

Similar Evolution of Cellular HIV-1 DNA Level in Darunavir/Ritonavir Monotherapy versus Triple Therapy in MONOI –ANRS136 Trial over 96 Weeks

Sidonie Lambert-Niclot^{1*}, Philippe Flandre¹, Marc-Antoine Valantin¹, Cathia Soulie¹, Slim Fourati¹, Marc Wirden¹, Sophie Sayon¹, Sophie Pakianather², Laurence Bocket³, Bernard Masquelier⁴, Georges Dos Santos⁵, Christine Katlama¹, Vincent Calvez¹, Anne-Genevieve Marcelin¹

1 AP-HP, Pitié-Salpêtrière Hospital, INSERM U 943 and Pierre et Marie Curie University Paris, France, **2** INSERM U 943, Paris, France, **3** CHRU de Lille, Virology, Lille, France, **4** AP-HP, Pellegrin Hospital, Bordeaux, France, **5** Centre Hospitalier et Universitaire de Fort-de-France, Fort de France, Martinique, France

Abstract

Background: A higher proportion of intermittent viremia (to have a HIV-1 RNA viral load >50 copies/mL not confirmed) was reported in the boosted protease inhibitor monotherapy arm in some studies including MONOI trial, and that could have an impact on the replenishment of the HIV-1 DNA reservoirs. The HIV-1 DNA level is an interesting marker which reflects the size of cellular HIV reservoir. Our objectives were to study the impact of 96 weeks of Darunavir/ritonavir monotherapy versus a triple standard combination on the HIV-1 blood reservoir and factors associated with HIV-1 plasma DNA at baseline in MONOI trial sub-study.

Methodology/Principal Findings: This sub-study is focused on 160 patients (79 patients in monotherapy arm and 81 in tritherapy arm) for whom blood cells were available both at baseline and at week 96 (W96). Baseline HIV-1 plasma DNA was associated with CD4 nadir, pre therapeutic HIV-1 RNA viral load and baseline HIV-1 RNA measured by ultrasensitive assay. A similar median delta HIV-DNA was observed between D0 and W96 in both arms; 0.35 log copies/10⁶ leucocytes in monotherapy arm versus 0.51 log copies/10⁶ leucocytes in tritherapy arm.

Conclusion/Significance: Despite a higher proportion of intermittent viremia in monotherapy arm, a similar evolution of cellular HIV-1 DNA level was observed between mono and triple therapy arm.

Trial Registration: ClinicalTrials.gov NCT00421551

Citation: Lambert-Niclot S, Flandre P, Valantin M-A, Soulie C, Fourati S, et al. (2012) Similar Evolution of Cellular HIV-1 DNA Level in Darunavir/Ritonavir Monotherapy versus Triple Therapy in MONOI –ANRS136 Trial over 96 Weeks. PLoS ONE 7(7): e41390. doi:10.1371/journal.pone.0041390

Editor: Mark Wainberg, McGill University AIDS Centre, Canada

Received: March 16, 2012; **Accepted:** June 20, 2012; **Published:** July 25, 2012

Copyright: © 2012 Lambert-Niclot et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This work was supported by the Agence Nationale de Recherches sur le SIDA (ANRS), the European Community's Seventh Framework Programme (FP7/2007–2013) under the project "Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)" – [grant agreement n° 223131] and ARVD (Association de Recherche en Virologie et Dermatologie). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: sidonie.lambert@psl.aphp.fr

Introduction

Different strategies of boosted protease-inhibitor (PI) monotherapy have been evaluated [1,2,3] to avoid nucleosides long term toxicities and to decrease cost of treatment. In this context, MONOI trial was designed to evaluate whether darunavir/ritonavir (DRV/r) monotherapy could maintain viral suppression showing non-inferior efficacy, compared with a triple therapy arm of two nucleoside analogues plus DRV/r [4,5]. Recently published European HIV treatment guidelines have introduced the option for a switch to PI monotherapy (either with DRV/r once daily or lopinavir/ritonavir twice daily) for patients who have HIV-RNA levels sustained <50 copies/mL and no history of virological failure (VF) [6]. However these studies reported a higher proportion of intermittent viremia (to have a viral load >50 copies/mL not confirmed) in the monotherapy arm [1,2,3,4,7]

that could have an impact on the replenishment of the HIV reservoirs.

One concern was, despite maintenance of viral suppression in plasma, the use of one drug compared to three drugs could lead to an increase in viral reservoirs including body compartments and target cells. The HIV-1 DNA level is an interesting marker which reflects the size of cellular HIV reservoir. Few studies have already addressed the HIV-1 DNA decrease in chronically infected patients receiving standard triple antiretroviral therapies [8,9]. Finally, no data are available regarding experienced patients receiving DRV/r monotherapy.

Monark study [10] had showed that non responders had significantly higher baseline HIV-1 DNA than responders in induction lopinavir/r monotherapy arm. In the MONOI study, factors associated with VF in patients receiving DRV/r monotherapy were having an initial blip, shorter time of ARV treatment

before monotherapy, an adherence <100% during monotherapy and higher HIV-1 DNA level at baseline [11]. Indeed, a low HIV-1 plasma DNA level, reflecting HIV-1 reservoir, could be related to a strongest control of residual viremia.

Our objectives here were to study the impact of 96 weeks of DRV/r monotherapy versus a triple standard combination on the HIV-1 blood reservoir and factors associated with HIV-1 plasma DNA at baseline in MONOI trial sub-study.

Methods

Participants

Overall 225 HIV-1 infected experienced patients were randomized in the MONOI trial (113 in the triple therapy arm and 112 in the monotherapy arm). This sub-study is focused on 160 patients (79 patients in monotherapy arm and 81 in tritherapy arm) for whom blood cells were available both at baseline and at week 96 (W96) (figure 1).

The MONOI study comprised a first phase where darunavir/ritonavir 600/100 mg bid was introduced for eight weeks as a component of a triple-drug regimen in replacement of the PI, non nucleoside reverse transcriptase inhibitors (NNRTI) or third nucleoside RTI (NRTI). Patients whose HIV-1 VL remained lower than 50 copies/mL four weeks after darunavir induction were randomly assigned 1:1 at day 0 (D0) to continue the triple drug darunavir-containing regimen (darunavir triple therapy) or to stop the two NRTIs (darunavir monotherapy). After screening (W-10), study evaluations were completed at W-4, randomization and at weeks 4, 8 and every 8 weeks thereafter for the duration of the study. The study population consisted of HIV-1 infected patients at least 18 years of age receiving a triple antiretroviral drug regimen. All patients had plasma HIV-1 RNA less than 400 copies/mL for the last eighteen months, based on at least four viral load measurements, and less than 50 copies/mL at screening. Patients had no history of virologic failure while receiving a PI containing regimen, a documented CD4 lymphocytes nadir greater than 50 cells/mm³, and acceptable laboratory results at screening. Patients with an history of HIV-related neurological disease or with hepatitis B co-infection could not be enrolled.

Ethics

The MONOI study was designed and conducted by members of the Agence Nationale de Recherche sur le SIDA et les Hépatites Virales, Paris, France (MONOI ANRS 136 trial). The protocol

was approved by the ethics committee CPP Paris VI Pitié-Salpêtrière and by the Agence Française de Sécurité Sanitaire des Produits de Santé (January 23, 2007 Clinical trial registration. NCT00421551). All patients provided written informed consent.

Description of Procedures

Residual plasma viremia was measured as previously described [12] with a limit of quantification of 1 copy/ml at W-10 and Day 0 (D0). According to the baseline ultrasensitive VL results, we defined 2 groups: W-10 and D0 values of ultrasensitive VL <1 copy/mL and others. Cellular HIV-1 DNA was quantified at D0 and at W96 as previously described [13]. The study definition for VF was: two plasma HIV-1 viral load >400 copies/mL on two weeks apart. We investigated whether some variables (age, sex, CD4 nadir, duration of HIV-infection, duration of previous NRTI, NNRTI, and PI exposure, pre therapeutic VL, baseline HIV-1 RNA measured by ultrasensitive assay, BMI) were associated with log₁₀ HIV-1 DNA at D0. Delta HIV DNA is the change in log₁₀ HIV-1 DNA from D0 to W96 (log copies/10⁶ leucocytes).

Statistical Methods

Between-group comparisons were carried out by the Kruskal-Wallis test for continuous variables and by the Fisher exact test for categorical variables. Pearson and Spearman correlation measure were used to quantify the association between log₁₀ HIV-1 DNA at D0 and some continuous variables (age, CD4 nadir, duration of HIV-infection, duration of previous NRTI, NNRTI, and PI exposure, pre therapeutic VL, BMI). The Pearson coefficient was used when the variance was constant over the errors (homoscedasticity) and the Spearman was used for variance not constant (heteroscedasticity). We investigated whether sex and baseline HIV-1 RNA measured by ultrasensitive assay (2 measures below 1 copy/ml vs. other) were associated with log₁₀ HIV-1 DNA at D0 by the Kruskal-Wallis test. We also investigated whether change in log₁₀ HIV-1 DNA from D0 to W96 was associated with randomized groups, VF and having two consecutive HIV-1 RNA plasma viral load >50 copies/ml by Kruskal-Wallis test. There were adequate number of participants in order to determine the magnitude of the outcome measures.

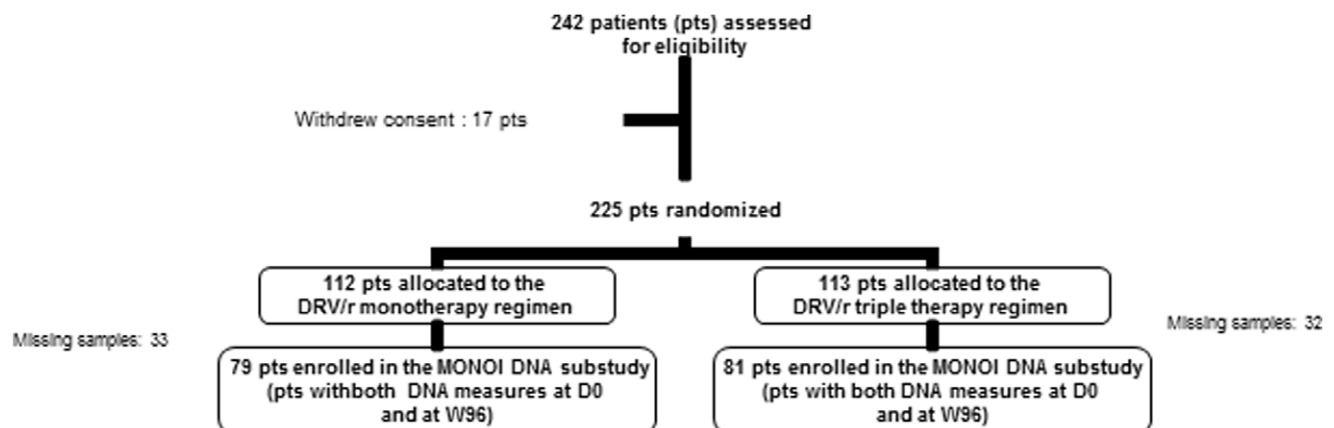


Figure 1. Flow chart.

doi:10.1371/journal.pone.0041390.g001

Results

The patient's characteristics are presented in table 1 and are not significantly different between monotherapy and tritherapy arms. These characteristics are similar to those of all patients included in MONOI (n=225)(all P-values >0.09) [4]. The difference of proportion of intermittent viremia between triple therapy arm and monotherapy arm was not significant (32% versus 46% respectively). The percentages of undetectable HIV-1 DNA viral load (<1.53 log₁₀ copies/10⁶ cellules) were 8% at baseline and 12% at W96, which is in the same order than previously described in patients after long term suppressive ARV therapy [14]. There was no significant difference of proportion of undetectable HIV-1 DNA viral load between monotherapy arm and tritherapy arm. A similar median delta HIV-DNA was observed between D0 and W96 in both arms; 0.35 log copies/10⁶ leucocytes in monotherapy arm versus 0.51 log copies/10⁶ leucocytes in tritherapy arm (p=0.22). The delta HIV-1 DNA viral load was not associated with VF (2 plasma HIV-1 viral load >400 copies/mL on two weeks apart) or to have two consecutive HIV-1 RNA plasma viral load>50 copies/ml. The evolution of HIV-1 DNA viral load was not associated with intermittent viremia and to have and baseline HIV-1 RNA measured by ultrasensitive assay. Factors associated with log₁₀ HIV-DNA at D0 were CD4 nadir (Spearman Test, p=0.001), pre therapeutic VL (Pearson test, p<0.001) and baseline HIV-1 RNA measured by ultrasensitive assay (Kruskal Wallis test, p=0.003) (Figure 2). Factor associated with baseline HIV-1 RNA measured by ultrasensitive assay was only log₁₀ HIV-DNA at D0.

Discussion

A higher proportion of intermittent viremia was reported in the boosted PI monotherapy arm in some studies including MONOI trial, and that could have an impact on HIV-1 DNA reservoirs [1,2,3,4,7]. So it was interesting to compare evolution of HIV-1 plasma DNA viral load between DRV/r monotherapy arm and a DRV/r based triple therapy.

Our results showed that DRV/r monotherapy may have the same impact on reservoirs as a triple regimen in experienced patients after 2 years of treatment. Indeed there was no difference of delta HIV-1 DNA viral load (D0–W96) between the two arms and the observed variations were closed to previously published range [15]. Few studies have already addressed the HIV-1 DNA decrease in chronically infected patients receiving standard triple antiretroviral therapies [8,9]. The MONARK data indicated that a lopinavir/ritonavir monotherapy regimen may have the same impact on reservoirs as a triple regimen in antiretroviral-naive patients after 1 year of treatment [10].

In MONOI trial, baseline HIV-1 DNA was identified as independent predictive factors of virological response in monotherapy arm [11]. This was in keeping with previous observations reporting that the baseline HIV-1 DNA level is associated with virological response on HAART [16,17,18] and in MONARK-study of induction lopinavir/ritonavir monotherapy [10]. A high baseline HIV-1 DNA level thus increased the risk of persistent low-level viremia during antiretroviral treatment [17]. Here we showed that baseline HIV-1 DNA was associated with CD4 nadir, pre therapeutic HIV-1 RNA viral load and residual viremia. Another

Table 1. Characteristics of patients.

	Darunavir/r triple therapy N=81	Darunavir/r monotherapy N=79
Age-year		
Median (IQR)	46 (40–56)	46 (41–51)
Male sex - n (%)	62 (77)	60 (76)
CD4 cells per mm ³ - median (IQR)		
Baseline	591 (393–859)	582 (456–727)
Nadir	208 (144–280)	219 (144–319)
3 classes experience - n (%)	33 (41)	29 (37)
Duration of HIV infection - year		
Median (IQR)	8.1 (4.1–16.3)	11.1 (6.4–16.0)
Duration of ART - year		
Median (IQR)	7.3 (2.8–11.2)	8.2 (4.5–10.7)
Prior PI exposure - n (%)	73 (90)	64 (81)
Regimen at screening - n (%)		
2 NRTIs + PI	64 (79)	50(63)
2 NRTIs + NNRTI	11 (14)	16 (20)
3 NRTIs	5 (6)	12 (15)
Baseline UltraSensitive HIV-1 RNA - n (%)		
<1 copy/ml	30 (37%)	38 (49%)
Other	51 (63%)	40 (51%)
Baseline log ₁₀ DNA/10 ⁶ cellules		
Median (IQR)	2.45 (2.1–2.8)	2.38 (2.1–2.8)
Patient presenting Intermittent viremia - n (%)	26 (32)	36 (46)

IQR: interquartile range; ART: antiretroviral therapy; PI: protease inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: non nucleoside reverse transcriptase inhibitor.

doi:10.1371/journal.pone.0041390.t001

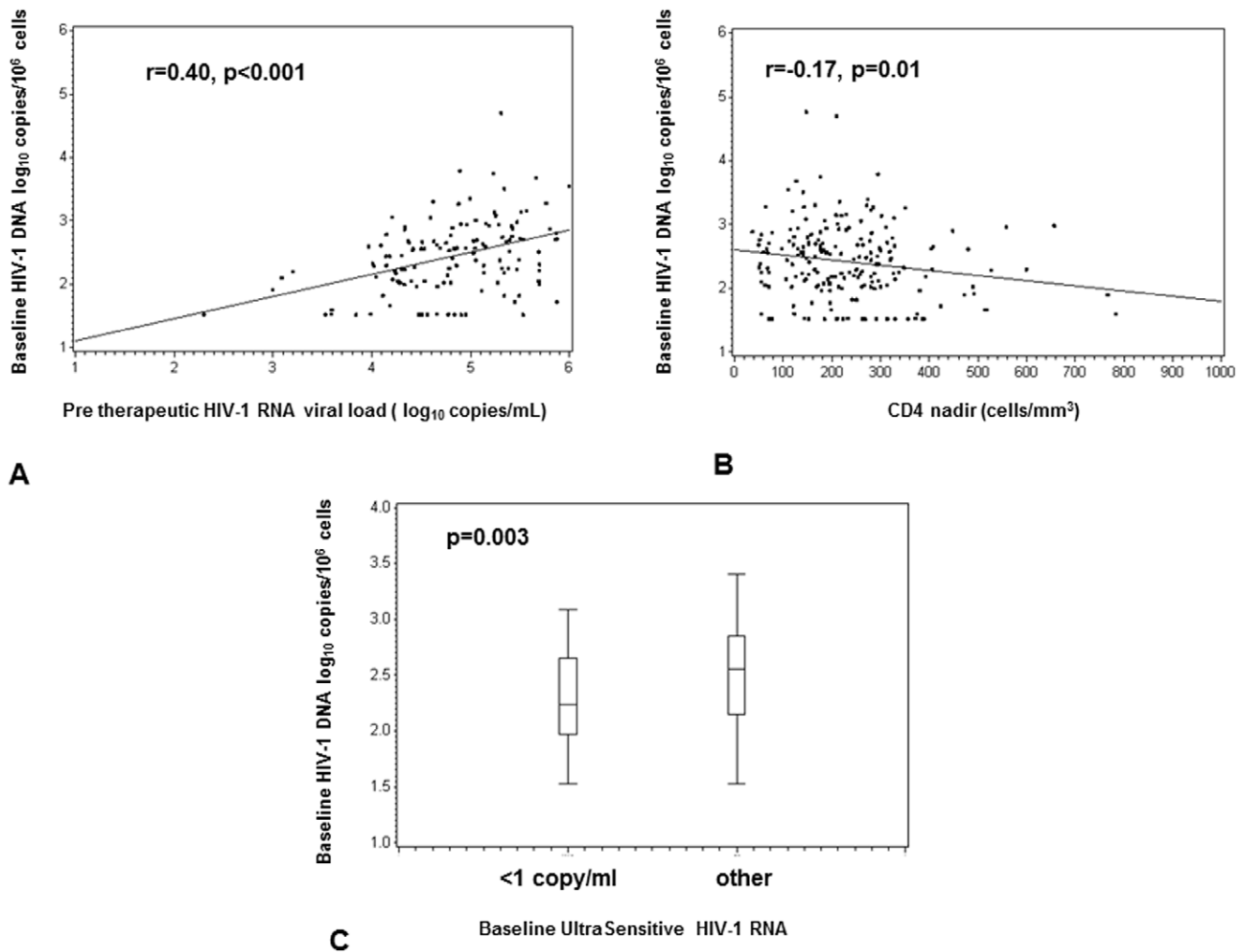


Figure 2. Factors associated with \log_{10} HIV-DNA at baseline. A. pre therapeutic VL (Pearson test); B. CD4 nadir (Spearman test); C. baseline HIV-1 RNA measured by ultrasensitive assay (Kruskal Wallis test). doi:10.1371/journal.pone.0041390.g002

study showed that whatever the duration of treatment HIV-1 DNA level was related with CD4 cell count nadir and plasma HIV-1 RNA zenith [14]. More recently, Chun et al demonstrated that residual plasma viremia correlated with the size of cellular HIV DNA reservoirs in infected patients receiving effective ARV therapy [19]. One limit of our study would be that quantification of total HIV-1 DNA in whole blood is not sensitive to measure the new event of cell infection during the intermittent viremia episodes. In conclusion, despite a higher proportion of intermittent viremia observed in some studies evaluating PI monotherapy strategy, we showed in MONOI trial that this does not seem to affect HIV-1 DNA level.

References

1. Arribas JR, Horban A, Gerstoft J, Fatkenheuer G, Nelson M, et al. (2010) The MONET trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV RNA below 50 copies/ml. *Aids* 24: 223–230.
2. Delraissy JF, Flandre P, Delaugerre C, Ghosn J, Horban A, et al. (2008) Lopinavir/ritonavir monotherapy or plus zidovudine and lamivudine in antiretroviral-naïve HIV-infected patients. *Aids* 22: 385–393.
3. Meynard JL, Bouteloup V, Landman R, Bonnard P, Baillat V, et al. (2010) Lopinavir/ritonavir monotherapy versus current treatment continuation for maintenance therapy of HIV-1 infection: the KALESOLO trial. *J Antimicrob Chemother.*
4. Katlama C, Valantin MA, Algarte-Genin M, Duvivier C, Lambert-Niclot S, et al. (2010) Efficacy of darunavir/ritonavir maintenance monotherapy in patients with HIV-1 viral suppression: a randomized open-label, noninferiority trial, MONOI-ANRS 136. *Aids* 24: 2365–2374.
5. Valantin MA, Lambert-Niclot S, Flandre P, Morand-Joubert L, Cabie A, et al. (2012) Long-term efficacy of darunavir/ritonavir monotherapy in patients with HIV-1 viral suppression: week 96 results from the MONOI ANRS 136 study. *J Antimicrob Chemother* 67(3): 691–5.
6. EACS European AIDS Clinical Society. April 2011, 5–4. 2011, posting date. Clinical Management and Treatment of HIV-infected Adults in Europe. <http://>

Acknowledgments

We thank G. Le Mallier and P. Grange for their technical assistance.

Author Contributions

Conceived and designed the experiments: CK VC AGM MAV PF. Performed the experiments: SLN CS SF MW SS. Analyzed the data: PF SP. Contributed reagents/materials/analysis tools: LB BM GDS SP MAV CK. Wrote the paper: SLN VC AGM PF.

- www.europeanclinicalsociety.org/guidelinespdf/1_Treatment_of_HIV_Infected_Adults.pdf Last accessed February 2012.
7. Pulido F, Arribas JR (2008) Noninferiority and lopinavir/ritonavir monotherapy trials. *Aids* 22: 1696–1697.
 8. Morand-Joubert L, Marcellin F, Launay O, Guiramand-Hugon S, Gerard L, et al. (2005) Contribution of cellular HIV-1 DNA quantification to the efficacy analysis of antiretroviral therapy: a randomized comparison of 2 regimens, including 3 drugs from 2 or 3 classes (TRIANON, ANRS 081). *J Acquir Immune Defic Syndr* 38: 268–276.
 9. Viard JP, Burgard M, Hubert JB, Aaron L, Rabian C, et al. (2004) Impact of 5 years of maximally successful highly active antiretroviral therapy on CD4 cell count and HIV-1 DNA level. *Aids* 18: 45–49.
 10. Avettand-Fenoel V, Flandre P, Chaix ML, Ghosn J, Delaugerre C, et al. (2010) Impact of 48 week lopinavir/ritonavir monotherapy on blood cell-associated HIV-1-DNA in the MONARK trial. *J Antimicrob Chemother* 65: 1005–1007.
 11. Lambert-Niclot S, Flandre P, Valantin MA, Peytavin G, Duvivier C, et al. (2011) Factors associated with virological failure in HIV-1-infected patients receiving darunavir/ritonavir monotherapy. *J Infect Dis* 204: 1211–1216.
 12. Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, et al. (2003) New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 41: 4531–4536.
 13. Avettand-Fenoel V, Chaix ML, Blanche S, Burgard M, Floch C, et al. (2009) LTR real-time PCR for HIV-1 DNA quantitation in blood cells for early diagnosis in infants born to seropositive mothers treated in HAART area (ANRS CO 01). *J Med Virol* 81: 217–223.
 14. Burgard M, Boufassa F, Viard JP, Garrigue I, Ruffault A, et al. (2009) Factors influencing peripheral blood mononuclear cell-associated HIV-1 DNA level after long-term suppressive antiretroviral therapy in 236 patients. *Aids* 23: 2165–2171.
 15. Burgard M, Izopet J, Dumon B, Tamalet C, Descamps D, et al. (2000) HIV RNA and HIV DNA in peripheral blood mononuclear cells are consistent markers for estimating viral load in patients undergoing long-term potent treatment. *AIDS Res Hum Retroviruses* 16: 1939–1947.
 16. Hatzakis AE, Touloumi G, Pantazis N, Anastassopoulou CG, Katsarou O, et al. (2004) Cellular HIV-1 DNA load predicts HIV-RNA rebound and the outcome of highly active antiretroviral therapy. *Aids* 18: 2261–2267.
 17. Havlir DV, Strain MC, Clerici M, Ignacio C, Trabattini D, et al. (2003) Productive infection maintains a dynamic steady state of residual viremia in human immunodeficiency virus type 1-infected persons treated with suppressive antiretroviral therapy for five years. *J Virol* 77: 11212–11219.
 18. Hoen B, Cooper DA, Lampe FC, Perrin L, Clumeck N, et al. (2007) Predictors of virological outcome and safety in primary HIV type 1-infected patients initiating quadruple antiretroviral therapy: QUEST GW PROB3005. *Clin Infect Dis* 45: 381–390.
 19. Chun TW, Murray D, Justement JS, Hallahan CW, Moir S, et al. (2011) Relationship between residual plasma viremia and the size of HIV proviral DNA reservoirs in infected individuals receiving effective antiretroviral therapy. *J Infect Dis* 204: 135–138.