Higher HIV-1 DNA associated with lower gains in CD4 cell count among patients with advanced therapeutic failure receiving optimized treatment (ANRS 123—ETOILE)

Véronique Avettand-Fenoël1,2*, Vincent Bouteloup3, Adeline Mélard2, Catherine Fagard3, Marie-Laure Chaix1,2, Pascale Leclercq4, Geneviève Chêne3, Jean-Paul Viard1,2 and Christine Rouzioux1,2 on behalf of the members of the ETOILE study†

1Université Paris-Descartes, EA3620 Paris, France; 2AP-HP Hôpital Necker-Enfants Malades, Paris, France; 3INSERM, U897, Bordeaux, France; 4CHU de Grenoble, Grenoble, France

*Corresponding author. Laboratoire de Virologie, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75015 Paris, France. Tel: +33-1-44-49-49-61; Fax: +33-1-44-49-49-60; E-mail: veronique.avettand@nck.aphp.fr

†Members are listed in the Acknowledgements section.

Received 15 March 2010; returned 10 May 2010; revised 23 June 2010; accepted 4 July 2010

Objective: To describe HIV-1 DNA levels from baseline (W0) to week 52 (W52) among patients receiving either interleukin-2 (IL-2)+optimized background therapy (OBT) or OBT as salvage treatment.

Methods: This was evaluated in a substudy of the ETOILE Agence Nationale de Recherches sur le SIDA et les hépatites virales (ANRS) 123 trial (patients with CD4 ≤ 200/mm3, HIV RNA > 4 log10 copies/mL and a genotypic score showing two or fewer active drugs). OBT included enfuvirtide whenever possible. HIV DNA was quantified with the ANRS assay.

Results: Blood samples were available for 21 patients in the IL-2+OBT arm and 23 in the OBT alone arm at baseline, and for 10 and 17 patients, respectively, at W52. Median baseline CD4 count was 47 cells/mm3 and 68 cells/mm3, respectively; median HIV RNA was 5.1 and 4.9 log10 copies/mL, respectively. Baseline median HIV DNA load was 3.44 log10 copies/10^6 peripheral blood mononuclear cells (PBMCs) (interquartile range 3.31–4.08) and 3.51 (3.18–3.82) log10 copies/10^6 PBMCs, respectively. At W52, it was 3.18 log10 copies/10^6 PBMCs (2.75–3.52) and 3.48 log10 copies/10^6 PBMCs (3.10–3.67), respectively. Cells were available at both W0 and W52 for 7 patients in the IL-2+OBT arm and 14 in the OBT arm. Change in HIV DNA load was not associated with IL-2 use, but decreased among the seven patients receiving enfuvirtide (−0.22 log10 copies/mL) compared with the other 14 patients (+0.20 log10; P = 0.046). A steeper decrease in HIV DNA was observed among patients who had a larger increase in CD4 count (Pearson coefficient r = 0.659, P = 0.001). Adjusted for enfuvirtide use, there was a trend for an association between upper baseline HIV DNA level and a less frequent CD4 gain ≥50 cells/mm3 at W52 (odds ratio = 0.17, P = 0.075).

Conclusions: HIV DNA levels were high in patients with advanced therapeutic failure. A larger viral reservoir may be associated with lower gains in CD4 count among patients receiving OBT. HIV DNA level could be a useful tool for the case management of patients in the late stages of disease.

Keywords: HIV reservoir, treatment failure, interleukin-2, enfuvirtide, HIV-1 DNA

Introduction

The cell-associated HIV DNA level reflects the cellular HIV reservoir. At baseline, it is a prognostic marker of response to combined antiretroviral treatment among chronically infected patients, independent of HIV RNA levels.1,2 Data are scarce in highly immunocompromised patients since previous studies mostly concerned patients with HIV RNA load <3 log10 copies/mL.3,4 We aimed to determine the cell-associated HIV-1 DNA load in immunocompromised multi-experienced patients with high HIV RNA load and any association of HIV DNA level change and CD4 T cell changes during 1 year of salvage therapy.
Patients and methods

Fifty-six patients with CD4 T cell counts \( \leq 200 \) cells/mm\(^3\), plasma HIV RNA \( >4 \log_{10} \) copies/mL and a genotypic score showing two or fewer active drugs were included and analysed in the randomized ETOILE ANRS 123 trial.\(^5\) The study protocol was approved by the local ethics committee. All patients provided written informed consent. In brief, patients received either interleukin-2 (IL-2) combined with an optimized background therapy (OBT) or OBT alone as salvage treatment. OBT included enfuvirtide whenever possible. This trial showed that IL-2 failed to increase CD4 T cells whereas enfuvirtide use, even in a poor OBT, was associated with treatment success.\(^5\)

Our current analysis of HIV DNA levels is a substudy focusing on a non-random set of patients for whom blood cells were available at baseline and/or at week 52: there were 21 patients in the IL2+OBT arm and 23 patients with OBT alone at baseline, and 10 and 17 patients respectively at week 52. Blood cells were available at both week 0 and week 52 for 7 patients in the IL2+OBT arm and 14 in the OBT arm. HIV-1 DNA was quantified in whole blood using real-time PCR, as described previously.\(^5\)

The comparisons between groups and the correlations were assessed using \( \chi^2 \), Wilcoxon and Pearson correlation coefficient tests, as appropriate. Correlations after adjustment were explored through a linear regression model. Any association between HIV DNA and CD4 T cell count change was assessed by logistic regression. Global type I error was equal to 0.05.

Results

Patients included in this substudy did not differ from other patients in baseline CD4 T cell count and HIV RNA load (data not shown).

In this substudy, patients from the two treatment arms had similar epidemiological characteristics [gender (\( P=1.00 \)), age (\( P=0.26 \)) and transmission risk group (\( P=0.47 \))]. Baseline immunological and virological characteristics were as follows [median and interquartile range (IQR)]: CD4 T cell count, 47 (10–100) cells/mm\(^3\) in the IL2+OBT arm and 68 (26–107) cells/mm\(^3\) in the OBT arm; HIV RNA, 5.1 (4.5–5.4) and 4.9 (4.4–5.1) \( \log_{10} \) copies/mL, respectively.

HIV DNA load at baseline [median (IQR)] was 3.44 (3.31–4.08) \( \log_{10} \) copies/10\(^6\) peripheral blood mononuclear cells (PBMCs) in the IL2+OBT arm and 3.51 (3.18–3.82) in the OBT arm. At week 52 it was 3.18 (2.75–3.52) and 3.48 (3.1–3.67) \( \log_{10} \) copies/10\(^6\) PBMCs, respectively.

Change in HIV DNA load from baseline to week 52 was not correlated with IL-2 use, but decreased among the 7 patients receiving enfuvirtide (\( -0.22 \log_{10} \) copies/10\(^6\) PBMCs) as compared with the other 14 patients (\( +0.20 \)) (\( P=0.046 \)).

A steeper decrease of HIV DNA was observed among patients who had a larger increase in CD4 T cell count from baseline to week 52 (Pearson coefficient \( r=0.659, P=0.001 \)) (Figure 1). After adjustment for baseline HIV-1 RNA level, this correlation remained statistically significant (adjusted \( r=0.682, P<0.001 \)). In contrast, the correlation became non-significant after adjustment for plasma HIV RNA change (\( r=0.13, P=0.58 \)).

Finally, when considering all patients with an available HIV-1 DNA load, an upper baseline HIV-1 DNA level was associated, though not significantly, with a less frequent CD4 T cell gain \( \geq 50 \) cells/mm\(^3\) from W0 to W52 [the odds ratio (OR) adjusted for enfuvirtide use was 0.17 per 0.5 \( \log_{10} \) copies/10\(^6\) PBMCs, \( P=0.075 \)]. When considering adjustment for enfuvirtide use and baseline HIV-1 RNA level, the association of baseline HIV-1 DNA load with a gain of CD4 of more than 50 cells/mm\(^3\) remained stable (adjusted OR=0.20) but was not significantly associated with CD4 T cell gain (\( P=0.14 \)). When considering adjustment for enfuvirtide use and change of HIV RNA load, the association also remained stable (OR=0.24) but not statistically significant (\( P=0.12 \)). This may be due to the lack of power linked to the small number of patients included in this substudy.

Discussion

To our knowledge, these are the first data evaluating the blood cellular HIV-1 reservoir in immunocompromised patients with multiple therapeutic failures and high HIV RNA loads. One limitation of our study is that stored cells were not available for all patients enrolled in the ETOILE trial, reducing the power of our analysis though not generating a selection bias since baseline immunovirological characteristics did not differ according to inclusion in this substudy.

HIV DNA levels are high in these patients with advanced therapeutic failure (median: 3.6 \( \log_{10} \)) as compared with untreated patients recruited at the time of primary infection (median: 3.2 \( \log_{10} \)) or untreated patients during the asymptomatic chronic phase of HIV disease (median: 2.7 \( \log_{10} \)).\(^8\) The proportion of infected cells is at its highest during AIDS, when there is a profound CD4 T cell depletion.

We showed that an optimized background regimen, even if not highly active, allowed the blood HIV reservoir to decrease in these immunocompromised patients. HIV DNA did not change much from baseline to week 52, but this marker has lower amplitude than HIV RNA. Consequently, even low variations should be taken into account when there is a significant difference between groups. This low variation is in keeping with...
previous observations that pretreated chronically infected patients had a small HIV DNA decrease compared with that observed in antiretroviral-naive chronically infected patients (0.32 log₁₀ copies/10⁶ PBMCs versus 0.52 log₁₀ copies/10⁶ PBMCs after treatment for 1 year, and 0.23 log₁₀ copies/10⁶ PBMCs versus 0.55 log₁₀ copies/10⁶ PBMCs respectively after treatment for 72 weeks). Interestingly, we found an association between immune restoration and cellular HIV reservoir diminution, even after adjustment for baseline plasma HIV RNA load. The absence of significance after adjustment for plasma HIV RNA change was expected because HIV reservoir decrease is not independent of HIV RNA decrease.

Lastly, although this association warrants confirmation, our results suggest that a higher HIV-1 DNA load at baseline may be associated with a smaller change of CD4 T cell counts among patients with advanced therapeutic failure receiving OBT. A larger blood viral reservoir could thus be deleterious to immune restoration.

In conclusion, this study suggests that measurement of HIV-1 DNA, in addition to other HIV biomarkers, might be useful for case management in HIV-infected patients at late stages of disease.

Acknowledgements
We would like to thank all the patients who participated in this study and all ETOILE study investigators and study personnel.

Investigators and participating centres (all in France) were as follows: M. T. Goërger-Saw, CHU Pointe à Pitre, Pointe à Pitre; A. Devidas, CH Sud Francilien, Corbeil-Essonnes; M. Bentata, Hôpital Avicenne, Bobigny; L. Gerard, Hôpital Saint-Louis, Paris; M. Dupon, Hôpital Pellegrin, Bordeaux; C. Lascoux-Combe, Hôpital Saint-Louis, Paris; J. P. Viard, Hôpital Necker, Paris; C. Goujard, Hôpital Bicêtre, Le Kremlin Bicêtre; P. Sellier, Hôpital Lariboisière, Paris; G. Pichoncourt, CH d’Avignon, Avignon; V. Jeantils, Hôpital Jean Verdier, Bondy; L. Weiss, Hôpital Européen Georges Pompidou, Paris; Y. Levy, Hôpital Henri Mondor, Créteil; M. A. Valantin, Hôpital Pitié-Salpêtrière, Paris; J. M. Molina, Hôpital Saint-Louis, Paris; P. Morlat, Hôpital Saint-André, Bordeaux; J. M. Ragnaud, Hôpital Pellegrin, Bordeaux; F. Jeanblanc, Hôpital Édouard Herriot, Lyon; L. Cotte, Hôpital Hôtel Dieu, Lyon; I. Poizot-Martin, Hôpital Sainte Marguerite, Marseille; E. Bouvet, Hôpital Bichat Claude Bernard, Paris; J. Reynes, Hôpital Gui de Chauliac, Montpellier; F. Raffi, Hôpital Hôtel Dieu, Nantes; C. Jacomet, Hôtel Dieu, Clermont-Ferrand; M. Duong, CHU Hôpital d’Enfants, Dijon; P. Leclercq, CH Grenoble, Grenoble.

Funding
This work was supported by the Agence Nationale de Recherches sur le SIDA et les hépatites virales (ANRS); Chiron Laboratories provided interleukin-2.

Transparency declarations
None to declare.

References
3 Avettand-Fenoel V, Prazuck T, Hocqueloux L et al. HIV-DNA in rectal cells is well correlated with HIV-DNA in blood in different groups of patients, including long-term non-progressors. AIDS 2008; 22: 1880–2.