

Role of Baseline HIV-1 DNA Level in Highly-Experienced Patients Receiving Raltegravir, Etravirine and Darunavir/Ritonavir Regimen (ANRS139 TRIO Trial)

Charlotte Charpentier^{1*}, Catherine Fagard^{2,3}, Céline Colin^{2,3}, Christine Katlama⁴, Jean-Michel Molina⁵, Christine Jacomet⁶, Benoit Visseaux¹, Anne-Marie Taburet⁷, Françoise Brun-Vézinet¹, Geneviève Chêne^{2,3,8}, Yazdan Yazdanpanah⁹, Diane Descamps¹, the ANRS139 TRIO Trial study group

1 Laboratoire de Virologie, Assistance Publique-Hôpitaux de Paris (AP-HP), Groupe Hospitalier Bichat-Claude Bernard, HUPNVS, Université Paris Diderot, Paris 7, PRES Sorbonne Paris Cité, EA4409, Paris, France, **2** Université de Bordeaux, ISPED, Centre INSERM U897-Epidémiologie Statistique, Bordeaux, France, **3** INSERM, ISPED, Centre INSERM U897-Epidémiologie Statistique, Bordeaux, France, **4** AP-HP, Hôpital Pitié-Salpêtrière, Service de Maladies Infectieuses, INSERM U943, UPMC, Paris 6, Paris, France, **5** AP-HP, Hôpital Saint-Louis, Service de Maladies Infectieuses, Université Paris-Diderot, Paris 7, Paris, France, **6** Hôpital Gabriel Montpied, Clermont-Ferrand, France, **7** AP-HP, Hôpital Bicêtre, Hôpitaux Universitaires Paris Sud, Pharmacie service de pharmacie clinique, CHU de Bicêtre, AP-HP, Le Kremlin Bicêtre, France, **8** CHU de Bordeaux, Pôle de Santé Publique, Service d'information médicale, Bordeaux, France, **9** AP-HP, Groupe Hospitalier Bichat-Claude Bernard, HUPNVS, Service de Maladies Infectieuses, Université Paris-Diderot, Paris 7, Paris, France

Abstract

Objective: In the ANRS 139 TRIO trial, the use of 3 new active drugs (raltegravir, etravirine, and darunavir/ritonavir), resulted in a potent and sustained inhibition of viral replication in multidrug-resistant treatment-experienced patients. The aim of this virological sub-study of the ANRS 139 TRIO trial was to assess: (i) the evolution of HIV-1 DNA over the first year; and (ii) the association between baseline HIV-1 DNA and virological outcome.

Methods: Among the 103 HIV-1-infected patients included in the ANRS-139 TRIO trial, HIV-1 DNA specimens were available for 92, 84, 88, and 83 patients at Week (W)0, W12, W24, and W48, respectively. Quantification of total HIV-1 DNA was performed by using the commercial kit "Generic HIV DNA Cell" (Biocentric, Bandol, France).

Results: Baseline median HIV-1 DNA of patients displaying virological success ($n = 61$), viral blip ($n = 20$), and virological failure ($n = 11$) were $2.34 \log_{10}$ copies/ 10^6 PBMC (IQR = 2.15–2.66), 2.42 (IQR = 2.12–2.48), and 2.68 (IQR = 2.46–2.83), respectively. Although not statistically significant, patients exhibiting virological success or viral blip had a tendency to display lower baseline HIV-1 DNA than patients experiencing virological failure ($P = 0.06$). Median decrease of HIV-1 DNA between baseline and W48 was $-0.13 \log_{10}$ copies/ 10^6 PBMC (IQR = -0.34 to $+0.10$), mainly explained by the evolution from W0 to W4. No more changes were observed in the W4–W48 period.

Conclusions: In highly-experienced multidrug-resistant patients, HIV-1 DNA slightly decreased during the first month and then remained stable during the first year of highly potent antiretroviral regimen. In this population, baseline HIV-1 DNA might help to better predict the virological response and to tailor clinical therapeutic management as more aggressive therapeutic choices in patients with higher baseline HIV-1 DNA.

Citation: Charpentier C, Fagard C, Colin C, Katlama C, Molina J-M, et al. (2013) Role of Baseline HIV-1 DNA Level in Highly-Experienced Patients Receiving Raltegravir, Etravirine and Darunavir/Ritonavir Regimen (ANRS139 TRIO Trial). PLoS ONE 8(1): e53621. doi:10.1371/journal.pone.0053621

Editor: Cristian Apetrei, University of Pittsburgh Center for Vaccine Research, United States of America

Received: October 17, 2012; **Accepted:** November 28, 2012; **Published:** January 17, 2013

Copyright: © 2013 Charpentier 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: The authors thank Merck Sharp & Dohme-Chibret for providing raltegravir; and Tibotec, a division of Janssen-Cilag, for providing etravirine. The research leading to these results has received funding from the ANRS, and the European Community's Seventh Framework Programme (FP7/2007-2013) under the project "Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)" (grant no. 223131). 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 the following interests. Merck Sharp & Dohme-Chibret provided raltegravir for this study which is marketed by them. Tibotec, a division of Janssen-Cilag, provided etravirine which is marketed by them. There are no further patents, products in development or marketed products to declare. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials, as detailed online in the guide for authors.

* E-mail: charlotte.charpentier@bch.aphp.fr

Introduction

HIV-1 DNA is a major and independent predictor of disease progression in untreated patients with primary or recent HIV infection [1,2]. Recently, it has been reported that higher baseline HIV-1 DNA was associated with a higher risk of virological rebound in virologically-controlled patients switching their com-

bined antiretroviral-based regimen to a protease inhibitor (PI) monotherapy with darunavir (ANRS-136 Monoï trial) [3]. However, few data are available about the predictive value of baseline HIV-1 DNA on virological response in highly-experienced patients receiving new antiretroviral drug classes such as integrase inhibitors, or new compounds in former classes such as etravirine (ETR) or darunavir (DRV).

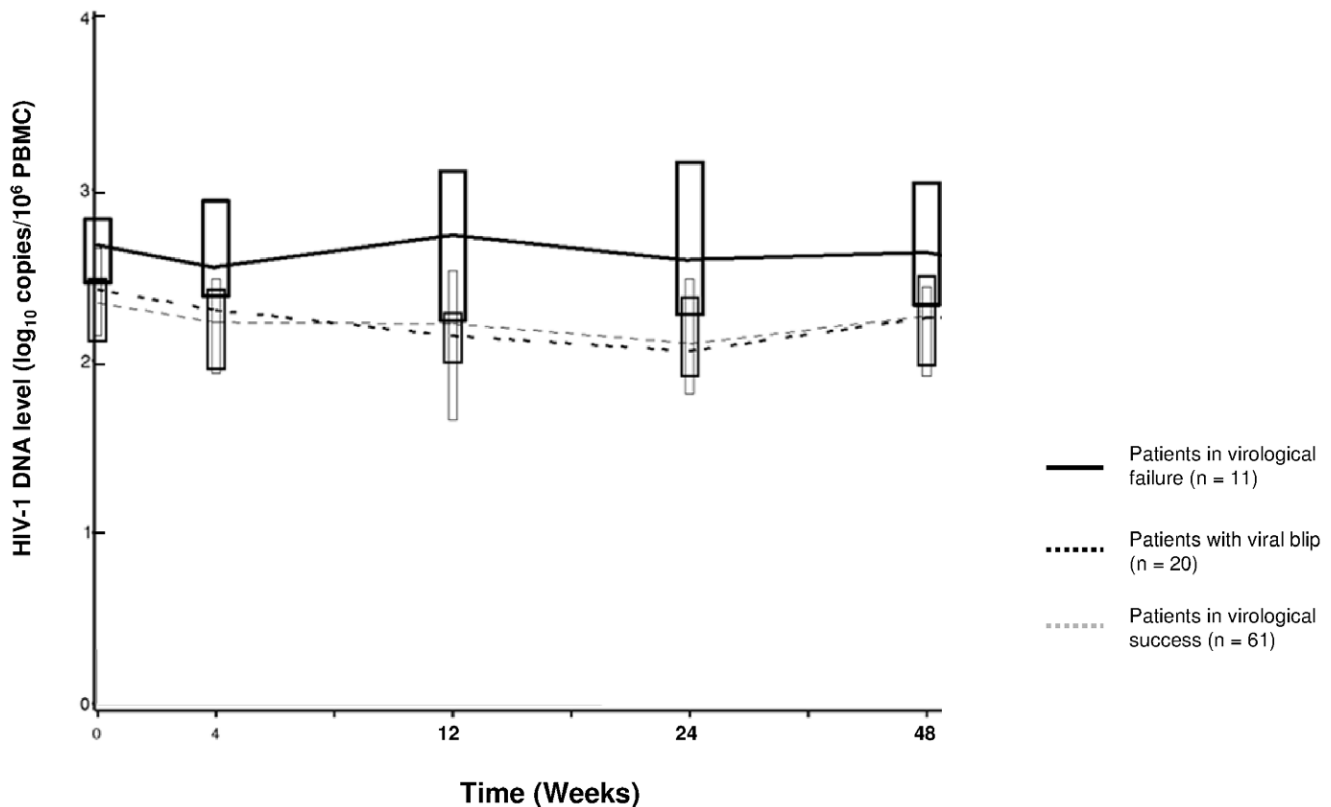


Figure 1. Longitudinal follow-up of HIV-1 DNA level during the ANRS 139 TRIO Trial.
doi:10.1371/journal.pone.0053621.g001

A non comparative study, the “Agence nationale de recherches sur le SIDA et les hépatites virales” (ANRS)-139 TRIO trial, showed that a regimen containing three new drugs: the integrase inhibitor raltegravir (RAL), ETR and DRV boosted with ritonavir (DRV/r), resulted in a sustained inhibition of viral replication in multidrug-resistant treatment-experienced patients, with 86% and 88% of patients displaying HIV-1 RNA below 50 copies/mL at one and two years, respectively [4,5].

The aim of this virological sub-study of the TRIO trial was to assess: (i) the evolution of HIV-1 DNA over the first year; and (ii) the association between baseline HIV-1 DNA and virological outcome.

Patients and Methods

Among the 103 HIV-1-infected patients included in the ANRS-139 TRIO trial [4], HIV-1 DNA specimens were available for 92, 84, 88, and 83 patients at Week (W)0, W12, W24, and W48, respectively. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque density gradient centrifugation.

Quantification of total HIV-1 DNA was performed by using the commercial kit “Generic HIV DNA Cell” (Biocentric, Bandol, France).

In the ANRS-139 TRIO trial, virological failure was defined as a plasma HIV-1 RNA level >50 copies/mL at W24; or >50 copies/mL on two consecutive specimens between W24 and W48 for those below 50 copies/mL at W24. A viral blip was defined as an isolated HIV-1 RNA measurement below 400 copies/mL.

HIV-1 DNA levels are described using median and interquartile range (IQR) and comparative analysis between subgroups of patients used non-parametric Kruskal-Wallis test. All statistical

analyses were performed using SAS, version 9.1.3 service pack 2 (SAS Institute).

Ethics Statement

Written informed consent was obtained from all patients. The protocol was reviewed and approved by an ethics committee (Comite de Protection des Personnes) and competent health authorities (Agence Française de Sécurité Sanitaire des Produits de Santé). The trial was conducted in accordance with the Declaration of Helsinki.

Results

At baseline, HIV-1 DNA was available in 92 patients who did not differ from the 11 other patients of the trial for the main demographic and immuno-virological characteristics (data not shown). At baseline, median HIV-1 DNA was 2.41 log₁₀ copies/10⁶PBMC (IQR = 2.17–2.67). Among these 92 patients, 61 exhibited virological success, 20 experienced a viral blip between W24 and W48, and a virological failure was reported for 11. Most of viral blips were at a low-level viremia (<100 copies/mL in 16 patients).

Baseline median HIV-1 DNA of patients displaying virological success, viral blip, and virological failure were 2.34 log₁₀ copies/10⁶PBMC (IQR = 2.15–2.66), 2.42 (IQR = 2.12–2.48), and 2.68 (IQR = 2.46–2.83), respectively. Although not statistically significant, patients exhibiting virological success or viral blip had a tendency to display lower baseline HIV-1 DNA than patients experiencing virological failure ($P = 0.06$).

Median decrease of HIV-1 DNA between baseline and W48 was -0.13 log₁₀ copies/10⁶ PBMC (IQR = -0.34 to +0.10), mainly

explained by the evolution in the first 4 weeks after inclusion with a median HIV-1 DNA of 2.28 log₁₀ copies/10⁶PBMC at W4 (IQR = 1.96–2.52). No more changes were observed in the W4–W48 period (median HIV-1 DNA level = 2.22 log₁₀ copies/10⁶PBMC at W12, 2.16 at W24, 2.26 at W48). Evolution of HIV-1 DNA in patients displaying virological success, viral blip or virological failure is depicted in Figure 1.

Discussion

In 92 highly-experienced patients receiving 3 active antiretroviral drugs, HIV-1 DNA showed only a modest decrease, mainly observed during the first 4 weeks of the trial. Furthermore, patients exhibiting virological success and/or viral blip had a tendency to display lower baseline HIV-1 DNA than patients experiencing virological failure during the first year of this regimen.

We acknowledge that the main limitation of our analysis is the lack of statistical power to assess the association between baseline HIV-1 DNA level and virological outcome.

In our study, median baseline HIV-1 DNA was 2.41 log₁₀ copies/10⁶ PBMC, close to the lower bound of HIV-1 DNA levels previously described in highly-experienced patients [6,7]. Indeed, in these viremic patients, HIV-1 DNA is reported to be around 3.5 log₁₀ copies/10⁶PBMC [6,7]. Moreover, in our analysis, no significant change of HIV-1 DNA was observed during the 48 weeks of follow-up, as expected and previously described in highly-experienced patients infected for a long time and with a large viral reservoir, where a median decrease of about 0.20 log₁₀ copies/10⁶PBMC was reported at 48 or 72 weeks [6,8,9]. In other studies, the most important decrease in HIV-1 DNA has been reported in the context of antiretroviral-treated acute infection, i.e. around –1 log₁₀ copies/10⁶CD4-T-cells [10].

HIV-1 DNA level is an independent predictor of disease progression in untreated patients with primary HIV infection and during the first 6 months after seroconversion [1,2]. However, in the population of antiretroviral-experienced patients, HIV-1 DNA is not suggested to be a predictive factor of virological outcome, except in a recent report of PI-monotherapy with DRV/r where higher baseline HIV-1 DNA was associated with a higher risk of virological rebound at W96 [3]. In our study, baseline HIV-1 DNA was only modestly associated with virological failure at one year, though the trend was not statistically significant. Larger studies are needed to confirm that HIV-1 DNA might represent a

parameter of interest even in highly-experienced patients with a large size of viral reservoir. Indeed, in viremic highly-experienced patients initiating a new antiretroviral-based therapy, in whom plasma viremia is frequently at low-level, our findings showed that baseline HIV-1 DNA might help to better predict the virological response.

In the present analysis, HIV-1 DNA in patients experiencing a viral blip, mostly below 100 copies/mL, behave closely to the evolution observed in patients experiencing virological success than those in virological failure. As in previous studies assessing the impact of viral blip on viral reservoir size, our findings might suggest that the occurrence of isolated viral blips is unlikely to have the same impact on the replenishment of viral reservoir in virologically-suppressed patients than in viremic highly-experienced patients with a large reservoir size [11,12].

In conclusion, HIV-1 DNA remains stable during the first year of highly potent antiretroviral regimen in highly-experienced patients. In this population, HIV-1 DNA might nevertheless be a biomarker of potential interest for clinical management if the association of baseline levels with further virological outcome is confirmed in larger studies.

Acknowledgments

We thank patients who participated in this trial and all study investigators, collaborators and participating centers. We thank Merck Sharp & Dohme-Chibret for providing raltegravir; and Tibotec, a division of Janssen-Cilag, for providing etravirine; M.J. Commo, S. Couffin-Cadiergues (ANRS), C. Jean-Marie, N. Agher and A. Beuscart (clinical research assistants, INSERM U897).

We thank Alexandre Storto for his excellent technical assistance.

The research leading to these results has received funding from the ANRS, and the European Community's Seventh Framework Programme (FP7/2007–2013) under the project “Collaborative HIV and Anti-HIV Drug Resistance Network (CHAIN)” (grant no. 223131).

Author Contributions

Performed plasma dosages of antiretroviral drugs: AMT. Performed statistical analysis: CF C. Colin GC. Performed the virological analysis: C. Charpentier BV. Recruited patients in the trial: CK JMM CJ. Conceived and designed the experiments: C. Charpentier CF YY DD. Analyzed the data: C. Charpentier CF FBV GC YY DD. Wrote the paper: C. Charpentier CF CK JMM CJ FBV GC YY DD.

References

- Goujard C, Bonarek M, Meyer L, Bonnet F, Chaix ML, et al. (2006) CD4 cell count and HIV DNA level are independent predictors of disease progression after primary HIV type 1 infection in untreated patients. *Clin Infect Dis* 42: 709–715.
- Rouzioux C, Hubert JB, Burgard M, Deveau C, Goujard C, et al. (2005) Early levels of HIV-1 DNA in peripheral blood mononuclear cells are predictive of disease progression independently of HIV-1 RNA levels and CD4+ T-cell counts. *J Infect Dis* 192: 46–55.
- 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.
- Yazdanpanah Y, Fagard C, Descamps D, Taburet AM, Colin C, et al. (2009) High rate of virologic suppression with raltegravir plus etravirine and darunavir/ritonavir among treatment-experienced patients infected with multidrug-resistant HIV: results of the ANRS 139 TRIO trial. *Clin Infect Dis* 49: 1441–1449.
- Fagard C, Colin C, Charpentier C, Rami A, Jacomet C, et al. (2012) Long-term efficacy and safety of raltegravir, etravirine, and darunavir/ritonavir in treatment-experienced patients: week 96 results from the ANRS 139 TRIO trial. *J Acquir Immune Defic Syndr* 59: 489–493.
- Charpentier C, Piketty C, Laureillard D, Tisserand P, Si-Mohamed A, et al. (2012) Dynamics of HIV-1 DNA level in highly antiretroviral-experienced patients receiving raltegravir-based therapy. *Eur J Clin Microbiol Infect Dis* 31: 129–133.
- Reigadas S, Andréola ML, Wittkop L, Cosnefroy O, Anies G, et al. (2010) Evolution of 2-long terminal repeat (2-LTR) episomal HIV-1 DNA in raltegravir-treated patients and in vitro infected cells. *J Antimicrob Chemother* 65: 434–437.
- Ngo-Giang-Huong N, Deveau C, Da Silva I, Pellegrin I, Venet A, et al. (2001) Proviral HIV-1 DNA in subjects followed since primary HIV-1 infection who suppress plasma viral load after one year of highly active antiretroviral therapy. *AIDS* 15: 665–673.
- Morand-Joubert L, Marcellin F, Launay O, Guiramand-Hugon S, Gérard 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.
- Yerly S, Perneer TV, Vora S, Hirschel B, Perrin L (2000) Decay of cell-associated HIV-1 DNA correlates with residual replication in patients treated during acute HIV-1 infection. *AIDS* 14: 2805–2812.
- Rong L, Perelson AS (2009) Modeling latently infected cell activation: viral and latent reservoir persistence, and viral blips in HIV-infected patients on potent therapy. *PLoS Comput Biol* 5: e1000533.
- Ramratnam B, Mittler JE, Zhang L, Boden D, Hurley A, et al. (2000) The decay of the latent reservoir of replication-competent HIV-1 is inversely correlated with the extent of residual viral replication during prolonged anti-retroviral therapy. *Nat Med* 6: 82–85.