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Selection of nonnucleoside reverse transcriptase inhibitor-associated mutations in HIV-1 subtype C: evidence of etravirine cross-resistance

Ujjwal Neogi[a,b,c], Anita Shet[a,b], Ranjani Shamsundar[a] and Maria L. Ekstrand[a,d]

Prevalence of etravirine genotypic resistance was assessed among 92 HIV-1C-infected patients failing nevirapine and efavirenz-based regimens from a cohort of 552 Indian patients. Overall, prevalence of etravirine cross-resistance identified using the Tibotec Weighted Score was 41% (31.5% intermediately-resistant and 9.8% fully-resistant). The most frequently described nonnucleoside reverse transcriptase inhibitor-associated mutations included Y181 (35.9%), K101 (20.7%), G190 (17.4%), and V108 (15.2%). The resistant group demonstrated higher viral load ($P = 0.01$) and longer duration of antiretroviral treatment ($P = 0.03$) compared with the susceptible group.

The low genetic barrier to development of resistance to first generation nonnucleoside reverse transcriptase inhibitors (NNRTIs) is compounded by cross-resistance across the class that makes sequential therapy with the NNRTIs therapeutically inappropriate. Current first-line NNRTI in most resource-constrained regions includes nevirapine, except in cases of intolerance or potential drug interaction when efavirenz is used [1]. Etravirine, a new generation NNRTI (TMC-125, Intelence; Tibotec Pharmaceuticals Ltd, Turnhoutseweg 30, 2340 Beerse, Belgium), was approved by US Food and Drug Administration for use in antiretroviral-experienced adults with resistance to first-line NNRTIs. Etravirine resistance-associated mutations (RAMs) in reverse transcriptase gene were identified, which are as follows: V90I, A98G, L100I, K101E/P/H, V106I, V179D/F, Y181C/I/V, G190A/S, E138A, V179T, and M230L [2–4]. The Tibotec Weighted Score was proposed with 17 etravirine RAMs and assigned differential weights based upon the impact on clinical response [5]. Alternatively, the Monogram Weighted Score included 30 etravirine RAMs based on the genotypic and phenotypic interrelationship [6]. Etravirine cross-resistance may be influenced by the prevailing HIV-1 subtype [7,8]. With a worldwide prevalence of 50% [9], and prevalence in India of 96%, HIV-1C undoubtedly has a significant impact on the evolution of the HIV epidemic globally. This study reports the selection of NNRTI RAMs and etravirine cross-resistance patterns among HIV-1C-infected patients failing first-line antiretroviral therapy (ART).

Among a total of 552 participants participating in a 2-year longitudinal cohort study [10], 18% ($n = 101$) with detectable viremia were assessed for presence of drug RAMs during their baseline visit [11]. Drug resistance genotyping was successfully done from 92 plasma samples from failing patients (viral load >1000 copies/ml) using a validated in-house method [12]. Drug-resistant strains previously reported from India ($n = 429$) from patients failing first-line ART were included as a second group in this study [13–19]. A third group of 1122 global HIV-1C sequences were obtained from HIVseq Program (http://hivdb.stanford.edu/; accessed 13 August 2010) reported from patients worldwide with a history of NNRTI drug treatment. Indian sequences and duplicates were excluded from global subtype C sequences. NNRTI DRMs in all these sequences were analyzed. Etravirine resistance was evaluated by Tibotec Etravirine Weighted Genotype Score [5]. Statistical analysis was performed in SPSS, version 11.5 (SPSS inc., Chicago, IL, USA).

Plasma virus was successfully genotyped in 92 failing patients; their mean age was 39.6 years (SD 10.2 years) and 67% were men, similar to the complete cohort. Among the 92 patients, 77% used nevirapine; 12% used efavirenz, and 10% changed from an initial nevirapine-based regimen to an efavirenz-based regimen for clinical reasons. The mean duration of nevirapine and efavirenz exposure was 23 and 14 months, respectively.

The overall prevalence of etravirine resistance was 41% (38 of 92). Single etravirine RAMs were seen in 13% and two etravirine RAMs were seen in 33% of the strains. Eleven percent (10 of 92) of strains harbored three or more etravirine RAMs. The Tibotec Weighted Score identified 58.7% of the strains to be susceptible to etravirine, whereas 31.5 and 9.8% displayed intermediate resistance and resistance, respectively. Alternative scoring methods showed comparable patterns (39% of strains had a monogram weighted score ≥ 4) indicating that a significant percentage of isolates had reduced efficacy to etravirine.

Genotypic analysis predicted that 41.6% (30 of 72) of samples from nevirapine-experienced and 9.1% (one of 11) from efavirenz-experienced patients were cross resistant to etravirine. The maximum level of cross-resistance (77.8%, seven of nine) was observed in those patients who had exposure of both the drugs. The most frequently described RAMs included amino acid

substitutions at positions Y181 (35.9%), K101 (20.7%), G190 (17.4%), and V108 (15.2%). Similar trends were observed in sequences reported previously from India ($n = 429$); however, among global subtype C sequences, K103N was the most frequent RAM (Fig. 1).

Compared to patients with susceptible virus, those who harbored etravirine-resistant virus were more likely to have been on ART for a longer duration ($P = 0.03$) and to have higher viral load ($P = 0.01$) (Supplementary digital content 1; http://links.lww.com/QAD/A126). There was no significant difference in age, CD4 cell count, time since diagnosis, or self-reported adherence in the last month measured by Visual Analogue Scale between the two groups.

Our report highlights the high prevalence of etravirine cross-resistance (41%) among the patients infected with HIV-1C viruses and failing first-generation NNRTI-based regimens in India. Etravirine RAMs have also been described in ART-naive patients from France, Mali, and India [20,21]. Our finding of etravirine resistance is higher than among HIV-infected patients harboring subtype B in UK (11.5%) and Spain (18.7%) [22,23]. A similar study from Thailand found 56% etravirine cross-resistance in HIV-1 CRF01_AE strains [24].

The high prevalence of Y181 and K101 found in our setting is also seen in other places where nevirapine is widely used as first-line NNRTI. Similar trends have been observed in patients with CRF01_AE strains from Thailand (50% Y181C/I/V and 18.7% K101E/H/P) [24] and the UK (17% Y181C in those failing efavirenz and 40.5% Y181C were in those failing nevirapine) [25], thus lending credence to the conclusion that Y181C is particularly selected during prolonged exposure to a failing nevirapine-containing regimen [11].

The association between etravirine resistance and higher viral loads in the study cohort may be reflective of the longer duration on poorly suppressive regimens experienced by these patients [26]. In settings like India where routine viral load monitoring is not a part of standard of care, the second-line ART regimens have to be designed with caution when including NNRTI drugs. As over 50% of failing isolates are susceptible to etravirine, it can be used as salvage therapy among those patients failing first-generation NNRTI-based regimens. Patients with high level of etravirine RAMs were also more likely to have tenofovir-associated mutations [27], which may raise challenges in designing an effective second-line regimen in resource-constrained settings like India. The presence of cross-resistance also highlights the need for developing effective and sustainable adherence interventions that target local adherence patterns and barriers in order to keep the limited first-line ART agents effective for as long as possible [10].

In summary, our analysis highlights the high level of etravirine cross-resistance in a cohort of ART-experienced patients failing NNRTI-containing first-line therapy in India. The pattern of NNRTI mutations in nevirapine-exposed patients also suggests the possible benefit of reconsidering the use of nevirapine in favor of efavirenz as first-line NNRTI choice in resource-constrained settings.

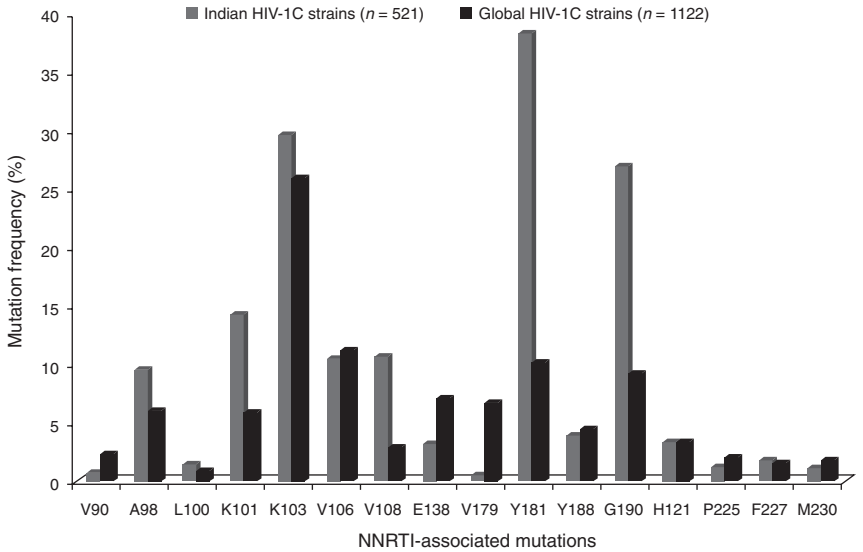

Fig. 1. Selection of nonnucleoside reverse transcriptase inhibitor mutations in antiretroviral therapy-experienced patients harboring HIV-1 subtype C viruses. Higher frequencies of nonnucleoside reverse transcriptase inhibitor (NNRTI) drug resistance mutations are present in residues Y181, K101, G190, and V108 in Indian sequences ($n = 521$, 92 primary isolates and 429 previously reported sequences) compared to global subtype C sequences ($n = 1122$) obtained from HIVseq Program from Stanford University HIV Drug resistance database (http://hivdb.stanford.edu/; accessed on 13 August 2010).

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[a]St John's National Academy of Health Sciences, Bangalore, India, [b]Division of Global Health, [c]Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden, and [d]Department of Medicine, Center for AIDS Prevention Studies, University of California, San Francisco, San Francisco, California, USA.

Correspondence to Maria L. Ekstrand, PhD, University of California, San Francisco, 50 Beale Street, Suite-1300, San Francisco, CA 94105, USA.
E-mail: maria.ekstrand@ucsf.edu

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HIV-1 decreases the levels of neurotrophins in human lymphocytes

Valeriya Avdoshina[a], Alfredo Garzino-Demo[b], Alessia Bachis[a], Maria C.G. Monaco[c], Pauline M. Maki[d], Rochelle E. Tractenberg[e], Chenglong Liu[f], Mary A. Young[f] and Italo Mocchetti[a]

Neurotrophins control cell survival. Therefore, we examined whether HIV-1 reduces neurotrophin levels. Serum of HIV-positive individuals exhibited lower concentrations of brain-derived neurotrophic factor (BDNF), but not of other neurotrophins, than HIV-negative individuals. In addition, R5 and X4 strains of HIV-1 decreased BDNF expression in T cells. Our results support the hypothesis that reduced levels of BDNF may be a risk factor for T-cell apoptosis and for neurological complications associated with HIV-1 infection.

Neurotrophins [1,2] are produced by immune organs and immunocompetent cells, including T cells [3] and macrophages [4], and are believed to play a role in various functions of the immune system, including lymphocyte proliferation [5,6]. Little is known about the effect of HIV-1 on neurotrophin levels. Loss of neurotrophin expression may impair the immune system and promote AIDS. In this study, we investigated whether HIV-1 reduces serum concentration of the neurotrophins and sought to establish a correlation between HIV infection and neurotrophin expression in T cells.

Serum levels of brain-derived neurotrophic factor (BDNF) were measured by an enzyme-linked immunosorbent assay in human samples collected between 1994 and 2007 at the Washington, District of Columbia site of the Women's Interagency HIV Study [7,8]. Because approximately 50% of these individuals were polydrug abusers, mainly cocaine, methamphetamine and heroin, a two-way analysis of variance (ANOVA) was used to examine a potential interaction between HIV-1 and drug use and to examine each factor independently. HIV-positive individuals exhibited significantly lower levels of BDNF compared with HIV-negative controls (Fig. 1a). Drug use significantly affected BDNF levels such that the amount of BDNF in the serum of HIV-positive drug users were higher than in HIV-positive nondrug users (Fig. 1a), suggesting that polydrug use may affect serum BDNF levels in HIV-1-positive individuals. There was no interaction between drug use and serostatus on BDNF levels ($P > 0.33$).

Drugs of abuse [9] or HIV-1 may influence the expression of other neurotrophins. To test this hypothesis, we measured nerve growth factor (NGF) and neurotrophin-3 (NT-3) levels in the same samples. The two-way ANOVAs analyzing associations of HIV status and drug use on NGF ($P = 0.516$) and NT-3 ($P = 0.382$) were not statistically significant, and no evidence of interaction between HIV and drug use was observed for either outcome. Although we found a tendency toward lower average NGF levels in the serum of HIV-positive individuals compared with controls, the effect was not significant ($P = 0.89$) nor did polydrug use affect NGF levels (data not shown). Results for NT-3 levels were similarly not statistically significant (data not shown).

The reduction of BDNF observed in HIV-1-positive individuals could be due to single nucleotide polymorphisms (SNPs) that alter intracellular packaging and secretion of BDNF [10]. rs6265 is a polymorphism in the *BDNF* gene that produces an amino acid substitution of valine to methionine in codon 66 (Val66Met); rs56164415 is located in the fifth of the seven noncoding exons of the *BDNF* gene [11] and appears to be moderately associated with substance abuse [12]. Therefore, these SNPs, either alone or in combination, might lead to a reduction in serum BDNF levels. To test this hypothesis, we examined the frequency of these polymorphisms in the same cohort, using DNA from the same sample of individuals. There was no significant difference in frequency of alleles in HIV individuals as compared with HIV-negative controls (rs6265, $P = 0.83$; rs56164415, $P = 0.72$). Therefore, mutation of the *BDNF* gene does not appear to account for difference in the levels of *BDNF* in these individuals.

Contributing factors that may account for the decrease in serum BDNF in HIV-positive individuals are not easily defined. BDNF and other neurotrophins are produced by immune organs and immunocompetent cells [13], as well as platelets [14]. Thus, a decrease in the number of platelets may explain the lower levels of BDNF in HIV-1-positive individuals. To determine whether BDNF from platelets constitutes a significant fraction of serum BDNF, we examined which blood cell type expresses BDNF. We found that platelets and T cells exhibited comparable levels of BDNF expression (Fig. 1b). Thus, platelets account for only for a fraction of serum BDNF. Nevertheless, to more directly examine the effect of HIV-1 on BDNF, we examined the ability of HIV-1 to decrease BDNF expression in T cells. T lymphocytes were prepared from healthy donors and were infected with X4 (IIIB) or R5 (BaL) HIVs. BDNF mRNA levels were then quantified 24 h after the infection. We observed an approximately 50% decrease in BDNF

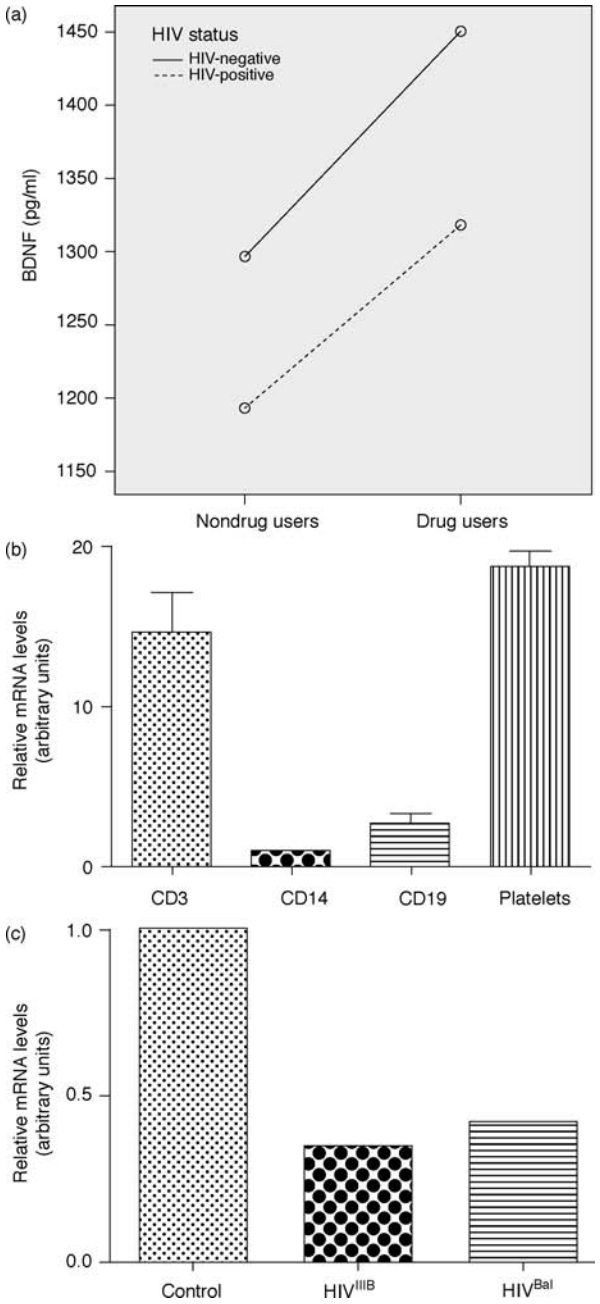

Fig. 1. HIV-1 and brain-derived neurotrophic factor levels. (a) The amount of brain-derived neurotrophic factor (BDNF) was determined in the serum of HIV-positive ($n = 109$) and HIV-negative ($n = 54$) individuals, nondrug or drug abusers by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Promega Corp., Madison Wisconsin, USA). The study was approved by the Georgetown University Institutional Review Board. HIV and drug use status did not interact [F (1, 155) = 0.076, $P = 0.783$]. However, main effects of HIV status [F (1, 155) = 5.203, $P = 0.024$] and drug use [F (1, 155) = 7.27, $P = 0.008$] were observed. (b) BDNF mRNA levels were determined by quantitative real-time PCR (RT-PCR) in the indicated cells obtained from peripheral blood (peripheral blood mononuclear cells) taken from healthy volunteers. Primers for BDNF were 5′-CATTGGCTGA CACTTTCGA-3′ and 5′-ACTGAGCATCACCCTGGAC-3′ (forward, reverse). Hypoxanthine phosphoribosyltransferase 1 was used as a housekeeping gene. Data are the mean ± SEM of three independent samples. (c) Human T cells (0.5×10^6 cells per well) prepared as previously described [22] were infected for 2 h with median tissue culture infective dose of approximately 100 $TCID_{50}$ of HIV_{BaL} (R5) or HIV_{IIIB} (X4). Infection was monitored by p24 ELISA (PerkinElmer Life Sciences Inc. Waltham, Massachusetts, USA). BDNF mRNA levels were determined 24 h after the infection by RT-PCR. Reverse transcriptase-negative controls were used to exclude genomic DNA contamination. Data, expressed as percentage of untreated cells, represent the mean of two independent preparations.

mRNA levels by both HIV-1 strains (Fig. 1c), further suggesting that HIV-1 is capable of reducing the expression of this neurotrophin in T cells.

Our main finding is that the serum of HIV-positive women is characterized by reduced levels of BDNF, but not of NGF or NT-3, irrespective of drug use status, suggesting that HIV-1 influences the expression of selected neurotrophins. This was confirmed by direct evidence that both R5 and X4 HIV-1 strains down-regulate BDNF mRNA levels in T cells. These results may contribute new insights into our understanding of the immune dysregulation of AIDS. In fact, given the well known antiapoptotic effect of the neurotrophins for T cells [6,13], we may speculate that a decrease in BDNF could be among the mechanisms employed by HIV-1 to induce apoptosis of T cells. On the contrary, experimental evidence has shown an inverse correlation between levels of BDNF and CXCR4 [15] and CCR5 [16] expression. These coreceptors are crucial for HIV-1 infection [17]. Therefore, reduced levels of BDNF may be a risk factor for increasing HIV infection.

HIV-1 also causes axonal injury, neuronal loss and dementia [18]. BDNF is critical for neuronal survival [19]. Blood neurotrophin levels have been used to investigate the role of the neurotrophins in the pathogenesis of various neurodegenerative diseases. In fact, recent data have shown a relationship between BDNF in blood and Alzheimer's disease [20] and age-related cognitive impairment [21]. Therefore, serum BDNF could be a predictor of risk for the development of neurological signs in HIV-positive individuals. Our findings of an association between HIV infection and serum BDNF levels, and of lowered BDNF mRNA levels in infected T cells, provide initial evidence in support of this hypothesis and suggest this neurotrophin as a possible biomarker for HIV dementia. Additional studies are needed to validate our results and extend them to both sexes, as we examined a relatively small cohort of women individuals. Also, a link between BDNF and cognitive performance needs to be established.

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[a]*Department of Neuroscience, Georgetown University Medical Center, Washington, District of Columbia,* [b]*Department of Microbiology and Immunology, Institute of Human Virology, University of Maryland, Baltimore,* [c]*Laboratory of Molecular Medicine and Neuroscience, National Institute of Neurological Disorders and Stroke/National Institute of Health, Bethesda, Maryland,* [d]*Departments of Psychiatry and Psychology, University of Illinois at Chicago, Chicago, Illinois,* [e]*Department of Neurology, and* [f]*Department of Medicine, Georgetown University Medical Center, Washington, District of Columbia, USA.*

Correspondence to Italo Mocchetti, PhD, Georgetown University Medical Center, New Research Building WP13, 3970 Reservoir Road NW, Washington, DC 20057, USA.
E-mail: moccheti@georgetown.edu

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High-sensitivity C-reactive protein levels fall during statin therapy in HIV-infected patients receiving ritonavir-boosted protease inhibitors

Elisabeth Aslangul[a,b], Soraya Fellahi[c,d,e], Lambert K. Assoumou[f,g], Jean-Philippe Bastard[c,d,e], Jacqueline Capeau[c,d,e] and Dominique Costagliola[f,g,h]

HIV-infected patients are at an increased risk of developing cardiovascular disease. Elevated levels of C-reactive protein (CRP) are associated with an increased risk of cardiovascular disease in the general population and are reduced by statin therapy. We examined the effect of pravastatin

and rosuvastatin on CRP levels in 58 dyslipidemic HIV-infected patients. A 45-day course of either statin reduced the median CRP level from 3.0 to 2.4 mg/l ($P < 0.001$) with no correlation with changes in lipid parameters.

Cardiovascular disease is more frequent in HIV-infected patients than in the general population, possibly owing to lipid disorders, viral infection, inflammation and antiretroviral therapy (ART), especially ritonavir (RTV)-boosted protease inhibitors [1–3]. Elevated levels of high-sensitivity C-reactive protein (hsCRP), a marker of persistent inflammation, are linked to an increased risk of cardiovascular events in the general population [4], whereas elevated hsCRP levels in HIV-infected patients are associated with a higher incidence of myocardial infarction [5] and death [6,7]. In the general population, rosuvastatin has been shown to reduce hsCRP levels by about one third and also to lower the risk of death and cardiovascular events [8]. Several studies have reported that hsCRP levels are higher in HIV-infected patients than those in the general population [9,10]. The role of combination ART (cART) is discussed [11–13]. Elevated CRP levels have also been linked to other cardiovascular risk factors, such as high low-density lipoprotein (LDL) levels, low high-density lipoprotein levels and smoking, in addition to ART [14]. The aim of this study was to examine changes in levels of hsCRP, soluble tumor necrosis factor-α receptors (TNFRs) and the endothelial markers intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) after a 45-day course of rosuvastatin or pravastatin in dyslipidemic HIV-infected patients participating in the Agence Nationale de Recherche sur le SIDA (ANRS126) VIHstatine trial; all the patients had good viral control on a RTV-boosted protease inhibitor regimen [15].

The VIHstatine randomized, double-blind, multicenter trial (NCT00117494) was designed to assess the impact of a 45-day course of rosuvastatin 10 mg per day or pravastatin 40 mg per day on lipid values in dyslipidemic (LDL > 4.1 mmol/l) HIV-infected patients receiving RTV-boosted protease inhibitors [15]. The present substudy focused on patients for whom frozen samples were available both at baseline and after the 45-day course of statin therapy, and who had a baseline CRP value below 10 mg/l, as values above 10 mg/l are suggestive of other inflammatory processes, as indicated by the American Heart Association [16]. Fifty-eight of the 83 patients enrolled in the VIHstatine trial were eligible for this substudy and were equally distributed between the two statin arms.

HsCRP was measured by immunonephelometry on an IMMAGE analyzer (Beckman-Coulter, Villepinte, France). sTNFR1, sTNFR2, ICAM-1 and VCAM-1 levels were measured with commercial ELISA kits from R&D Systems (Oxford, UK), using the manufacturer's protocols.

Results are reported as median [interquartile range (IQR)]. Baseline hsCRP, sTNFR1, sTNFR2, ICAM-1 and VCAM-1 levels, and changes between baseline and day 45, were compared between the two statin arms by using the Mann–Whitney nonparametric test, whereas changes between baseline and day 45 were compared with the nonparametric paired Wilcoxon test. Correlations between changes in parameters were tested with Spearman's nonparametric test. All reported *P*-values are two tailed. The Bonferroni rule was used to take multiplicity issues into account: we used nine Mann–Whitney tests and nine Wilcoxon tests, yielding the significance threshold at *P*-value less than 0.0055, and 30 Spearman correlation tests, yielding the significance threshold at *P*-value less than 0.0017. The SPSS software package version 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA) and SAS statistical software version 9.1 (SAS Institute Inc., Cary, North Carolina, USA) were used for all analyses.

The patients were mainly men (74%) and white (91%) and had a median age of 49 years (IQR 42–56). Plasma HIV-1-RNA levels were below 400 copies/ml in 90% of patients and the median CD4 cell count was 490 cells/μl (IQR 314–704). The median duration of cART was 9 years (IQR 5–13). Baseline values of lipid, inflammation and endothelial parameters are reported in Table 1. After 45 days of statin therapy, the median change in the hsCRP concentration was −20% overall (−0.6 mg/l, $P < 0.001$), and, respectively, −22 and −16% in the pravastatin and rosuvastatin groups ($P = 0.932$). There was no significant change in sTNFR1 and sTNFR2 levels or in ICAM-1 and VCAM-1 levels (Table 1).

The LDL-cholesterol level fell by a median of 19% in the pravastatin group and 37% in the rosuvastatin group ($P < 0.001$ for difference between groups), whereas triglyceride levels fell by, respectively, 3% and 26% ($P = 0.008$ for difference between groups), values similar to those obtained in the original VIHstatine trial [15]. As shown in Table 1, LDL-cholesterol, total cholesterol and triglyceride levels also fell significantly in the entire substudy. There was no correlation between the change in the hsCRP level and changes in the markers of lipid, endothelial and inflammatory status [LDL-cholesterol ($r = -0.071$, $P = 0.598$); total cholesterol ($r = -0.176$, $P = 0.188$); and triglycerides ($r = -0.273$, $P = 0.038$)].

This is the first study of the effect of statins on inflammatory status in HIV-infected patients on effective ART. We observed a reduction in the CRP level, but not in the levels of sTNFR1 and sTNFR2, two other inflammatory markers. It is interesting to note that sTNFR1 and sTNFR2 levels were reported to fall in

Table 1. Lipid, inflammatory and endothelial markers at baseline and after 45 days of statin therapy by treatment group and overall.

	Pravastatin			Rosuvastatin			*P*-value[a] Pravastatin vs. rosuvastatin	Total		*P*-value[b] Day 0 vs. day 45
	n	Day 0	Percentage of change at day 45	*n*	Day 0	Percentage of change at day 45		Day 0	Percentage of change at day 45	
Total cholesterol (mmol/l), median (IQR)	29	7.14 (6.35–8.01)	−14% (−21 to −1)	29	7.32 (6.42–7.94)	−29% (−32 to −17)	<0.001	7.28 (6.38–7.91)	−20% (−29 to −11)	<0.001
LDL cholesterol (mmol/l), median (IQR)	29	4.81 (4.23–5.69)	−19% (−29 to 0)	29	4.94 (4.27–5.89)	−37% (−42 to −19)	<0.001	4.90 (4.24–5.79)	−28% (−37 to −13)	<0.001
HDL cholesterol (mmol/l), median (IQR)	29	1.43 (1.17–1.65)	0% (−6 to 12)	29	1.31 (1.15–1.56)	8% (−2 to 13)	0.249	1.35 (1.17–1.61)	3% (−4 to 13)	0.029
Triglycerides (mmol/l), median (IQR)	29	2.07 (1.65–2.64)	−3% (−23 to 11)	29	2.82 (1.90–3.45)	−26% (−41 to −9)	0.008	2.33 (1.72–3.43)	−16% (−33 to 0)	<0.001
High-sensitivity C-reactive protein (mg/l), median (IQR)	29	2.8 (1.7–4.0)	−22% (−41 to 23)	29	3.2 (2.0–4.7)	−16% (−54 to 11)	0.932	3.0 (1.9–4.5)	−20% (−44 to 17)	0.001
Soluble tumor necrosis factor-α receptor (sTNFR)1 (ng/ml), median (IQR)	27	1235 (1011–1661)	−6% (−15 to 8)	28	1469 (1309–1665)	−5% (−11 to 8)	0.501	1411 (1122–1661)	−6% (−15 to 8)	0.140
sTNFR2 (ng/ml), median (IQR)	27	2124 (1810–2615)	−3% (−8 to 10)	28	2717 (2159–3045)	−4% (−11 to 12)	0.490	2332 (1935–2823)	−3% (−8 to 11)	0.738
Intercellular adhesion molecule-1 (ng/ml), median (IQR)	24	265 (218–296)	4% (−5 to 14)	27	295 (228–393)	3% (−3 to 10)	0.497	279 (220–339)	3% (−4 to 12)	0.044
Vascular cell adhesion molecule-1 (ng/ml), median (IQR)	27	866 (747–1069)	−1% (−10 to 8)	29	900 (753–1168)	−4% (−13 to 8)	0.676	885 (749–1079)	−3% (−11 to 7)	0.151

HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein.
[a]Mann–Whitney test.
[b]Paired Wilcoxon test.

HIV-infected patients starting first-line ART, whereas CRP levels were unaffected [12]. Together, these data suggest that these markers reflect different phenomena: sTNFRs may reflect control of HIV infection, whereas CRP elevation could result from other proinflammatory mechanisms not resolved by the control of viral load.

We show here that pravastatin 40 mg per day and rosuvastatin 10 mg per day induce similar significant falls in hsCRP, even during brief administration. In addition, the fall in hsCRP did not correlate with the improvement in lipid parameters or with modifications in endothelial status, as reflected by ICAM-1 and VCAM-1 serum concentrations. The fact that the effect of a statin on the level of hsCRP do not correlate with its effect on LDL-cholesterol is already known [17]; this may explain why, although rosuvastatin and pravastatin did not have the same effect on lipid parameters in this trial, their effect was similar on the hsCRP level. The impact of statin on immune activation, which has been recently shown in HIV-naive patients, might be the mechanism by which the hsCRP level was influenced [18].

In the general population, statins induce a dose-dependent fall in hsCRP levels [19]. HsCRP continues to fall during the course of statin treatment, especially at higher doses [20]. In the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial, 20 mg per day rosuvastatin reduced hsCRP levels by 37% after 1.9 years of treatment [8]. Thus, the fall in CRP observed here in HIV-infected patients during short-term statin therapy could be accentuated during long-term treatment.

In conclusion, a 45-day course of rosuvastatin or pravastatin reduced not only lipid levels but also hsCRP levels in HIV-infected patients treated with RTV-boosted protease inhibitors. Clinical trials are warranted to determine the potential benefit of long-term statin treatment on the risk of cardiovascular events and death in at-risk HIV-infected patients.

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Statistical analysis was performed by L.K.A. and D.C.

Interpretation of the data was performed E.A., S.F., L.K.A., J.-P.B., J.C. and D.C.

Drafting of the article was done by E.A., L.K.A., J.-P.B., J.C. and D.C.

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[a]Université Paris Descartes, [b]AP-HP, Hôtel Dieu, Service de Médecine Interne, [c]AP-HP, Hôpital Tenon, Service de Biochimie et Hormonologie, [d]INSERM U938, CDR Saint-Antoine, [e]UPMC Univ Paris 06, UMR-S938, [f]INSERM U943, [g]UPMC Univ Paris 06, UMR-S943, and [h]AP-HP, Hôpital Pitié-Salpétrière, Service de Maladies Infectieuses et Tropicales, Paris, France.

Correspondence to Dominique Costagliola, INSERM U943, 56 Boulevard Vincent Auriol, BP 335, 75625 Paris Cedex 13, France.
Tel: +33 1 4216 4282;
e-mail: dominique.costagliola@upmc.fr.

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Impact of *IL28B* polymorphisms on response to peginterferon and ribavirin in HIV–hepatitis C virus-coinfected patients with prior nonresponse or relapse

Pablo Labarga[a], Pablo Barreiro[a], José A. Mira[b], Eugenia Vispo[a], Norma Rallón[a], Karin Neukam[b], Angela Camacho[c], Antonio Caruz[d], Sonia Rodriguez-Novoa[a], Javier Pinilla[e], Antonio Rivero[c], José M. Benito[a], Juan A. Pineda[b] and Vincent Soriano[a]

IL28B polymorphisms predict treatment response in chronic hepatitis C. However, no information exists in prior treatment failures. A total of 62 HIV/hepatitis C virus (HCV) patients who completed retreatment with peginterferon-α/ribavirin were examined, of whom 25 (40%) had been cured. Predictors of response [odds ratio, OR (95% confidence interval, CI)] were HCV genotypes 2/3 [16.1 (2.7–90.9)], prior relapse [9.6 (1.5–62.4)]

and ribavirin plasma trough concentrations at week 4 [4.9 (1.3–18.4)]. IL28B-CC only predicted response in prior nonresponders carrying HCV genotypes 1/4 [25.1 (1.9–337)].

While awaiting for the arrival of new direct hepatitis C virus (HCV) antivirals, the accelerated course of liver fibrosis in HIV/HCV-coinfected individuals [1] makes treatment of chronic hepatitis C a priority [2], being patients who have failed interferon (IFN)α-based therapy in the past no exception. Besides the strong influence of HCV genotypes, the chances of a sustained virological response (SVR) after treatment rechallenge seem to mostly depend on patient's characteristics rather than viral factors, that is, extent of liver fibrosis or ribavirin (RBV) plasma exposure [3]. Single nucleotide polymorphisms (SNPs) nearby the *IL28B* gene are currently known to be strong predictors of response to first-line pegIFNα–RBV therapy in both HCV-monoinfected [4–6] and HCV/HIV-coinfected individuals [7,8]. At this time, the impact of *IL28B* variants on treatment rechallenge is unknown.

We have assessed the influence of *IL28B* rs12979860 SNPs in 62 HIV/HCV-coinfected patients who received a second course of therapy with pegIFNα-2a (180 μg/week) and RBV (1000–1200 mg/day) for 48 weeks, after having failed to suboptimal IFNα-based regimens in the past (i.e., conventional IFNα with/without RBV or pegIFNα and fixed 800 mg/day RBV dosing). Participants without a decline of more than 2 logs in serum HCV-RNA at week 12 or with serum HCV-RNA more than 10 IU/ml at week 24 were considered as virological failures and discontinued therapy [3]. Likewise, participants who showed HCV-RNA rebound after discontinuing treatment with undetectable viremia were considered as relapsers. Plasma HCV-RNA was measured using a real-time PCR assay (lower limit of detection of 10 IU/ml). HCV genotyping was performed using a commercial RT-PCR hybridization [9]. Plasma trough concentrations of RBV were measured at week 4 using high-performance liquid chromatography (HPLC) [10]. The *IL28B* rs12979860 SNP was examined in peripheral blood mononuclear cells using the 5′ nuclease assay with allele-specific TaqMan probes (ABI TaqMan allelic discrimination kit) and ABI7900HT Sequence Detection System (Applied Biosystems, Carlsbad, California, USA) [11].

In the study population, mean age was 43 years, most were men (82%) and were former injection drug users (IDUs; 97%); active alcohol abuse was rare (8%) and most patients were on antiretroviral therapy (94%), with undetectable plasma HIV-RNA (95%) and mean CD4 cell counts of 657 cells/μl. Most participants had serum HCV-RNA levels more than 500 000 IU/ml (73%) and were infected with HCV genotypes 1 or 4 (76%). More than a half of patients had advanced liver fibrosis (53%) and had failed to pegIFNα and low-dose RBV (58%). Nonresponse (63%) was the most frequent type of virological failure to first hepatitis C therapy, being HCV relapse recognized in only 21% of cases. In the remaining 16%, the prior course of therapy had been prematurely interrupted due to toxicity. Overall, 47% of patients had the *IL28B* rs12979860 CC genotype.

A total of 25 (40%, by on-treatment analysis) attained SVR after completion of pegIFNα–RBV retreatment. Patients who achieved SVR had lower baseline serum HCV-RNA (5.8 vs. 6.2 log IU/ml, $P = 0.06$) and were less frequently infected with HCV genotype 1 or 4 (48% vs. 95%, $P < 0.01$) than failures. The likelihood of achieving SVR was significantly greater in prior relapsers than in nonresponders (85% vs. 31%; $P < 0.001$). Participants carrying *IL28B* CC more likely attained SVR than non-CC carriers (57% vs. 24%, respectively; $P = 0.006$). However, when the population was split out according to HCV genotype, the impact of *IL28B* on SVR was only seen in HCV genotypes 1 or 4 carriers (44% for CC vs. 14% for non-CC, $P = 0.02$), being not recognized in participants infected with HCV genotype 2 or 3 (82% SVR for CC vs. 100% for non-CC, $P = 0.36$). Patients who attained sustained HCV clearance had greater mean RBV plasma trough concentrations at week 4 of therapy than patients who failed therapy (2.41 vs. 1.75 μg/ml; $P = 0.02$). The best discriminatory RBV threshold was 2.0 μg/ml, which displayed a positive predictive value of 69% and a negative predictive value of 70% for SVR ($P = 0.02$).

Two models for the multivariate analysis were built considering or not RBV plasma trough concentrations at week 4 among the predictors of SVR. HCV genotype 2 or 3, relapse after prior IFNα-based therapy, and RBV plasma concentrations were all associated with SVR (Table 1). Interestingly, the impact of *IL28B* polymorphisms on SVR was only recognized in the subset of patients more difficult to treat, namely those infected with HCV genotypes 1 or 4 and with true nonresponse to a first course of therapy. In the multivariate analysis for this subpopulation, adjusting for sex, use of antiretroviral therapy, serum HCV-RNA levels, and liver fibrosis staging, the subset of patients carrying the CC genotype had a higher likelihood of response than CT/TT carriers [odds ratio (OR), 25.07 (95% confidence interval, CI 1.86–337), $P = 0.01$]. Moreover, in these patients, RBV plasma trough concentrations at week 4 did not predict SVR [OR, 2.79 (95% CI 0.58–13.03), $P = 0.2$].

The finding of a restricted influence of the favorable *IL28B* genotype in patients with history of true nonresponse instead of relapsers is in line with the recognition by others of a strong association between *IL28B* rs12979860 SNPs and early viral kinetics on therapy but not with prevention of viral rebound upon completion of treatment [12]. In contrast, RBV plasma trough concentrations predicted SVR to re-treatment of

Table 1. Predictors of sustained virological response in the study population (multivariate analysis).

	OR (95% CI) *P*	
	Considering RBV	Not considering RBV
Male sex	7.66 (0.13–456) 0.33	2.88 (0.38–21.69) 0.30
Under HAART	0.07 (0.003–1.51) 0.09	0.09 (0.007–1.09) 0.06
HCV-RNA <500 000 IU/ml	0.54 (0.03–9.70) 0.68	2.61 (0.53–12.79) 0.24
HCV genotype 2–3 vs. 1–4	52.63 (2.35–1000) 0.01	16.13 (2.75–90.91) 0.002
Advanced liver fibrosis	0.51 (0.06–4.57) 0.55	0.41 (0.01–1.75) 0.23
Prior relapse	13.01 (0.61–275) 0.09	9.65 (1.49–62.44) 0.02
IL28B rs12979860 CC vs. CT/TT	2.02 (0.18–23.12) 0.57	2.51 (0.54–11.69) 0.24
RBV (per μg/ml)	4.92 (1.31–18.45) 0.02	–

RBV represents ribavirin plasma trough concentration. CI, confidence interval; HAART, highly active antiretroviral therapy; HCV, hepatitis C virus; OR, odds ratio.

hepatitis C in the subset of patients with prior HCV relapse and/or HCV genotypes 2 or 3. In them, RBV trough concentrations more than 2 μg/ml were associated with SVR [OR, 11.67 (95% CI 0.92–147), $P = 0.06$] with almost statistical significance in the multivariate analysis.

In summary, re-treatment of chronic hepatitis C in HIV–HCV-coinfected patients must ensure optimal RBV exposure, especially in prior relapsers and/or in patients infected with HCV genotype 2 or 3. In contrast, in prior true nonresponders infected with HCV genotype 1 or 4, which is the most prevalent and difficult-to-treat population, optimization of RBV exposure seems to have little impact on SVR, while a favorable *IL28B* genotype plays a major role in the outcome of re-treatment.

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[a]*Hospital Carlos III, Madrid,* [b]*Hospital Valme, Seville,* [c]*Hospital Reina Sofía, Córdoba,* [d]*Universidad de Jaén, Jaén, and* [e]*Hospital San Pedro, Logroño, Spain.*

Correspondence to Dr Pablo Barreiro, Department of Infectious Diseases, Hospital Carlos III, Calle Sinesio Delgado 10, Madrid 28029, Spain.
Tel: +34 91 4532500; fax: +34 91 7336614;
e-mail: pm.barreiro@gmail.com

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