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### ► To cite this version:

Philippe Morlat, Edwidge Pereira, Pascal Clayette, Nathalie Derreudre-Bosquet, Jean-Luc Ecobichon, et al.. Early evolution of plasma soluble TNF-alpha p75 receptor as a marker of progression in treated HIV-infected patients.. *AIDS Research and Human Retroviruses*, Mary Ann Liebert, 2008, 24 (11), pp.1383-9. 10.1089/aid.2007.0293 . inserm-00350155

**HAL Id: inserm-00350155**

**<https://www.hal.inserm.fr/inserm-00350155>**

Submitted on 6 Jan 2009

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# Early Evolution of Plasma Soluble TNF- $\alpha$ p75 Receptor as a Marker of Progression in Treated HIV-Infected Patients

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## Abstract

We evaluated the prognostic value of different mediators of inflammation: TNF- $\alpha$  and its soluble receptor p75, platelet-activating factor, and glutathione tripeptide in a case-control study nested within a cohort of 1281 patients infected by the human immunodeficiency virus (HIV) started on highly active antiretroviral treatment (HAART). During the first year of HAART, 16 cases experienced an AIDS-defining event and 6 experienced an evolution of T CD4<sup>+</sup> cell count <100/mm<sup>3</sup>. Forty-four controls who did not progress during the same follow-up period were matched for age, baseline CD4<sup>+</sup>, and HIV-RNA. In the control group, plasma levels of TNF- $\alpha$  and its soluble receptor p75 decreased significantly from baseline to month 4: from 11.0 to 8.7 pg/ml ( $p < 0.001$ ) and from 27.3 to 22.8 pg/ml ( $p < 0.003$ ), respectively. Furthermore the decrease of TNF- $\alpha$  and its soluble receptor p75 was larger in nonprogressors than in progressors ( $p = 0.003$ ). Measurement of TNF- $\alpha$  soluble receptor p75 may be of interest as an additional marker of early antiretroviral effect.

## Introduction

THE CHRONIC IMMUNE ACTIVATION SPECIFIC TO HIV DISEASE contributes to HIV disease pathogenesis. Several cohorts have already shown the prognostic value of plasma levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and especially of its soluble TNF- $\alpha$  p75 receptor (sR-TNF- $\alpha$  p75) for HIV disease progression.<sup>1-7</sup> These studies were mainly conducted before the era of highly active antiretroviral therapy (HAART) and information is lacking on the relationship between sR-TNF- $\alpha$  p75 or other immune markers with clinical progression in patients receiving HAART. In a previous study, we showed a correlation between the evolution of plasma levels of immune markers, especially sR-TNF- $\alpha$  p75, and simultaneous immunovirological responses in HAART-treated patients. However, this study did not assess the prognostic role of early variations of these markers on HIV disease progression.<sup>8</sup>

In addition, the prognostic role of other inflammatory mediators remains currently unknown. The platelet-activating

factor (PAF; 1-O-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) may play a role in inflammatory and immune responses<sup>9</sup> and some of its antagonists had *in vitro* antiretroviral properties.<sup>10,11</sup> Inflammatory processes triggered by PAF are mediated by interactions with a specific PAF receptor<sup>12</sup> and restrained by a enzymatic PAF-inactivating system, including plasma PAF acetylhydrolases (PAF-AH) that catalyzes the hydrolysis of the *sn*-2 ester bond of PAF.<sup>13</sup> Because PAF-AH is important for regulation of plasma PAF concentration, the dosage of plasma PAF activity is considered more reliable than PAF. Oxidative stress may also contribute to inflammatory processes in HIV disease and may be measured either by the major antioxidant in the organism, the glutathione tripeptide (GSH or L- $\gamma$ -glutamyl-L-cysteinylglycine),<sup>14</sup> whose low level is associated with poor survival in HIV-infected patients,<sup>15</sup> or the activity of GSH peroxidases (GSH-Px), which oxidizes GSH in its disulfide form (GSSG). GSH-Px associated with superoxide dismutases (SOD) is an important initial component in cellular defenses against reactive oxygen species (ROS) toxicity, e.g.,

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superoxide anions ( $O_2^{\ominus}$ ) and hydrogen peroxide ( $H_2O_2$ ). Moreover, HIV, by subverting the intracellular cysteine pool for its own replication, is able to decrease GSH biosynthesis<sup>16</sup> and to generate a production of inflammatory mediators such as TNF- $\alpha$ .<sup>17</sup>

In the context of the well-validated surrogacy value of CD4<sup>+</sup> lymphocyte count and plasma HIV-RNA levels, which are the cornerstones of the laboratory follow-up in HIV-infected treated patients, we assessed the potential additive prognostic value of plasma levels of TNF, sR-TNF- $\alpha$  p75, PAF-AH, GSH, and GSH-Px on the progression of HIV infection in HAART-treated patients.

## Materials and Methods

The study was a case-control study nested within the APROCO-COPILOTE cohort. This cohort is a prospective study of the French National Agency for Research on AIDS and viral hepatitis (ANRS CO8) that enrolled consecutively adult HIV-infected patients who for the first time started a protease inhibitor (PI)-containing regimen in 1997-1998 and were followed in 47 French clinical wards.<sup>18</sup> Standardized clinical and biological data are collected at baseline, 1 and 4 months after enrolment, and every 4 months thereafter. Investigators are requested to report clinical progression and deaths to the coordinating center as soon as they are known. In the current analysis, cases (i.e., rapid progressors) were defined as patients who experienced HIV disease progression during the first year of treatment. Disease progression was defined as an occurrence of a new AIDS-defining event according to the CDC definition<sup>19</sup> or a T CD4<sup>+</sup> cell count decline below 100/mm<sup>3</sup> (two repeated measurements). Two control subjects (i.e., nonprogressors) per case were matched for age ( $\pm 5$  years), baseline T CD4<sup>+</sup> cell count ( $\pm 80$ /mm<sup>3</sup>), and plasma HIV-1 RNA ( $\pm 0.3$  log<sub>10</sub> copies/ml); these patients did not experience any clinical or biological progression as defined previously during their first year of HAART. All eligible cases and controls were selected if they had available plasma frozen at inclusion, at month 1 (M1), and at month 4 (M4) follow-up visits.

### Measurement of virological and immunological parameters

CD4<sup>+</sup> counts were measured by flow cytometry and plasma HIV-1 RNA levels were assessed using the local available assays, mainly bDNA Chiron 2.0 (detection threshold of 500 copies/ml or 2.7 log<sub>10</sub> copies/ml) or PCR by Amplicor-Roche (detection threshold of 200 copies/ml or 2.3 log<sub>10</sub> copies/ml).

Plasma concentrations of TNF- $\alpha$  and its soluble p75 receptor were measured using specific R&D Systems ELISA (Nivelles, Belgium), whereas plasma PAF-AH activity and plasma GSH and GSH-Px levels were quantified using Cayman Chemical assays (Ann Arbor, MI). These dosages were used according to the manufacturer's recommendations.

### Statistical analysis

Comparisons between progressors and nonprogressors were performed by a Chi-square test for proportions and by a Wilcoxon test for median values. Evolution of immune markers was studied between baseline and M4 using a multivariate linear mixed model making it possible to test for ef-

fect of time and of disease progression. Models were adjusted for age, history of AIDS, and baseline CD4<sup>+</sup> cell count, plasma HIV RNA level, and hemoglobin. Before entering the values of immune markers in the model, they were log<sub>e</sub> transformed to normalize the distribution. Analyses were performed using the Statistical Analysis System Software (version 8.2, SAS Institute, Cary, NC).

## Results

### Study population and baseline characteristics

Among the 26 patients of the APROCO-COPILOTE cohort who had clinical or laboratory progression during the first year of follow-up, 22 had a complete collection of frozen blood samples (baseline, M1, and M4) and were retained in the analysis as cases: 16 had experienced at least one clinical AIDS-defining event (Table 1) and 6 experienced biological progression only (the lowest levels of CD4<sup>+</sup> lymphocyte count experienced by these patients were 19, 21, 65, 82, 69, and 54/mm<sup>3</sup>). Among the 22 progressors, 8 progressed early (between M1 and M4) and 14 progressed later (between M4 and M12). Forty-four nonprogressors were appropriately matched for age, baseline CD4<sup>+</sup> cell count, and plasma HIV-1 RNA.

On average, patients were 36 years old and were mainly infected through sexual contacts (Table 2). The median CD4<sup>+</sup> cell count was 120/mm<sup>3</sup> and HIV-1 RNA 4.5 log<sub>10</sub> copies/ml. Progressors and nonprogressors differed only for median baseline hemoglobin level, 12.1 vs. 13.8 g/dl, respectively ( $p = 0.02$ ).

### Evolution of laboratory markers during the first 4 months of HAART

**CD4<sup>+</sup> lymphocyte count.** From baseline to M1 and M4, median CD4<sup>+</sup> lymphocyte count increased from 125 to 201 (at M1) and 197/mm<sup>3</sup> (at M4) in nonprogressors ( $p < 10^{-4}$ ) but only from 113 to 148 (at M1) and 151/mm<sup>3</sup> (at M4) in those patients who rapidly progressed ( $p = 0.22$ , NS).

**Plasma HIV-1 RNA.** During the same follow-up period, median plasma HIV-1 RNA level decreased from 4.6 to 2.7 in nonprogressors ( $p < 10^{-3}$ ) and from 4.5 to 2.5 log<sub>10</sub>

TABLE 1. CLINICAL EVENTS OCCURRING DURING THE FIRST YEAR OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY IN 22 CASES, ANRS CO8 APROCO-COPILOTE

Description of event	Number of patients
Cytomegalovirus retinitis	3
Kaposi sarcoma	3
Non-Hodgkin lymphoma	2
<i>Pneumocystis carinii</i> pneumonia	2
Toxoplasmic encephalitis	1
Progressive multifocal leukoencephalopathy	1
HIV encephalitis	1
Pulmonary tuberculosis	1
Esophageal candidiasis + disseminated tuberculosis	1
Cerebral nocardiosis	1

TABLE 2. BASELINE CHARACTERISTICS OF CASES AND CONTROLS INCLUDED IN THE ANALYSIS, ANRS CO8 APROCO-COPILOTE

	Cases Progression within the first year of HAART <sup>a</sup> (n = 22)	Controls Nonprogression within the first year of HAART <sup>a</sup> (n = 44)	p
Median age in years (range)	35.1 (28.2–40.7)	37.7 (33.0–40.5)	0.61
HIV transmission category, n (%)			
Heterosexuals	9 (40.9)	14 (31.8)	0.62
Men who have sex with men	8 (36.4)	17 (38.6)	
Intravenous drug users	3 (13.6)	11 (25.0)	
Other	2 (9.1)	2 (4.6)	
CDC <sup>b</sup> clinical stage, n (%)			
A	5 (22.7)	17 (38.6)	0.21
B	4 (18.2)	11 (25.0)	
C	13 (59.1)	16 (36.4)	
Median CD4 <sup>+</sup> cells/mm <sup>3</sup> (range)	113 (60–252)	125 (61–249)	0.72
Median HIV1-RNA (log <sub>10</sub> copies/ml) (range)	4.5 (3.4–5.5)	4.6 (3.3–5.5)	0.95
Median plasma haemoglobin (g/dl) (range)	12.1 (10.1–13.8)	13.8 (11.9–15.1)	0.02

<sup>a</sup>HAART, highly active antiretroviral therapy.

<sup>b</sup>CDC, Centers for Disease Control.

copies/ml in progressors ( $p = 3.10^{-4}$ ); these decreases were not statistically different between the two groups ( $p = 0.32$ ).

In nonprogressors, HIV-1 RNA reached undetectable levels (below 2.3 log<sub>10</sub> copies/ml) in 33% at M1 and 45% at M4 vs. 37% and 55% in progressors; these variations were not significantly different between the two groups ( $p = 0.8$  and  $p = 0.5$ , respectively).

**Plasma sR-TNF- $\alpha$  p75 and TNF- $\alpha$  levels.** Among non-progressors, median plasma sR-TNF- $\alpha$  p75 and TNF- $\alpha$  decreased significantly from baseline to M4: from 11.0 to 8.7 pg/ml [ $-0.10 \log_e$  pg/ml/month (adjusted  $p < 0.001$ )] and from 27.3 to 22.8 pg/ml [ $-0.06 \log_e$  pg/ml/month (adjusted  $p < 0.003$ )], respectively (Table 3 and Fig. 1). Adjusted  $p$ -values of the variations between M0 and M4 did not change if undetectability of viral load at M4 was included as an additional variable in the models. Conversely, no significant decrease was observed in progressors ( $-0.007 \log_e$  pg/ml/month for sR-TNF- $\alpha$  p75 and  $-0.04 \log_e$  pg/ml/month for TNF- $\alpha$ ).

Among nonprogressors, median plasma sR-TNF- $\alpha$  p75 decreased significantly from baseline to M4 either in the 19 patients who achieved viral suppression at M4 or in the 25 who did not ( $p < 0.001$  in both subgroups). Among progressors, no significant variation of plasma sR-TNF- $\alpha$  p75 was observed either in the 12 patients who achieved viral suppression at M4 or in the 10 who did not.

Finally, the decrease of plasma sR-TNF- $\alpha$  p75 between M0 and M4 was larger in nonprogressors than in progressors ( $p = 0.003$ ) but no significant difference was found for variations of TNF- $\alpha$  between the two groups (Table 3).

Between M0 and M1 we noticed that the median evolution of sR-TNF- $\alpha$  p75 was  $-2$  pg/ml in nonprogressors and  $+0.5$  pg/ml in progressors: the differences between these variations were also highly significant ( $p = 0.01$ ). For TNF- $\alpha$  ( $-3$  pg/ml in nonprogressors and  $-1.8$  pg/ml in progressors) no significant difference was found between the two groups.

**Plasma GSH level.** A significant increase of plasma GSH level was observed in nonprogressors from baseline to M4:  $3.9$ – $4.6 \mu\text{mol/ml}$  ( $+0.04 \log_e \mu\text{mol/ml/month}$ , adjusted  $p = 0.008$ ). No significant evolution was reported in progressors. Nevertheless, the overall evolution did not differ between patients who progressed and those who did not progress.

Between M0 and M1 we did not notice significant variations of median plasma GSH either in nonprogressors ( $+0.5 \mu\text{mol/ml}$ ) or in progressors ( $+1 \mu\text{mol/ml}$ ) and no significant difference was found between the two groups.

**Plasma GSH-Px and PAF-AH levels.** Plasma GSH-Px did not significantly change between baseline and M4, either in nonprogressors ( $p = 0.99$ ) or progressors ( $p = 0.07$ ). Similar trends were found for the evolution of plasma PAF-AH. No significant change within or between groups was observed between M0 and M1 for both markers.

## Discussion

The decrease of plasma sR-TNF- $\alpha$  p75 was larger in non-progressors than in progressors while the two groups were appropriately matched for baseline CD4<sup>+</sup> lymphocyte count and plasma HIV-1 RNA and while differences of virological responses between groups did not explain the differences of HIV disease progression. As a result of our study design the sR-TNF- $\alpha$  p75 plasma level might be considered as a prognostic marker, in addition to baseline CD4<sup>+</sup> and plasma HIV RNA in HAART-treated patients. Whereas the prognostic value of sR-TNF- $\alpha$  p75 plasma level variations within the first 4 months is obvious, the simultaneous variations of plasma HIV-RNA did not make it possible to predict HIV disease progression in our study.

If plasma levels of HIV-RNA and CD4<sup>+</sup> cell count are highly predictive of progression to AIDS or death, they do not explain all the variability of disease progression.<sup>3,20,21</sup> We assume, as do others, that if an additional marker is required

TABLE 3. EVOLUTION OF HIV-1 RNA AND IMMUNE MARKERS BETWEEN BASELINE AND MONTH 4 IN CASES (PROGRESSION DURING THE FIRST YEAR OF HIGHLY ACTIVE ANTIRETROVIRAL TREATMENT) AND CONTROLS (NONPROGRESSION), APROCO-COPILOTE ANRS CO8

	Progressor group (n = 22)	Nonprogressor group (n = 44)	p <sup>a</sup>
Median HIV-1 RNA log <sub>10</sub> copies/ml (range)			
Baseline	4.5 (3.4–5.6)	4.6 (3.3–5.5)	0.95
Month 1	2.7 (2.5–3.8)	2.7 (2.6–3.1)	0.64
Month 4	2.5 (2.3–3.4)	2.7 (2.3–2.7)	0.93
HIV RNA <500 copies/ml, n (%)			
Baseline	3 (13.6)	2 (4.6)	0.32
Month 1	8 (36.4)	14 (32.6)	0.76
Month 4	12 (54.6)	19 (45.2)	0.48
Median sR-TNF- $\alpha$ p75 (pg/ml) (range)			
Baseline	14.1 (8.3–20.3)	11.0 (7.5–22.4)	0.73
Month 1	14.6 (8.3–19.9)	9.0 (6.6–13.8)	0.07
Month 4	11.5 (6.1–20.3)	8.7 (5.9–11.5)	0.05
Median TNF- $\alpha$ (pg/ml) (range)			
Baseline	35.7 (20.0–44.5)	27.3 (19.9–42.3)	0.53
Month 1	33.9 (23.8–45.4)	24.3 (18.6–35.7)	0.09
Month 4	28.7 (18.6–37.8)	22.8 (18.4–29.2)	0.12
Median PAF-AH ( $\mu$ mol/min/ml) (range)			
Baseline	10.0 (7.0–12.6)	10.6 (8.2–13.0)	0.72
Month 1	11.8 (7.7–14.0)	10.3 (7.4–12.6)	0.21
Month 4	10.5 (7.9–14.4)	9.9 (8.5–12.8)	0.66
Median GSH ( $\mu$ mol/ml) (range)			
Baseline	3.9 (2.9–6.1)	4.0 (3.4–5.2)	0.88
Month 1	4.9 (3.4–6.7)	4.5 (3.1–6.1)	0.63
Month 4	4.6 (3.5–6.5)	4.7 (3.4–5.6)	0.82
Median GSH-Px (ng/ml) (range)			
Baseline	604 (471–729)	652 (546–800)	0.37
Month 1	609 (376–706)	656 (505–825)	0.11
Month 4	642 (430–728)	700 (568–788)	0.33

<sup>a</sup>Comparison between progressors and nonprogressors.

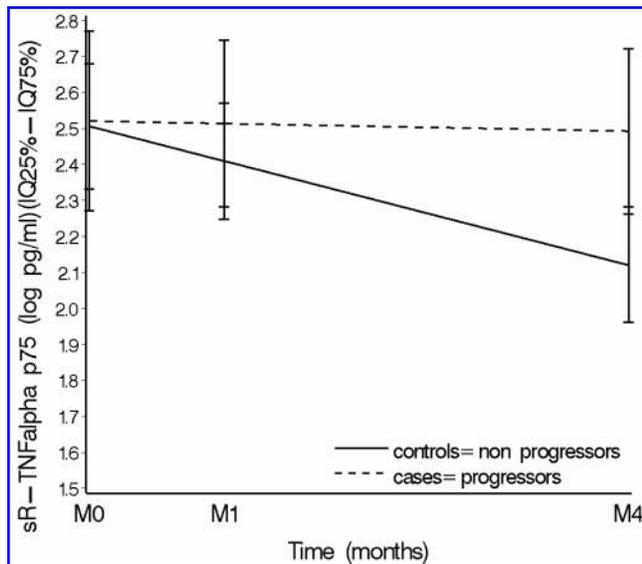
to predict more accurately HIV disease progression, the use of sR-TNF- $\alpha$  p75 might then be taken into account.<sup>22</sup>

Other inflammatory markers evaluated, i.e., TNF- $\alpha$ , PAF-AH, GSH, and GSH-Px levels, were not associated with disease progression. Although not established as having a prognostic value, the increase in GSH level in no progressing patients is nevertheless consistent with the deleterious effect of glutathione deficiency in progression of HIV disease.<sup>15,16</sup>

In our study, TNF- $\alpha$  plasma level was not associated with HIV disease progression. TNF- $\alpha$  itself may be difficult to detect in the circulation because of its rapid clearance and the use of assays unable to detect TNF bound to sR-TNF- $\alpha$  p75.<sup>23</sup> In contrast, sR-TNF- $\alpha$  p75 is easily detectable in the plasma of HIV-infected patients and plasma sR-TNF- $\alpha$  p75 may be considered as a long-term marker of TNF effects.<sup>24,25</sup>

The limited size and the case-control design of our study comparing two groups matched for age, baseline CD4<sup>+</sup> count, and HIV-1 RNA restrict the interpretation of our results to the additive role of plasma immune markers to already well-known surrogate markers as we cannot make inferences regarding the intrinsic prognostic role of the markers. Other observational studies are necessary to confirm the prognostic role of sR-TNF- $\alpha$  p75 on disease progression under HAART and to reevaluate those of other inflammatory markers, particularly for components of the GSH

system; these further evaluations could also be of value to assess if markers of immune status, such as CD8 T cell activation, are prognostic of the failure of HAART, which is still a controversial issue.<sup>26–28</sup> During the chronic phase of HIV infection, particularly in progressors and even in patients undergoing HAART, the functionality of HIV-specific CD8 T cells is gradually altered, partly due to persistent antigenic stimulation and progressive loss of CD4 T cells.<sup>29</sup> The TNF system, by its ability to interfere with HIV replication, apoptosis, and T cell activation, could play a major role in this functionality of CD8 T cells<sup>30</sup> and the relationship between the TNF system and the activation status of CD8 T cells would then be particularly interesting to study. Concerning the GSH system, the depletion of GSH and GSH-Px in blood was confirmed during the acute infection of monkeys by SHIV.<sup>31</sup> Nevertheless, even if a correlation was observed between GSH in blood and lymphocyte proliferative responses in HIV-infected patients, this correlation is not associated with variations for HIV viral load and CD4 and CD8 counts.<sup>32</sup> Moreover, during chronic infection, no significant associations were found by Stephensen *et al.*<sup>33</sup> between GSH and GSH-Px concentrations in blood and the severity of HIV disease. As a consequence, it seems difficult at present to consider these elements of the antioxidant defenses as markers of HAART failure.



**FIG. 1.** Evolution of plasma sR-TNF $\alpha$  p75 (with 95% confidence interval) in cases (clinical or laboratory progression within 1 year after starting highly active antiretroviral therapy) and controls (who did not progress within the first year of HAART), modeling using a linear mixed model, ANRS CO8 APROCO-COPILOTE.

Our findings may be explained by the relations between the TNF system and HIV replication. HIV enhances TNF- $\alpha$  production in various cells and, in turn, this activation induces activation of NF- $\kappa$ B, enhancing HIV replication through an amplification circle.<sup>34-36</sup> Therefore, the increased activation of the TNF system may be either the cause or the result of an increase of HIV-RNA. It has already been suggested that persistent activation of the TNF system may be involved in treatment failure<sup>7</sup> and that decreased TNF activity may lead to low-grade viral replication in HAART-treated patients.<sup>5,37</sup> In this context the impact of antiinflammatory or anti-TNF drugs might be of interest; thalidomide induced, *in vitro*, a decrease of HIV type 1 replication in human macrophages, but its use for countering oral esophageal ulcers was not associated with a reduction of plasma levels of HIV RNA, TNF- $\alpha$ , or its soluble receptors.<sup>38-40</sup> Conversely, pentoxiphylline therapy improved constitutional manifestations and sustained CD4 counts among asymptomatic HIV-infected individuals.<sup>41</sup>

If sR-TNF- $\alpha$  p75 plasma levels reflect the activation of the TNF system, we may hypothesize that it also reflects an activity of HIV disease that is not explored by the measurement of plasma HIV RNA: in this way activation of inflammatory pathways has recently been considered as having a potential deleterious impact on morbidity in HIV-infected patients.<sup>42</sup> Optimization of antiretroviral therapy might then be considered in patients with high sR-TNF $\alpha$  p75 and low HIV-RNA plasma levels and, conversely, therapeutic reductions could be assessed in patients with low HIV-RNA and low sR-TNF- $\alpha$  p75 plasma levels.

CD4<sup>+</sup> cell count is the only immune marker routinely measured in HIV-infected subjects, but its evolution under treatment is slow and widely varies among HAART-treated patients. An increase of plasma soluble markers may be an

earlier marker of poor progression by preceding the increase of HIV RNA and the decrease of CD4<sup>+</sup> lymphocyte counts for several months.<sup>43</sup> Therefore, our results suggest that plasma sR-TNF- $\alpha$  p75 could be an early marker for treatment failure, allowing treatment adaptations as soon as possible. Moreover, CD4<sup>+</sup> cell count does not reflect the activation status of the immune system, although it is of particular interest in the case of discordant immunovirological responses to treatment, especially when the CD4<sup>+</sup> count and plasma HIV-1 RNA are both low. Measurement of plasma sR-TNF- $\alpha$  p75 might then be an earlier and additive marker of the immunological effect of antiretroviral therapy. If the prognostic value of plasma sR-TNF- $\alpha$  p75 level to predict failure of HAART is confirmed by further evaluations, the use of this immune marker should be considered during the development of new antiretrovirals or immune therapies.

## Appendix

### APROCO-COPILOTE ANRS CO8 Study Group

Scientific Committee: Steering Committee: *Principal Investigators*: C. Lepout, F. Raffi; *Methodology*: G. Chêne, R. Salomon; *Social Sciences*: J-P. Moatti, J. Pierret, B. Spire; *Virology*: F. Brun-Vézinet, H. Fleury, B. Masquelier; *Pharmacology*: G. Peytavin, R. Garraffo; *Other members*: D. Costagliola, P. Dellamonica, C. Katlama, L. Meyer, D. Salmon, A. Sobel; *Events Validation Committee*: L. Cuzin, M. Dupon, X. Duval, V. Le Moing, B. Marchou, T. May, P. Morlat, C. Rabaud, A. Waldner-Combernoux; *Project Coordination*: F. Collin-Filleul; *ANRS Representatives*: Nadine Job-Spira, Marcia Trumeau; *Observer*: M. Garré; *Clinical Research Group*: V. Le Moing, C. Lewden.

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*Promotion*: Agence Nationale de Recherches sur le Sida et les hépatites virales (ANRS, Action Coordonnée no. 7).

*Other Supports*: Collège des Universitaires de Maladies Infectieuses et Tropicales (CMIT ex APPIT), Sidaction Ensemble contre le Sida, et laboratoires: Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Glaxo-SmithKline, Roche.

*Clinical Centers (coordinators)*: Amiens (Pr. J.L. Schmit), Angers (Dr. J.M. Chennebault), Belfort (Dr. J.P. Faller), Besançon (Pr. J.L. Dupond, Dr. J.M. Estavoyer, Dr. Drobachef), Bobigny (Pr. O. Bouchaud), Bordeaux (Pr. M. Dupon, Pr. Longy-Boursier, Pr. P. Morlat, Pr. J.M. Ragnaud), Bourg-en-Bresse (Dr. P. Granier), Brest (Pr. M. Garré), Caen (Pr. R. Verdon), Compiègne (Dr. D. Merrien), Corbeil Essonnes (Dr. A. Devidas), Créteil (Pr. A. Sobel), Dijon (Pr. H. Portier), Garches (Pr. C. Perronne), Lagny (Dr. P. Lagarde), Libourne (Dr. J. Ceccaldi), Lyon (Pr. D. Peyramond), Meaux (Dr. C. Allard), Montpellier (Pr. J. Reynes), Nancy (Pr. T. May), Nantes (Pr. F. Raffi), Nice (Pr. J.G. Fuzibet, Pr. P. Dellamonica), Orléans (Dr. P. Arsac), Paris (Pr. E. Bouvet, Pr. F. Bricaire, Pr. P. Bergmann, Pr. J. Cabane, Dr. J. Monsonego, Pr. P.M. Girard, Pr. L. Guillevin, Pr. S. Herson, Pr. C. Lepout, Pr. MC. Meyohas, Pr. J.M. Molina, Pr. G. Pialoux, Pr D.

Salmon), Poitiers (Pr. B. Becq-Giraudon), Reims (Pr. R. Jaus-saud), Rennes (Pr. C. Michelet), Saint-Etienne (Pr. F. Lucht), Saint-Mandé (Pr. T. Debord), Strasbourg (Pr. J.M. Lang), Toulon (Dr. J.P. De Jaureguiberry), Toulouse (Pr. B. Marchou), Tours (Pr. J.M. Besnier).

### Acknowledgments

The authors wish to acknowledge the work of Geneviève Chêne for study design and helpful discussion and of Cécile Droz and Céline Aubenque for their technical assistance. The authors acknowledge the sponsorship of the French Research Agency for Research on AIDS (Agence Nationale de Recherches sur le Sida, ANRS, Action Coordonnée no. 7), financial support from the ANRS, and support from the Collège des Enseignants de Maladies Infectieuses et Tropicales (CMIT). Support for this specific study was provided by a grant from Paris 7 University (FAC-SIDA) for the measurements of plasma levels of immune markers

### Disclosure Statement

Support was provided by associated pharmaceutical companies: Abbott, Boehringer-Ingelheim, Bristol-Myers Squibb, Glaxo-SmithKline, Merck Sharp and Dohme, and Roche.

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