HIV-1 antibodies on the WB, the steeper was the decrease in CA-HIV-DNA levels during the first 8 months of cART.

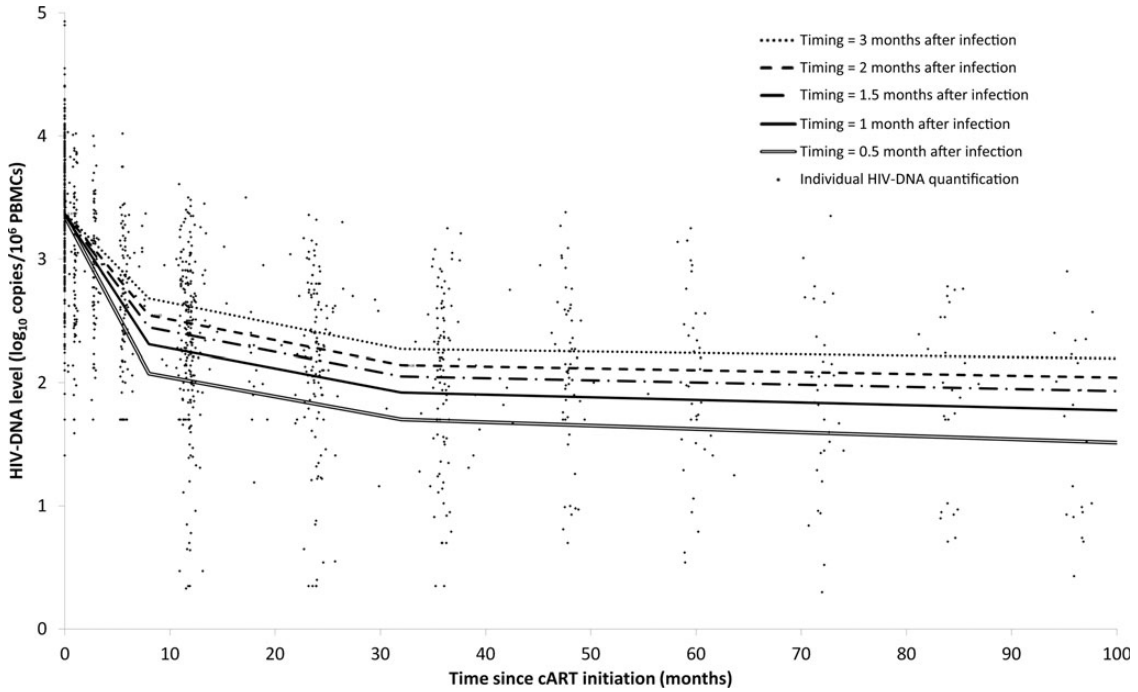

Figure 1. Slopes of decay of cell-associated human immunodeficiency virus (HIV) DNA under uninterrupted combination antiretroviral therapy (cART) with successful virologic response (<50 copies/mL from 6 months) predicted by a mixed-effects model, according to time from HIV infection to cART initiation, in the ANRS PRIMO cohort (N = 327; 1305 measurements). Abbreviation: PBMCs, peripheral mononuclear blood cells.

DISCUSSION

This study highlights that the earlier cART is initiated after HIV infection, the faster the CA-HIV-DNA reservoir decreases during the first 8 months following cART initiation. The decrease in CA-HIV-DNA reservoir was still ongoing even after 8 months, the decay being then slower and not dependent on the timing of treatment. Thus, the benefit of an earlier cART initiation persisted for several years, unlike the blunted decrease after 4–5 years when cART is initiated during the chronic phase of infection [15, 16]. The predicted mean CA-HIV-DNA level reached when cART was initiated 15 days after infection and maintained for at least 5 years was close to that observed in HIV controllers and posttreatment controllers [9, 10].

Previous studies have addressed the issue of treating patients during PHI [17, 29, 30]. Two of these studies compared patients in whom cART was initiated during PHI with patients in whom it was initiated during CHI [17, 29]. Both found a significantly faster decay in CA-HIV-DNA reservoir among PHI patients. Beyond comparing PHI and CHI patients, our findings emphasize that, among patients with PHI, the earlier cART is initiated, the more cART is efficient on CA-HIV-DNA decay, and therefore on CA-HIV-DNA levels reached several years after cART initiation. Recently, Okoye et al compared the reduction of simian immunodeficiency virus (SIV)-DNA reservoir under cART in rhesus macaques in which cART was initiated 7, 10, or 42 days after infection [19]. The reduction in SIV-DNA reservoir was greater for a cART initiation between 7 and 10 days than between 10 and 42 days. Whitney et al even found that, in rhesus monkeys, initiation of cART on day 3 blocked the emergence of proviral SIV-DNA in peripheral blood [20]. These findings are consistent with ours, and also reinforce our choice of a logarithmic transformation of the timing of cART initiation instead of a linear function. Our findings are also in keeping with a recent study in humans which found that CA-HIV-DNA reduction was stronger among a small number of PHI patients who initiated cART while in Fiebig stage III–IV (n = 3) than in Fiebig stage V (n = 6) [18].

The impact of the timing of cART initiation on the decrease in CA-HIV-DNA can be interpreted in light of the different subsets of CD4 T cells infected as PHI progresses. Indeed, transitional and effector memory T cells have shorter half-lives and are infected earlier than naive and central memory T cells. This could be explained notably because of the higher level of immune activation and expression of CCR5 coreceptors on transitional and effector memory T cells than on naive and central memory cells [31, 32]. Thus, the fast first period decay can be interpreted as the impact of cART on the clearance of activated and productive infected T cells, as

previously suggested for plasma HIV-RNA decay [33]. Mostly, the higher benefit on CA-HIV-DNA reduction when cART is initiated earlier can be interpreted as a lower infection rate and the protection, thanks to cART, of long half-life cells [34], also preserving immune functions and innate immunity. Indeed, recent studies showed that deferral of cART initiation after seroconversion had an impact on the normalization of $CD4^+$ T-cell counts, CD4/CD8 ratio, and immune function, and increases immune activation [35–37].

Our study was conducted in patients enrolled in the ANRS PRIMO cohort. To the best of our knowledge, it is the largest cohort of patients followed since PHI and for whom frozen blood samples are available throughout follow-up. The centralization and use of a sensitive and reproducible method for CA-HIV-DNA quantifications permitted us to decrease measurement errors. We conducted several sensitivity analyses that yielded similar results compared to the main analysis, thus strengthening our conclusions.

Our study has several limitations, one of them being that we could not analyze the impact of different treatment options because of the wide diversity in antiretroviral combinations over the study period. Adjusting the model for a calendar effect did not modify the estimates, suggesting that the efficacy of cART was independent of the antiretroviral regimen. We assessed the impact of ritonavir-boosted protease inhibitors containing regimens on CA-HIV-DNA decay, and we did not find any difference compared to the impact of other regimens (data not shown).

We chose to estimate the size of HIV-1 DNA reservoirs by measuring the total CA-HIV-DNA load and not the integrated HIV-DNA load, as CA-HIV-DNA quantification is a well standardized technique and is, to date, the only marker of reservoirs feasible on such a large number of samples [5]. Also, it has been shown that, in patients who had achieved prolonged viral suppression on cART, the level of total CA-HIV-DNA was similar to that of integrated HIV-DNA [38, 39]. Besides, findings from the recent SPARTAK trial suggested that CA-HIV-DNA could be a better predictor of time to rebound after treatment interruption than integrated HIV-DNA [40].

Our study provides new and strong arguments in favor of cART initiation at the earliest possible time point after infection. Not only is it more efficient to initiate cART during PHI compared to CHI on CA-HIV-DNA reduction, but it is also more efficient to initiate cART as soon as possible after infection during the PHI period. This strong benefit of cART timing on HIV reservoirs may have an impact on the HIV diagnosis strategy: It adds further weight for promoting early HIV diagnosis, especially in individuals at high risk of acquiring HIV infection, and, subsequently, early cART initiation as soon as possible after HIV infection. Finally, in some patients in whom cART was initiated very early during PHI, and who did not discontinue it for several years, cART reduction or interruption could be considered if the CA-HIV-DNA load is low enough, opening perspectives for functional remission [10].

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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Potential conflicts of interest. J. G. is a board member of Gilead, ViiV Healthcare, Bristol-Myers Squibb (BMS), and Merck, has received travel expenses from Gilead; his institution has received grants from BMS, Merck, and Roche. P.-M. G. has received payment for development of educational presentations from BMS, Gilead, Janssen, and ViiV. C. G. is a board member of Gilead, has received expert testimony expenses from Janssen Cilag, and is on the speaker's bureau of ViiV Healthcare and Janssen Cilag. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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