Intensive five-drug antiretroviral therapy regimen versus standard triple-drug therapy during primary HIV-1 infection (OPTIPRIM-ANRS 147): a randomised, open-label, phase 3 trial

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Summary

Background Early combination antiretroviral therapy (cART) initiation at the time of primary HIV-1 infection could restrict the establishment of HIV reservoirs. We aimed to assess the effect of a cART regimen intensified with raltegravir and maraviroc, compared with standard triple-drug cART, on HIV-DNA load.

Methods In this randomised, open-label, phase 3 trial, we recruited patients from hospitals across France. Inclusion criteria were primary HIV-1 infection (an incomplete HIV-1 western blot and detectable plasma HIV-RNA), with either symptoms or a CD4+ cell count below 500 cells per µL. Patients were randomly assigned (1:1) to an intensive, five-drug cART regimen (raltegravir 400 mg and maraviroc 150 mg twice daily, and a fixed-dose combination of tenofovir disoproxil fumarate 300 g plus emtricitabine 200 g, darunavir 800 g, and ritonavir 100 g once daily) or a standard triple-drug cART regimen (tenofovir disoproxil fumarate 300 g plus emtricitabine 200 g, darunavir 800 g, and ritonavir 100 g once daily) using a predefined randomised list generated by randomly selected variable block sizes. The primary endpoint was the median number of HIV-DNA copies per 10^6 peripheral blood mononuclear cells (PBMC) at month 24, analysed in the modified intention-to-treat population, defined as all patients who started their assigned treatment. This study is registered with ClinicalTrials.gov, number NCT01033760.

Findings Between April 26, 2010, and July 13, 2011, 110 patients were enrolled, of whom 92 were randomly assigned and 90 started treatment (45 in each treatment group). Six (13%) patients in the intensive cART group and two (4%) in the standard cART group discontinued before month 24. At month 24, HIV-DNA loads were similar between groups (2·35 [IQR 2·05–2·50] $\log_{10}$ per 10^6 PBMC in the intensive cART group *vs* 2·25 [1·71–2·55] in the standard cART group; p=0·21). Eight grade 3–4 clinical adverse events were reported in seven patients in the intensive cART group and seven grade 3–4 clinical adverse events were reported in seven patients in the standard cART group. Three serious clinical adverse events occurred: two (pancreatitis and lipodystrophy) in the standard cART group, which were regarded as treatment related, and one event (suicide attempt) in the intensive cART group that was unrelated to treatment.

Interpretation After 24 months, cART intensified with raltegravir and maraviroc did not have a greater effect on HIV blood reservoirs than did standard cART. These results should help to design future trials of treatments aiming to decrease the HIV reservoir in patients with primary HIV-1 infection.

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Introduction

In France, guidelines now recommend initiation of combination antiretroviral therapy (cART) during primary HIV-1 infection,[1] which is when HIV reservoirs are established.[2] The resulting viral invasion leads to harmful activation of the immune system, and the extent of this activation is related to disease progression.[3] HIV-DNA loads in peripheral blood mononuclear cells (PBMC) give an estimation of the size of HIV reservoirs, and are predictive of both immunological and clinical outcomes in people infected with HIV.[4–6] Early treatment during primary HIV-1 infection might restrict the size of HIV reservoirs,[6,7] ensuring optimal immune restoration[7] and lessening T-cell activation.[8]

HIV blood reservoirs are generally larger at primary HIV-1 infection[9] than during the chronic phase, but the same first-line drugs are recommended for both situations. One challenge is to develop treatment strategies for primary HIV-1 infection that can block HIV expansion and attenuate the so-called cytokine storm.[10]

The OPTIPRIM–Agence Nationale de Recherche sur le Sida et les Hépatites Virales (ANRS) 147 trial was designed to establish whether intensive cART—specifically, darunavir, ritonavir, tenofovir disoproxil fumarate plus emtricitabine, raltegravir, and maraviroc—started early during primary HIV-1 infection has a greater effect on HIV reservoir status than does the recommended triple-drug regimen. We also aimed to assess

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whether patients receiving the intensive cART regimen could achieve so-called post-treatment controller status after interruption of a 2 year treatment course. We chose raltegravir for its potent antiviral effect[11] and maraviroc for both its antiviral activity and its positive effect on the CD4+ T-cell count.[12]

Methods

Study design and participants

OPTIPRIM was a randomised, open-label, phase 3 trial done in 33 French hospitals (appendix). Recruitment began in April, 2010, for a planned period of 2 years.

Patients were eligible for the trial if they presented with primary HIV-1 infection with either symptoms or a CD4+ cell count below 500 cells per µL (ie, they met criteria to receive treatment as recommended by the 2010 national guidelines).[13] Primary HIV-1 infection was defined as detectable plasma HIV-RNA and an incomplete HIV-1 western blot (four or fewer antibody bands), irrespective of ELISA result (positive or negative) and p24 antigenaemia (positive or negative), documented within the 8 days before inclusion.[14] The date of infection was estimated as the date of symptom onset minus 14 days or, in asymptomatic patients, the date of the first incomplete western blot minus 1 month.[5]

The main non-inclusion criteria were pregnancy, breastfeeding, HIV-2 infection, ongoing malignancy, cirrhosis, a hepatic aminotransferase or total bilirubin concentration of ten times the upper limit of normal, a prothrombin time of less than 50%, a glomerular filtration rate estimated by the Cockcroft-Gault method of less than 60 mL/min, hepatitis B surface antigen positivity, and post-exposure prophylaxis received less than 6 months previously. A full list of inclusion and non-inclusion criteria is provided in the appendix.

The study was approved by the Sud-Méditerranée-1 Ethics Committee and the French Health Products Safety Agency, and complied with the Helsinki Declaration. All participants gave written informed consent.

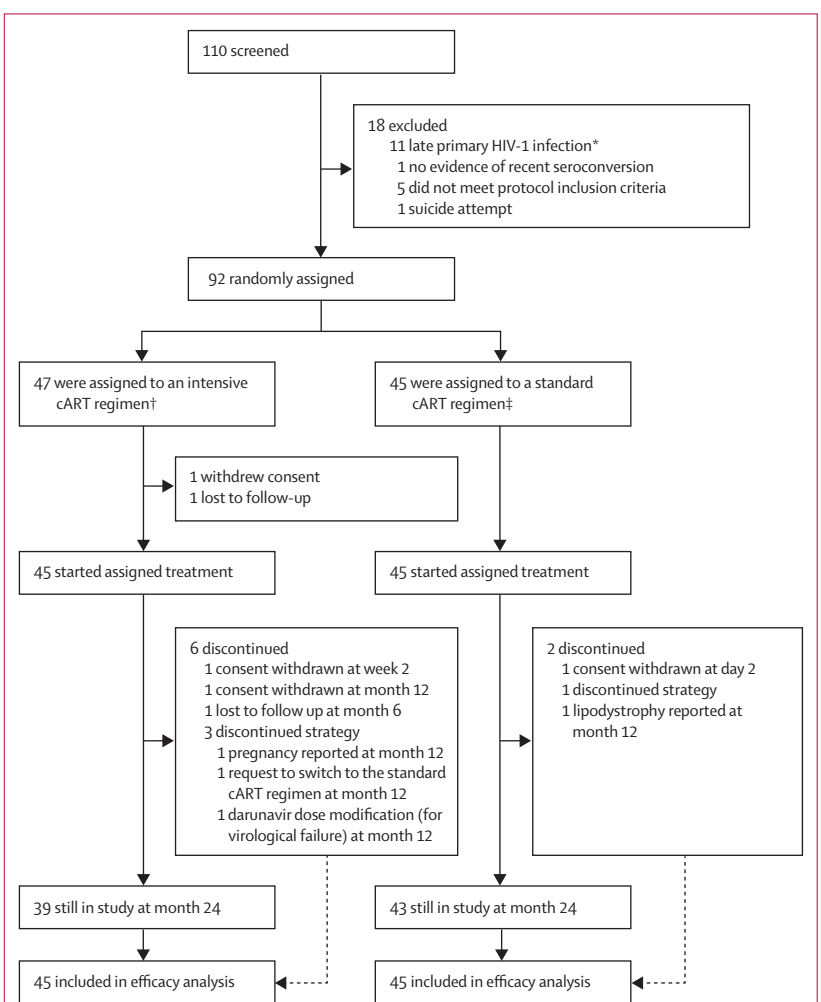

***Figure 1*: Trial profile**
*At least five antibodies on HIV-1 western blot. †Consisting of raltegravir 400 mg, maraviroc 150 mg, tenofovir disoproxil fumarate 300 g plus emtricitabine 200 g, darunavir 800 g, and ritonavir 100 g. ‡Consisting of tenofovir disoproxil fumarate 300 g plus emtricitabine 200 g, darunavir 800 g, and ritonavir 100 g.

Randomisation and masking

We used central computer-generated randomisation to randomly assign patients (1:1) to an intensive, five-drug cART regimen or a standard triple-drug ART regimen using a predefined randomised list generated by randomly selected variable block sizes. Randomisation could not be stratified by site because of the large number of sites relative to the sample size. Patients and care providers were aware of the treatment allocation. Investigators analysing blood samples for HIV-DNA and ultrasensitive HIV-RNA loads were masked to treatment assignment.

Procedure

Patients allocated to the intensive cART regimen were assigned eight pills per day, consisting of raltegravir 400 mg (twice daily), maraviroc 150 mg (twice daily), a fixed-dose combination of tenofovir disoproxil fumarate 300 g plus emtricitabine 200 g (once daily), darunavir 800 g (once daily; two pills), and ritonavir 100 g (once daily). Those allocated to the standard triple-drug regimen were assigned four pills per day, consisting of tenofovir disoproxil fumarate 300 g plus emtricitabine 200 g (once daily), darunavir 800 g (once daily; two pills), and ritonavir 100 g (once daily). To see whether post-treatment control could be observed within a randomised clinical trial, treatment interruption was proposed at month 24 in patients who had less than 50 HIV-RNA copies per mL and a CD4+ cell count of 500 cells per µL or more, or at least 30%. Treatment resumption was recommended if the HIV-RNA load rose to 50 000 copies per mL or more, or the CD4+ cell count fell below 500 cells per µL or below 30%. To follow up patients after this trial, patients were asked whether they would coenrol in the ANRS C06 PRIMO cohort, which is being studied to assess HIV-1 disease from primary infection.

Clinical examinations and laboratory analyses were done at inclusion and at 1, 3, 6, 12, 18, 24, 25, 26, 28, and 30 months. HIV-RNA load, CD4 and CD8 T-cell counts, blood cell counts, and biochemical values were measured locally at each site. Quantification of total cell-associated HIV-DNA was centralised in the virology laboratory at Necker Hospital (Paris, France). Thawed whole frozen blood was analysed with an ultrasensitive real-time PCR method (Generic HIV-DNA assay, Biocentric, Bandol, France) with a detection limit of five copies per PCR.[15] Each entire DNA extract was tested in two to four replicates. Results were reported as the number of HIV-DNA copies per 10^6 PBMC, per 10^6 CD4+ cells, and per mL of whole blood. Samples taken at month 24 were analysed for HIV-RNA with an ultrasensitive method (Ultrasensitive Generic HIV-1 RNA Charge Virale, Biocentric); 3–5 mL of plasma was ultracentrifuged and tested in two replicates, giving a detection threshold of 5–12 copies per mL.

Genotypic resistance tests were done at baseline, using the AC11 ANRS technique. HIV-1 coreceptor use was predicted by a genotypic method based on the SMV Geno2pheno algorithm. HIV-1 subtypes were identified by phylogenetic analysis.[16]

Outcomes

The primary endpoint was HIV-DNA copies per 10^6 PBMC at month 24, analysed in the modified intention-to-treat population, defined as all randomly assigned patients who started their assigned treatment. Other prespecified, secondary endpoints for effectiveness at month 24 included change in the HIV-DNA load from baseline, proportion of patients with plasma HIV-RNA loads below the ultrasensitive detection threshold (5–12 copies per mL), CD4+ and CD8+ cell counts, and change from baseline in the proportion of patients with plasma HIV-RNA loads of less than 50 and less than 400 copies per mL. Two other major secondary endpoints were the proportion of patients who had less than 400 HIV-RNA copies per mL 6 months after treatment interruption and the proportion of post-treatment controllers (defined as having <400 copies per mL 12 months after treatment interruption). A list of other secondary endpoints not presented here can be found in the appendix.

The main safety endpoints were the incidence and severity of adverse events, assessed using the ANRS severity scale,[17] and changes in laboratory variables, analysed in the modified intention-to-treat population. Other safety endpoints were the incidence of disease progression, and self-reported treatment adherence (ANRS questionnaire) at week 2 and months 1, 3, 6, 12, 18, and 24. Patients were asked whether they had missed taking any pills during the previous 4 days or had missed taking at least one pill on the previous Saturday and Sunday, and whether they had followed the correct drug intake during the previous 4 weeks.

An unmasked independent data and safety monitoring board reviewed the data twice, as planned.

Statistical analysis

Using data from the ANRS C06 PRIMO cohort of patients with primary HIV-1 infection,[9] we calculated that we would need to enrol 60 patients to achieve 80% power to detect a difference in HIV-DNA loads of at least $0 \cdot 50$ $\log_{10}$ copies per 10^6 PBMC between the two groups at month 24, with an SD of $0 \cdot 70$, at a significance level of 5% in a two-sided test. We increased this number to 90 to take into account an expected dropout of 5% and to ensure that we would have the required number of patients for substudy analyses.

In the modified intention-to-treat analysis, we used the last observation carried forward method to handle missing data for continuous variables (HIV-DNA, HIV-RNA, and CD4+ and CD8+ cell counts), whereas we regarded missing binary variables (ie, <50 HIV-RNA copies per mL or <400 copies per mL) as a treatment failure. Patients with protocol deviations were analysed in

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See **Online** for appendix

For more on the **AC11 ANRS technique** see www.hivfrenchresistance.org

For more on the **Geno2pheno algorithm** see http://coreceptor.geno2pheno.org/

	Intensive cART regimen (n=45)	Standard cART regimen (n=45)	Total (n=90)
Men	43 (96%)	40 (89%)	83 (92%)
MSM	33 (73%)	35 (78%)	68 (76%)
Age, years	36 (30–47)	35 (26–43)	35 (28–44)
Place of birth			
Europe	37 (82%)	34 (76%)	71 (79%)
Sub-Saharan Africa	4 (9%)	2 (4%)	6 (7%)
Other	4 (9%)	9 (20%)	13 (14%)
Symptomatic primary infection	43 (96%)	44 (98%)	87 (97%)
Acute primary infection*	17 (38%)	21 (47%)	38 (42%)
Time between PHI and enrolment, days	20 (14–28)	20·5 (12–28·5)	20 (13–28)
Time between estimated date of infection and enrolment, days	35 (30–42)	36 (31– 44)	35·5 (30– 43)
CD4 count, cells per μL	481 (356–653)	471 (388–604)	472 (368–640)
CD8 count, cells per μL	1227 (697–1797)	1108 (793–1550)	1135·5 (750–1772)
CD4-to-CD8 ratio	0·39 (0·26–0·72)	0·45 (0·28–0·68)	0·42 (0·26–0·72)
HIV-1 RNA, $\log_{10}$ copies per mL	5·53 (4·99–6·04)	5·20 (4·81–5·80)	5·40 (4·90–5·88)
HIV-1 DNA, $\log_{10}$ copies per 10^6 PBMC	3·66 (3·41–4·06)†	3·52 (3·26–3·94)	3·60 (3·35–4·02)
HIV-1 subtype			
B	31 (69%)	27 (60%)	58 (64%)
Non-B	14 (31%)	18 (40%)	32 (36%)
HIV-1 tropism			
CCR5	43 (96%)	38 (84%)	81 (90%)
CXCR4	2 (4%)	7 (16%)	9 (10%)
Genotypic resistance to drug regimen	0	1 (2%)‡	1 (1%)‡

Data are number (%) or median (IQR). cART=combination antiretroviral therapy. MSM=men who have sex with men. PHI=primary HIV-1 infection. PBMC=peripheral blood mononuclear cells. *Acute HIV infection was defined by the presence of one band or fewer on HIV-1 western blot plus detectable plasma HIV-RNA. †n=44; HIV-DNA data was missing for one participant due to a non-amplificable HIV-1 N subtype. ‡Resistance to raltegravir.

***Table 1*: Demographic and baseline characteristics**

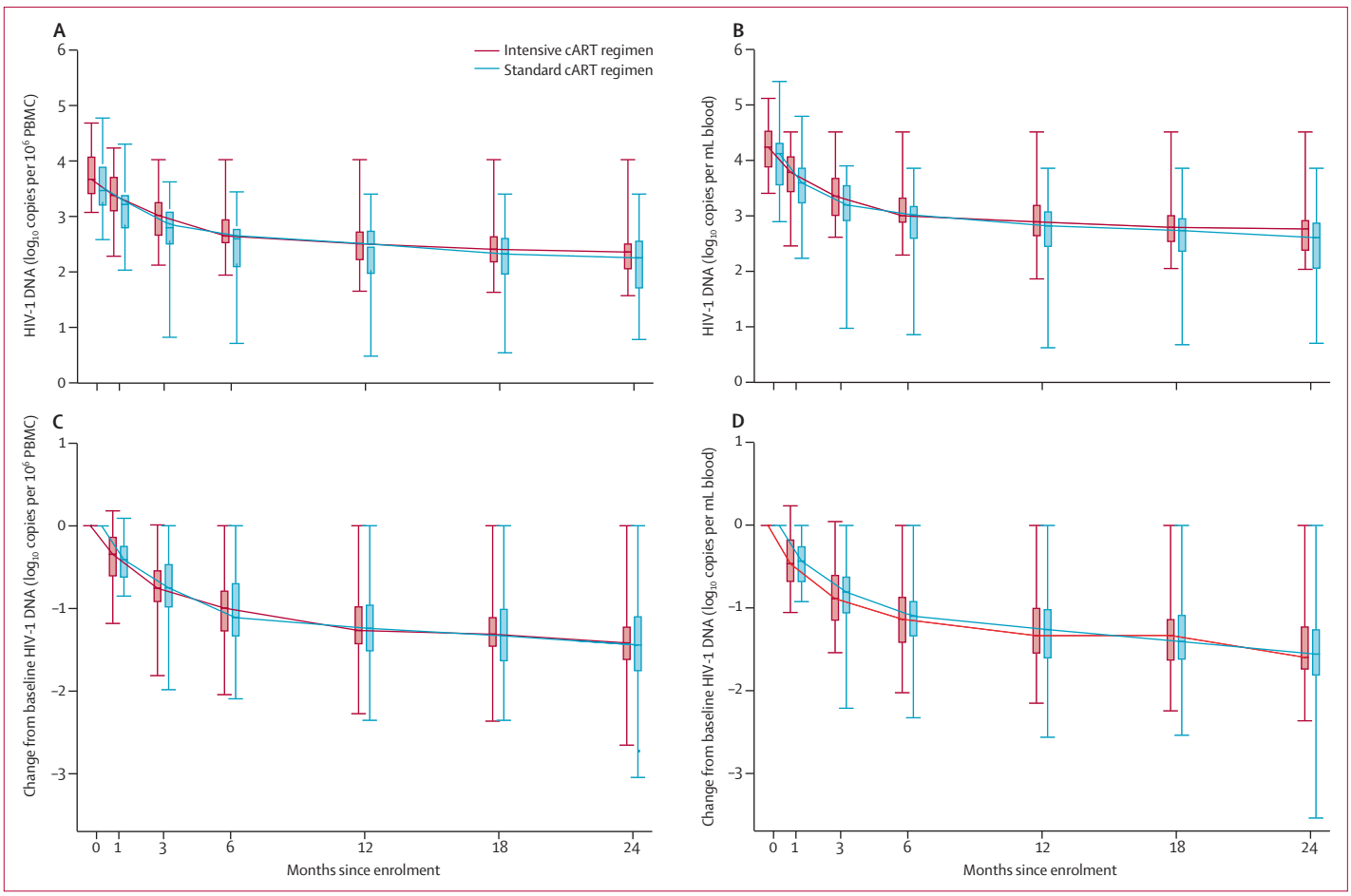

***Figure 2*: Median HIV-DNA load and change from baseline to month 24 in the modified intention-to-treat population**
Boxes show IQR and bars show range. cART=combination antiretroviral therapy. PBMC=peripheral blood mononuclear cells.

	Intensive cART regimen (n=45)	Standard cART regimen (n=45)	p value
HIV-DNA, log_{10} copies per 10^6 PBMC	2·35 (2·05 to 2·50)*	2·25 (1·71 to 2·55)	0·21
HIV-DNA change, log_{10} copies per 10^6 PBMC†	−1·41 (−1·61 to −1·22)*	−1·44 (−1·75 to −1·10)	0·74
HIV-DNA, log_{10} copies per 10^6 CD4	2·82 (2·56 to 3·06)*	2·77 (2·27 to 3·00)	0·17
HIV-DNA change, log_{10} copies per 10^6 CD4†	−1·71 (−1·92 to −1·46)*	−1·66 (−1·97 to −1·28)	0·96
HIV-DNA, log_{10} copies per mL blood	2·76 (2·38 to 2·91)*	2·61 (2·06 to 2·87)	0·09
HIV-DNA change, log_{10} copies per mL blood†	−1·59 (−1·73 to −1·22)*	−1·55 (−1·80 to −1·26)	0·66
Patients with <50 plasma HIV-1 RNA copies per mL	41 (91%; 78 to 97)	42 (93%; 82 to 98)	0·99
CD4 count, cells per μL	731 (616 to 877)	711 (550 to 858)	0·78
CD4 count change, cells per μL†	223 (95 to 463)	211 (96 to 359)	0·53
CD4-to-CD8 ratio	1·06 (0·82 to 1·38)	1·24 (1·03 to 1·45)	0·04

Data are median (IQR) or number (%; 95% CI). We compared differences in HIV-1 DNA, HIV-1 DNA change, CD4, CD4 change, and ratio of CD4 to CD8 between groups with the Wilcoxon rank test. We compared proportions of patients with <50 HIV-1 RNA copies per mL with Fisher's exact test. cART=combination antiretroviral therapy. PBMC=peripheral blood mononuclear cells. *n=44; HIV-DNA data was missing for one participant due to a non-amplificable HIV-1 N subtype. †Between month 24 and baseline.

***Table* 2: Outcomes at month 24 in the modified intention-to-treat population**

the modified intention-to-treat analysis as having missing data from the time of the protocol deviation and were excluded from the per-protocol analysis. Demographic and clinical characteristics were summarised within each treatment group using median and IQR for continuous variables, and frequency and percentage for categorical variables.

We used the Wilcoxon rank sum test to compare the distribution of HIV-DNA loads (log_{10} per 10^6 PBMC) at month 24 between treatment groups. We also used this test to analyse the distribution of changes in the HIV-DNA load from baseline to months 1, 3, 6, 12, 18, and 24 between the two groups. We used the χ^2 test or Fisher's exact test to compare the proportions of patients with plasma HIV-RNA of less than 400 and less than 50 copies per mL in the two groups at months 1, 3, 6, 12, 18, and 24, and the proportion of patients with HIV-RNA below the ultrasensitive detection threshold at month 24. We also used the Wilcoxon rank sum test to compare the distribution of CD4 cell counts and the CD4 to CD8 ratio at month 24 between treatment groups.

We estimated the proportion of patients in whom HIV-RNA remained at less than 400 copies per mL after treatment interruption using Kaplan-Meier curves, and compared the differences between the two groups with a log-rank test. We did not regard transient blips (ie, >400 copies per mL that returned to <400 copies per mL on the subsequent sample 1 month later) as treatment failures. We used the SAS software package version 9.2 and STATA/IC for Windows (32-bit) for all analyses.

This study is registered with ClinicalTrials.gov, number NCT01033760.

Role of the funding source

The study was funded by ANRS. ViiV Healthcare, Gilead, Janssen, and Merck Sharp & Dohme acted as cofunders through an ANRS contract. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All the authors accept responsibility for the veracity and completeness of the data reported. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April 26, 2010, and July 13, 2011, 110 patients were enrolled, of whom 92 were randomly assigned to a treatment group. Two patients in the intensive cART group dropped out before starting treatment, so 45 patients in each group were analysed in the modified intention-to-treat population (figure 1).

A symptomatic primary HIV-1 infection was noted in 87 (97%) of 90 patients, a median of 20 days (IQR 13–28) after symptoms onset (table 1). The median time from the estimated date of infection was 35·5 days (IQR 30–43). HIV-1 western blot confirmed acute primary HIV-1 infection (one or fewer antibody bands) in 38 (42%) of 90 patients. Results of genotypic studies showed that antiretroviral resistance (to raltegravir; 157Q integrase mutation) occurred in one patient in the standard cART group.

At month 24, median total HIV-DNA loads did not differ significantly between treatment groups (2·35 [IQR 2·05–2·50] log_{10} copies per 10^6 PBMC in the intensive cART group *vs* 2·25 [1·71–2·55] log_{10} copies per 10^6 PBMC in the standard cART group; p=0·21; figure 2A, table 2). Similar results were obtained when HIV-DNA load was expressed per mL of blood (figure 2B) or per 10^6 CD4 T cells (table 2), and also in the per-protocol analysis (data not shown). The largest decrease in HIV-DNA load occurred during the first 3 months in both groups: at month 3 the median change from baseline was –0·76 (IQR –0·92 to –0·55) per 10^6 PBMC in the intensive cART group and –0·75 (–0·98 to –0·47) per 10^6 PBMC in the standard cART group (figure 2C); similar results were obtained when change in HIV-DNA load was expressed per mL of blood (figure 2D). HIV-DNA loads continued to decline until month 24, at a similar rate in

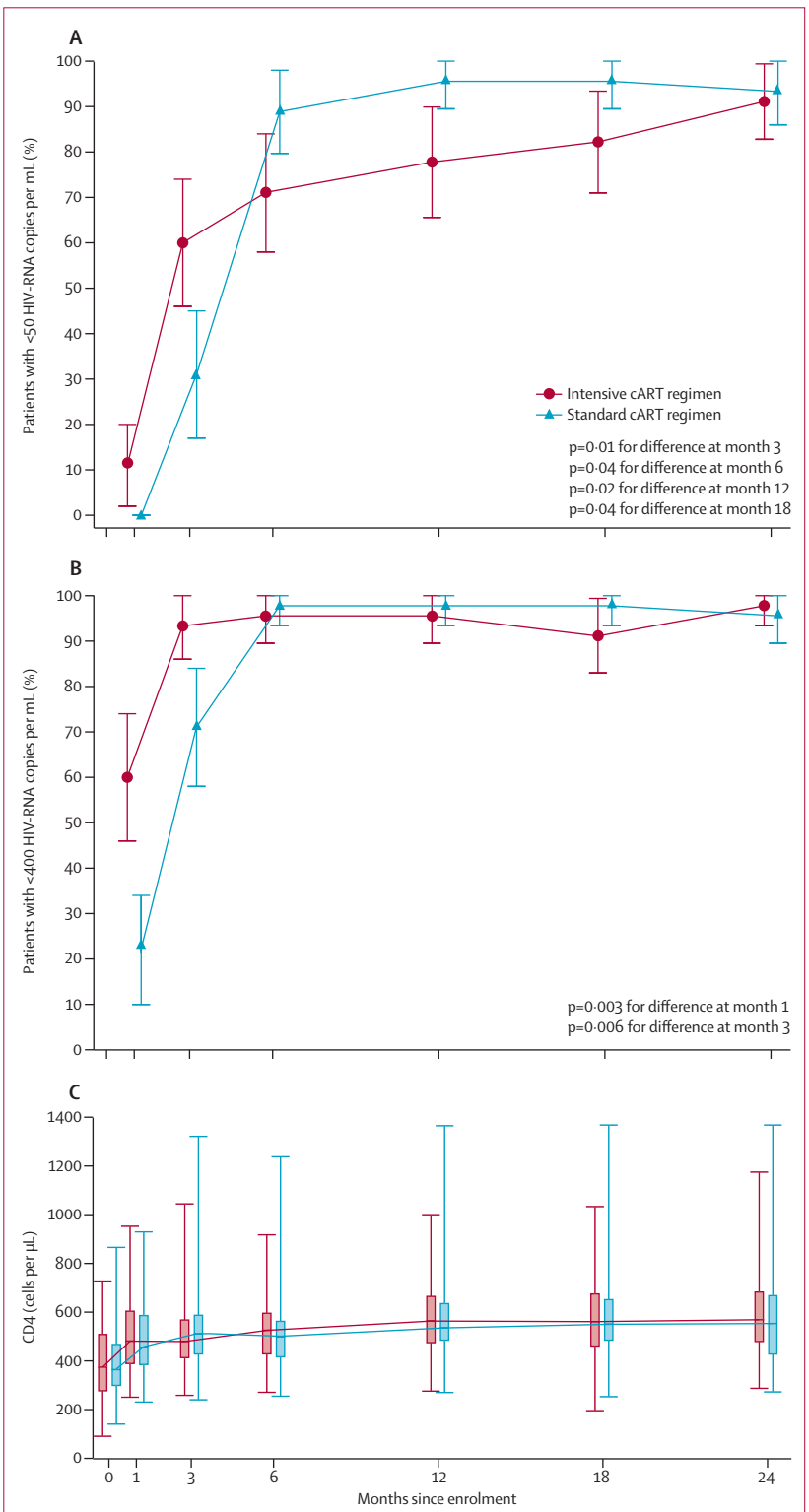

***Figure 3*: Changes in HIV-RNA loads and CD4 cell counts in the modified intention-to-treat population**
Proportion of patients in the intention-to-treat population who had less than 50 HIV-RNA copies per mL (A) and the proportion of those who had less than 400 HIV-RNA copies per mL (B); error bars show 95% CI. (C) Median CD4 cell count; boxes show IQR and bars show range. cART=combination antiretroviral therapy.

both groups. Median change from baseline in HIV-DNA load was –1·26 (IQR –1·43 to –0·98) log_{10} copies per 10^6 PBMC in the intensive cART group versus –1·24 (–1·51 to –0·96) log_{10} copies per 10^6 PBMC in the standard cART group at month 12, and –1·41 (–1·61 to –1·22) and –1·44 (–1·75 to –1·10) log_{10} copies per 10^6 PBMC, respectively, at month 24 (figure 2C).

The proportion of patients with less than 50 HIV-RNA copies per mL at month 24 was similar in the two groups (table 2), as was the proportion of patients with HIV-RNA loads below the ultrasensitive detection threshold (26 [58%] of 45 in the intensive cART group *vs* 31 [69%] of 45 in the standard cART group; p=0·27). At month 3, 27 (60%) of 45 patients in the intensive cART group had less than 50 HIV-RNA copies per mL compared with 14 (31%) of 45 in the standard cART group (p=0·01; figure 3A). Conversely, the proportion of patients with less than 50 HIV-RNA copies per mL was significantly lower in the intensive cART group than in the standard cART group at month 6 (32 [71%] of 45 *vs* 40 [89%] of 45; p=0·04), month 12 (35 [78%] *vs* 43 [96%]; p=0·02), and month 18 (37 [82%] *vs* 43 [96%]; p=0·04). In the per-protocol population, HIV-RNA load was between 400 and 5000 copies per mL in only one (3%) of 39 patients in the intensive cART group at month 6, and in only one (3%) of 39 patients in the intensive cART group at month 18, whereas all other patients who had at least 50 HIV-RNA copies per mL from month 6 onwards also had fewer than 400 copies per mL; figure 3B shows results in the modified intention-to-treat population.

The CD4 T-cell count increased within the first 3 months in both groups. At month 24 the increase was similar between groups, with gains from baseline in median CD4 count of 223 cells per µL (IQR 95–463) in the intensive cART group and 211 cells per µL (96–359) in the standard cART group (figure 3C). The CD4-to-CD8 ratio was slightly higher in the standard cART group than in the intensive cART regimen (p=0·04), and median ratios were greater than 1 in both groups (table 2).

The mean duration of study drug exposure was similar in both groups (table 3). Most adverse events were grade 1 or 2 (data not shown). Two patients stopped the study treatment prematurely at month 12 (one patient in the intensive cART group stopped treatment because of pregnancy and one in the standard cART group stopped treatment because of severe lipodystrophy). Biological abnormalities were also similar (table 3). Small increases in serum creatinine (grade 1) occurred in two patients in the intensive cART group and three in the standard cART group. Two patients in the intensive cART group had grade 3 hypophosphataemia. Grade 3–4 clinical adverse events affected seven patients in each group (one patient in the intensive cART group had two grade 3–4 events; table 3). Three serious clinical adverse events occurred: two (pancreatitis and lipodystrophy) in the standard cART group that were regarded as treatment related, and one event (suicide attempt) in the intensive cART group that was unrelated to treatment. The proportion of patients who completed 24 months of treatment (per-protocol population) who reported that they had taken their prescribed dose of treatment during the previous 4 days did not differ significantly between the two groups, although adherence was slightly lower, although not significantly so, in the intensive cART group at month 12 than in the standard cART group (32 [82%] of 39 *vs* 41 [95%] of 43; p=0·08). The proportion of patients in the per-protocol population who stated that they had not missed a dose on the previous weekend was at least 90% at all visits except in the intensive cART group at month 18 (34 [87%] of 39 *vs* 43 [100%] of 43 in the standard cART group; p=0·02) and month 24 (32 [82%] *vs* 40 [93%]; p=0·18). The proportion of patients who stated that they had strictly followed their prescribed drug intake during the previous 4 weeks was significantly lower in the intensive cART group at month 24 (22 [56%] of 39 *vs* 35 [81%] of 43; p=0·01), but not at month 12 (26 [67%] of 39 *vs* 36 [84%] of 43; p=0·07), or any other timepoint (data not shown). We noted no significant relation between the proportion of patients with undetectable HIV-RNA and the different measures of adherence in either group (data not shown).

At month 24, only four (5%) of the 82 patients still in the study (two in each group) had not reached the

	Intensive cART regimen (n=45)	Standard cART regimen (n=45)
Treatment exposure, weeks	94·6 (4·2–05·4)	97·6 (0·2–107)
Grade 3–4 clinical adverse events	8	7
Upper respiratory tract infection	2	0
Pulmonary infection	0	2
Gastrointestinal infection	1	0
Inguinal lymph infection	0	1
Upper abdominal pain (pancreatitis)	0	1*†
Renal colic pain	1	0
Lipodystrophy	0	1*
Secondary syphilis	1	0
Hepatitis C	0	1
Suicide attempt	1	0
Condyloma surgery	1	1
Depression symptoms	1	0
Grade 3–4 laboratory abnormalities		
Alanine aminotransferase, >5×ULN	0	1
Creatine phosphokinase, >5×ULN	2	3
Hypophosphataemia (<0·452 mmol/L)	2	0
γ-Glutamyl transferase, >3·5×ULN	1	2
Lipasaemia (>3×ULN)	0	1*†

Data are mean (range) or number of events. *Serious adverse event at least possibly related to study treatment . †These two events occurred in the same patient. ULN=upper limit of normal.

***Table* 3: Clinical adverse events and laboratory abnormalities until month 24 in the modified intention-to-treat population**

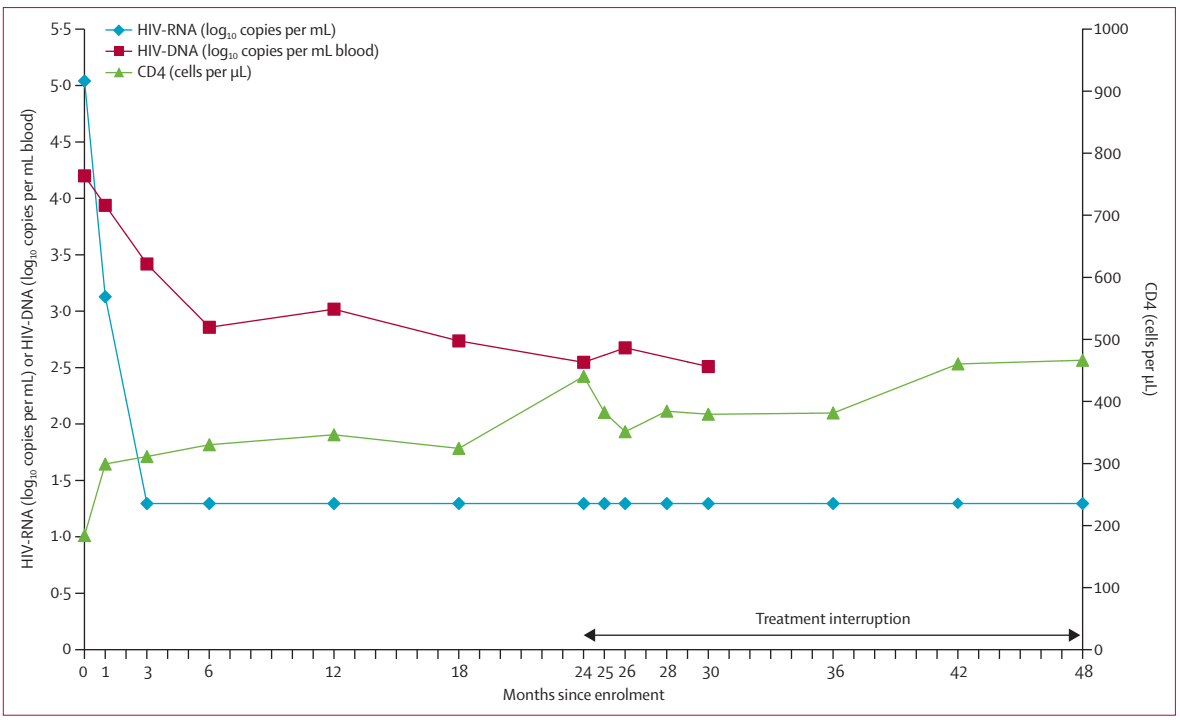

Figure 4: **HIV-RNA, HIV-DNA, and CD4 concentration in one patient in the standard cART group who achieved post-treatment controller status**
This patient maintained a viral load of less than 50 copies per mL for at least 24 months after treatment interruption. cART=combination antiretroviral therapy.

treatment interruption criteria (two patients in the standard cART group had viral loads of 1·89 log_{10} copies per mL and 3·00 log_{10} copies per mL, respectively; in the intensive cART group, one patient had a viral load of 2·00 log_{10} copies per mL, and one had a CD4 count of 427 cells per µL). 29 (78%) of the 37 eligible patients in the intensive cART group and 34 (83%) of the 41 eligible patients in the standard cART group agreed to stop their treatment (appendix). Of these, 19 (66%) of 29 and 11 (32%) of 34 patients, respectively, resumed treatment within 6 months. One case of viral transmission was reported (in the standard cART group); it occurred at 1 month after treatment interruption, despite repeated counselling. 6 months after treatment interruption, viral load remained at less than 400 copies per mL in four untreated patients who stopped treatment at month 24 (one [3%] of 29 patients in the intensive cART group *vs* three [9%] of 34 in the standard cART group; log-rank p=0·62; appendix). 12 months after treatment interruption, viral load remained at less than 400 copies per mL in two patients (one in each group), defining them as post-treatment controllers; one (in the intensive cART group) subsequently lost post-treatment controller status. The other post-treatment controller (from the standard cART group) still had a viral load of less than 50 copies per mL at the most recent follow-up visit, 24 months after treatment interruption (figure 4).[18] This patient was infected by R5 HIV-1 subtype B, and did not have protective HLA B-27/B-57 alleles.

Discussion

Compared with early standard cART, early intensive cART including raltegravir and maraviroc had no additional effect on HIV-DNA load after 24 months of treatment. A marked decline in the size of HIV blood reservoirs was reported at month 24 in both groups—larger than previously reported in patients with chronic HIV on treatment.[19] At month 12, the median change in HIV-DNA load was –1·26 (IQR –1·43 to –0·98) log_{10} copies per 10^6 PBMC in the intensive cART group and –1·24 (–1·51 to –0·96) log_{10} copies per 10^6 PBMC in the standard cART group compared with –1·1 (–1·6 to –0·8) log_{10} copies per 10^6 PBMC in the QUEST study (at 48 weeks), in which patients received zidovudine, lamivudine, abacavir, and amprenavir therapy during primary HIV-1 infection.[20] The marginal difference in effect between our study and QUEST might be due to better diffusion in lymphatic tissues of the drugs assessed in our study, as suggested by Fletcher and colleagues.[21] Of note, most of our patients were recruited at the time of symptomatic primary HIV-1 infection and therefore had high HIV-DNA and HIV-RNA loads at treatment initiation.[9]

The strong effect of both cART regimens on the HIV reservoir might have been also due to the very potent backbone of darunavir and tenofovir disoproxil fumarate plus emtricitabine. Because cART does not eradicate infected T cells that are already quiescent early during primary HIV-1 infection, the maximum HIV-DNA

Panel: Research in context

Systematic review

We did a systematic review to address a clearly defined question: has the effect of intensive highly active antiretroviral therapy (ART) started during early primary HIV-1 infection on HIV blood reservoirs already been assessed? We searched PubMed for articles published in English up to Nov 14, 2014, using the terms "maraviroc", "raltegravir", "reservoir", "acute", and "primary HIV infection". We retrieved four articles[23-26] and manually searched them. All these studies had a different design from ours. The first[26] was a non-randomised trial that included 15 patients presenting with acute primary HIV-1 infection, in which combination ART (tenofovir, emtricitabine, efavirenz, raltegravir, and maraviroc) had a greater effect on HIV-DNA load than during the chronic phase of infection. The second study[24] involved recently infected patients (<6 months), but not acutely infected patients, who were randomly assigned to raltegravir, tenofovir, and emtricitabine (n=15) or to the same regimen intensified with maraviroc (n=15) for 48 weeks. Results were not significant and maraviroc had no added effect on HIV-DNA decrease. The third trial[25] enrolled 34 patients with primary HIV-1 infection who were randomly assigned either to raltegravir and maraviroc plus protease inhibitor and nucleoside inhibitor or to protease inhibitor and nucleoside inhibitor. Results showed that the proportion of patients with a viral load of less than 50 HIV-RNA copies per mL tended to be lower in the raltegravir and maraviroc-intensified regimen group than in the standard regimen group; however, possibly due to small sample size and rate of retention, the results were not conclusive. The fourth[23] assessed outcomes in eight patients with primary HIV-1 infection and eight patients with chronic HIV infection, both of whom received combination ART containing raltegravir, and showed that HIV-DNA loads were lower in patients with primary HIV-1 infection than in those with chronic infection at the end of the study period.

Interpretation

Our study, the largest reported to date, did not show any difference in HIV-DNA load at month 24 between patients assigned to an intensified ART regimen and those assigned to a standard ART regimen. HIV-DNA declined substantially in both groups; more than 90% of patients had less than 50 HIV-RNA copies per mL at month 24. The intensive regimen was particularly effective at reducing the HIV-RNA load in the first 3 months, but a significant proportion of patients had low but persistent viral replication until month 18. This paradoxical result might be due to the transient effect of maraviroc on immune cell trafficking through CCR5 blockade[27] and confirms findings in the previously cited trials.[24,25] We also showed, for the first time in the context of a randomised trial, that a standardised interruption of an early combination ART regimen can lead to post-treatment controller status. The continuous decrease of HIV-DNA until month 24 suggests that more than 2 years of treatment initiated at primary HIV-1 infection would increase the effect of combination ART on HIV in patients with primary HIV-1 infection. Together, these results reinforce the recommendation that treatment started at the time of primary infection is essential, and the findings should contribute to the design of trials aiming to decrease the HIV reservoir and achieve lifelong HIV remission in patients with primary HIV-1 infection.

decrease could have been nearly reached with the standard triple-drug therapy alone. We also noted a strong effect on viral load: more than 90% of patients in both groups had viral load suppression (<50 copies per mL) at month 24. Similarly, the CD4+ cell count rose rapidly and the median CD4-to-CD8 ratio was higher than 1 in both groups. The tolerability was good, with only two treatment-related serious adverse effects (in the standard cART group).

The 90 patients were enrolled within less than 16 months, more rapidly than planned. Thus, this very early treatment initiation was well accepted, even at this sensitive period when patients have just been diagnosed with HIV. Treatment adherence was satisfactory in both groups, despite being slightly lower in the intensive cART group than in the standard cART group. The proportion of patients who were followed up was very high in both groups, with 82 (91%) of the 90 patients still receiving their assigned treatment at month 24.

Despite these good outcomes at month 24, the changes in HIV-RNA load in the intensive cART group were somewhat paradoxical. A greater proportion of patients in the intensive cART group had less than 50 HIV-RNA copies per mL during the first 3 months, possibly due to the presence of raltegravir in the regimen, as previously reported in chronically infected patients[22] and in patients with primary HIV-1 infection (panel).[23] Surprisingly, this superiority did not persist, with a lower proportion of patients in the intensive cART group than in the standard cART group at 12 and 18 months presenting with less than 50 HIV-RNA copies per mL. This result has several possible explanations. First, we cannot exclude the fact that lower adherence was noted in the intensive cART group (possibly due to the twice-a-day prescription compared with the once-a-day prescription in the standard cART group), although we did not note a relation between self-reported adherence and viral load response. Additionally, most patients had less than 400 HIV-RNA copies per mL from 6 months onwards. Secondly, previously unidentified pharmacological interactions could have interfered with the efficacy of the intensive cART regimen. Another possibility is a transient and versatile effect of maraviroc on immune cell trafficking, through CCR5 blockade. This effect of maraviroc on immune activation is controversial.[24,27,28] Moreover, maraviroc has been shown to induce persistent low-level viral replication in vitro.[29] In our study, any such immunomodulatory effect of maraviroc would have diminished over time, because most patients had less than 50 HIV-RNA copies per mL at month 24. Patients in this study received maraviroc for 2 years in the context of disturbed immune homoeostasis.[30] As a result, the expected benefit of the first rapid HIV-RNA decrease might have been eliminated, thus counteracting the effect of raltegravir, by maintaining a low viral replication and limiting the reservoir decrease.[31] A similar trend for a lower HIV-RNA decrease was reported by Markowitz and colleagues,[25] although it was non-significant, probably due to a smaller sample size.

We chose to estimate the size of the HIV reservoir by quantifying total cell-associated HIV-DNA, because it is a standardised and reproducible marker.[15] This robust surrogate marker of HIV reservoir size is well correlated with integrated HIV-DNA and to the productive reservoir measured in infectious units per million cells in effectively treated patients.[32] Total HIV-DNA quantification is better

adapted to studies with a large number of patients than is the labour viral outgrowth assay.[7,9,32] Moreover, its clinical relevance has been shown in several reports.[4–6,26,33]

One patient in the standard cART group had long-term control after 24 months of treatment interruption (<50 copies per mL), which corresponds to the definition of a sustained post-treatment controller.[18] This randomised trial is the first to show that a standardised treatment interruption can lead to post-treatment controller status as defined in the ANRS VISCONTI study.[18] Nevertheless, the case of sexual transmission that we reported reinforces the need to be very cautious with the use of treatment interruption. Moreover, the continuous decline in HIV-DNA reported until month 24 suggests that a longer cART effect on HIV reservoirs would be beneficial for all patients whose treatment is initiated during primary HIV-1 infection. Together, these results should help to guide ART initiated at the time of primary HIV infection and contribute to the design of novel trials aiming to decrease HIV reservoirs and achieve lifelong HIV remission.

Contributors

AC, LM, and CR were the chief investigators, designed the trial, and developed the protocol. AM, CR, VA-F, and M-LC were responsible for the virological investigations. AC, CL, LS, PM, PY, SA, J-MM, CK, CG, FR, JR, IR, BH, and J-FD enrolled patients. LM and GN coordinated the data collection and regulatory requirements, and were responsible for the data analysis. AC, CR, and LM interpreted the data. GN, LM, and AC generated the tables and figures. AC, CR, and LM wrote the manuscript, and all authors reviewed, revised, and approved the final manuscript. AC, CR, LM, CL, GN, VA-F, M-LC, CG, BH, IR, CT, AV, and AL were part of the OPTIPRIM scientific committee.

Declaration of interests

AC reports grants from Merck, and personal fees from Gilead, ViiV, and Janssen. LS reports personal fees from ViiV, Bristol-Myers Squibb, Gilead, and the AIDS International Education Project. V-AF reports grants from Agence Nationale de Recherche sur le Sida (ANRS) and conference fees from Gilead. M-LC reports grants from ANRS. J-MM reports grants and personal fees from Gilead and Merck Sharp & Dohme, and personal fees from Janssen, ViiV, and Bristol-Myers Squibb. CG has received personal fees and other financial support from Janssen, Gilead, ViiV, Abbott, and Merck Sharp & Dohme. FR reports personal fees from Abbvie and Bristol-Myers Squibb, grants and personal fees from Gilead Sciences, Janssen, and Merck Sharp & Dohme, and personal fees from ViiV. JR reports personal fees and travel grants from Abbvie, Bristol-Myers Squibb, Gilead Sciences, Janssen, Merck, Pfizer, Splicos, and ViiV. CR reports grants from ANRS, and has received conference fees from ViiV and Abbvie. All other authors declare no competing interests.

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