



HAL
open science

A High HIV DNA Level in PBMCs at Antiretroviral Treatment Interruption Predicts a Shorter Time to Treatment Resumption Independently of CD4 Nadir

Christophe Piketty, Laurence Weiss, Lambert Assoumou, Marianne Burgard, Aurelie Melard, Jean-Michel Ragnaud, Michele Bentata, Pierre Marie Girard, Christine Rouzioux, Dominique Costagliola

► **To cite this version:**

Christophe Piketty, Laurence Weiss, Lambert Assoumou, Marianne Burgard, Aurelie Melard, et al.. A High HIV DNA Level in PBMCs at Antiretroviral Treatment Interruption Predicts a Shorter Time to Treatment Resumption Independently of CD4 Nadir. *Journal of Medical Virology*, 2010, 82 (11), pp.1819. 10.1002/jmv.21907 . hal-00577327

HAL Id: hal-00577327

<https://hal.science/hal-00577327>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



A High HIV DNA Level in PBMCs at Antiretroviral Treatment Interruption Predicts a Shorter Time to Treatment Resumption Independently of CD4 Nadir

Journal:	<i>Journal of Medical Virology</i>
Manuscript ID:	JMV-10-1755.R1
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	18-Jun-2010
Complete List of Authors:	Piketty, Christophe; Service d'Immunologie Clinique, Hôpital Européen Georges Pompidou, Paris Weiss, Laurence assoumou, lambert Burgard, Marianne; Hop necker melard, aurelie; Hop necker ragnaud, jean-michel bentata, michele Girard, Pierre Marie; Universitary hospital, Infectious disease Rouzioux, Christine; Hop necker costagliola, dominique
Keywords:	HIV, treatment interruption, HIV-DNA, CD4 nadir, cART



1
2
3
4 1 **A High HIV DNA Level in PBMCs at Antiretroviral**
5
6
7 2 **Treatment Interruption Predicts a Shorter Time to**
8
9
10 3 **Treatment Resumption, Independently of the CD4 Nadir**
11
12
13 4
14
15 5 **Christophe Piketty¹, Laurence Weiss^{1,2}, Lambert Assoumou^{3,4},**
16
17
18 6 **Marianne Burgard⁵, Aurélie Mélard⁵, Jean-Michel Ragnaud⁶,**
19
20
21 7 **Michele Bentata⁷, Pierre-Marie Girard⁸, Christine Rouzioux^{2,5},**
22
23
24 8 **Dominique Costagliola^{3,4,9} and the ANRS 116 SALTO study**
25
26
27 9 **group**
28
29
30
31

32 11 ¹AP-HP, Service d'Immunologie Clinique, Hôpital Européen Georges
33 12 Pompidou, Paris;

34 13 ²Université Paris Descartes, Paris ;

35 14 ³INSERM U 943, Paris F-75013, France

36 15 ⁴UPMC Univ Paris 06, UMR S 943, Paris F-75013, France;

37 16 ⁵AP-HP, Laboratoire de Virologie, Hôpital Necker, Paris,

38 17 ⁶Service des Maladies Infectieuses, Hôpital Pellegrin, Bordeaux,

39 18 ⁷AP-HP, Service de Médecine Interne, Hôpital Avicenne, Bobigny,

40 19 ⁸AP-HP, Service des Maladies Infectieuses, Hôpital Saint-Antoine, Paris ;

41 20 ⁹AP-HP, Groupe hospitalier Pitié-Salpêtrière, Service de Maladies Infectieuses
42 21 et Tropicales, Paris F-75013, France
43 22
44 23

45 24 **Running head: CD4-guided treatment interruption**
46 25
47 26
48 27
49 28
50 29
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Corresponding author:**

2 Christophe Piketty, MD, PhD

3 Service d'Immunologie Clinique, Hôpital Européen Georges Pompidou,

4 20 Rue Leblanc

5 75908 Paris Cedex 15, France.

6 Phone: 33 (1) 56 09 32 93; FAX: 33 (1) 56 09 30 26;

7 E-mail: christophe.piketty@egp.aphp.fr

8

9

10

For Peer Review

1 Abstract

2
3 This study aimed to evaluate the safety of antiretroviral treatment interruption
4 (TI) in HIV-infected patients who started treatment based on earlier guidelines,
5 and to identify baseline factors predictive of the time to reach fixed criteria for
6 treatment resumption.

7 Prospective, open-label, multicentre trial. Patients were eligible if they had a
8 CD4 cell count $>350/\text{mm}^3$ and plasma HIV RNA <50000 copies/ml when they
9 first started antiretroviral therapy (ART); and if they had a CD4 count $>450/\text{mm}^3$
10 and stable plasma HIV RNA <5000 copies/mL for at least six months prior to
11 enrolment. The criteria for ART resumption were a CD4 cell count $<300/\text{mm}^3$
12 and/or a CDC stage B or C event.

13 116 patients had received ART for a median of 5.3 years. The median CD4 cell
14 count and plasma HIV RNA values at inclusion were $809/\text{mm}^3$ and 2.6 log
15 copies/mL, respectively. Median HIV DNA load at inclusion was 2.3 log
16 copies/ 10^6 PBMCs. Thirty-six months after TI, 63.9% of the patients had not yet
17 reached the criteria for ART resumption, and 55.9% of patients had not
18 resumed ART. In Cox multivariable analysis, a high HIV DNA level at TI, a low
19 CD4 nadir, and pre-existing AIDS status were the only significant risk factors for
20 reaching the criteria for ART resumption (hazards ratio: 2.15(1.02-4.53),
21 4.59(1.22–17.24) and 5.74(1.60–20.56), respectively).

22 Patients who started ART with a CD4 cell count above $350/\text{mm}^3$ were able to
23 interrupt treatment for long periods without a high absolute risk of either AIDS or
24 severe non-AIDS morbidity/mortality. A high PBMC HIV DNA level at treatment
25 interruption was a strong predictor for more rapid treatment resumption.

26

1
2
3 1 (ClinicalTrials.gov Identifier, NCT00118677)
4

5
6 2 **Key words:** HIV, treatment interruption, HIV-DNA, CD4 nadir, ART
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1 Introduction

2 Combined antiretroviral therapy (ART) increases CD4 cell numbers, reduces
3 plasma HIV RNA levels and improves patient survival [Hogg et al., 1998; Hogg
4 et al., 2001; Lee et al., 2001; Li et al., 1998; Mocroft et al., 2000; Murphy et al.,
5 2001; Palella et al., 1998; Palella et al., 2003]. However, efficacy can be
6 undermined by suboptimal adherence to treatment and by adverse effects.
7 Treatment interruption appears to be feasible and safe in patients with high CD4
8 cell counts, a high CD4 nadir, and long-term undetectable plasma HIV RNA, as
9 suggested by several small uncontrolled studies [Boschi et al., 2004; Fagard et
10 al., 2003; Fernandez Guerrero et al., 2005; Giuntini et al., 2005; Krolewiecki et
11 al., 2006; Maggiolo et al., 2004; Parish et al., 2002; Pellegrin et al., 2005;
12 Pogany et al., 2007; Ruiz et al., 2000; Skiest et al., 2004; Skiest et al., 2007;
13 Tarwater et al., 2003] and by two controlled trials [Ananworanich et al., 2006;
14 Maggiolo et al., 2008]. However, recent large randomized trials indicate that,
15 compared with patients who continue ART, unselected patients undergoing
16 treatment interruption experience significantly worse clinical outcomes,
17 including HIV disease progression and cardiovascular events [Danel et al.,
18 2006; El-Sadr et al., 2006].

19 In view of the results of these latter studies, treatment interruptions are no
20 longer recommended. However, many patients are concerned with the toxicity
21 and potential long-term adverse effects of ART, and spontaneously stop taking
22 their treatment. Factors predictive of treatment resumption would therefore be
23 useful for clinicians managing such patients, but have not been studied
24 extensively.

1
2
3 1 The aim of this study was to evaluate the safety of treatment interruption in
4
5
6 2 chronically HIV-infected patients who started ART based on earlier guidelines
7
8 3 and who had a high CD4 nadir. Factors potentially predictive of the length of
9
10 4 treatment interruption, and changes in metabolic parameters were also
11
12
13 5 examined.
14
15
16 6
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

1 Patients and Methods

2

3 *Study design*

4 The ANRS 116 SALTO trial was a prospective, open-label, multicenter trial of
5 treatment interruption in patients who had started treatment early in the course
6 of HIV infection, based on contemporary guidelines, and who wished to
7 discontinue ART. The study protocol was approved by the ethics committee of
8 Pitié Salpêtrière Hospital, Paris, France, and all the patients gave their written
9 informed consent before entering the study. The study conformed to the
10 International Conference on Harmonization guidelines for Good Clinical
11 Practices, and with the Helsinki Declaration.

13 *Study population*

14 The following patients were eligible: HIV-infected men and non pregnant women
15 at least 18 years of age; who had a CD4 cell count above 350/mm³ and plasma
16 HIV RNA below 50 000 copies/ml (4.7 log₁₀ copies/ml) at ART initiation; who
17 had been on a stable ART regimen (at least two drugs) for 6 months prior to
18 inclusion; who had had no more than one treatment failure; and who, at
19 baseline, had a CD4 cell count above 450/mm³ and stable plasma HIV RNA
20 below 5000 copies/ml.

21 The exclusion criteria were chronic hepatitis B treated with lamivudine or
22 tenofovir disoproxil fumarate; acute opportunistic infections; and anticancer
23 chemotherapy or immunotherapy.

24

1 **Assessment and monitoring**

2 3 *Study design*

4 ART was interrupted at enrolment in the study and the patients were monitored
5 for up to 36 months. Patients taking a non nucleoside reverse transcriptase
6 inhibitor were advised to discontinue it 7 days before their other antiretroviral
7 drugs.

8 The patients were evaluated at the screening visit (week -4), then at day 0 (T1),
9 week 2, months 1, 2, 4 and 6, and every 3 months thereafter until month 36.

10 Absolute numbers of CD4 T lymphocytes were determined by flow cytometry in
11 whole blood. The plasma HIV RNA level was determined with the techniques
12 routinely used in each participating center, all of which had a detection limit of
13 400 genome copies/mL. Each patient's samples were all tested with the same
14 technique. The plasma HIV RNA level and the CD4 cell count were determined
15 at each study visit.

16 The HIV DNA level in peripheral blood mononuclear cells (PBMCs) was
17 measured in blood samples taken at day 0 and at months 6, 12 and 18. PBMC
18 HIV-1 DNA was quantified by real-time PCR with amplification of the LTR gene,
19 using the 8E5 cell line as an external standard [Avettand-Fenoel et al., 2007;
20 Goujard et al., 2006; Rouzioux et al., 2005]. Briefly, PBMCs were separated by
21 Ficoll-Hypaque gradient centrifugation and aliquots were frozen until use. Total
22 DNA extracted from each aliquot of PBMCs was first quantified with the
23 Hoechst dye method (Pharmacia), 1 µg of DNA being considered equivalent to
24 ~150 000 cells. This HIV-DNA PCR method quantifies total HIV-DNA (both
25 integrated and non integrated). Final results were expressed as the number or

1
2
3 1 \log_{10} number of HIV-1 DNA copies per 10^6 PBMCs (quantification limit: 5
4
5 2 copies/PCR, i.e. 30 copies/ 10^6 PBMCs).
6
7

8 3 Frozen plasma samples were collected at months 1, 6, 12, 18 and 24. HIV
9
10 4 genotypic resistance mutations were studied before treatment resumption, as
11
12 5 described elsewhere [Mouroux et al., 2001].
13
14

15 6 Safety was assessed at each visit by means of an interview, a physical
16
17 7 examination, and collection of adverse events. Adverse events were coded with
18
19 8 the Medical Dictionary for Regulatory Activities (MedDRA) and their severity
20
21 9 was graded from 1 to 4 with the ANRS Adverse Event Grading Scale.
22
23

24 10 Lipodystrophy was scored clinically, and anthropometric measurements were
25
26 11 made (waist and hip circumference, waist/hip ratio, thigh circumference and
27
28 12 body mass index) on day 0 and at months 6, 24 and 36.
29
30

31 13 Total cholesterol, HDL cholesterol, LDL cholesterol and triglyceride serum
32
33 14 levels were measured after an overnight fast on day 0 and every 6 months
34
35 15 thereafter.
36
37

38 16 The criteria for ART resumption were a fall in the CD4 cell count to below
39
40 17 $300/\text{mm}^3$ in two consecutive samples collected 15 days apart, and/or the
41
42 18 occurrence of a stage B or C AIDS-defining event. Immunologic and virologic
43
44 19 responses were assessed 1, 3 and 6 months after ART resumption.
45
46
47
48
49
50

51 ***Statistical analysis***

52 *Sample size*

53
54
55
56 23 To estimate the percentage of patients who did not reach the ART resumption
57
58 24 criteria 12 months after antiretroviral treatment interruption, with a precision of
59
60 25 10%, 100 patients needed to be enrolled.

1

2 *Statistical Analysis*

3 The results are reported as proportions (and 95% confidence intervals when
4 appropriate) for categorical variables, and as medians and interquartile ranges
5 for continuous variables. Changes in the CD4 cell count during treatment
6 interruption were analyzed by using mixed linear models, testing for changes in
7 the slopes during the interruption, with cut points chosen after visual inspection
8 of the median CD4 cell count at each time point. In these analyses, patients
9 resuming ART were censored at the time of treatment resumption. The primary
10 endpoint was the time to the first of two consecutive CD4 cell counts below
11 $300/\text{mm}^3$, 15 days apart, or a CDC stage B or C AIDS-defining event. All events
12 were reviewed by the Data and Safety Monitoring Board. Patients who reached
13 the primary endpoint were analyzed as such even if they did not resume
14 treatment, while patients who resumed treatment without reaching the primary
15 endpoint were censored at time of treatment resumption in the intent-to-treat
16 analysis. In the analysis of observational data, all patients resuming therapy
17 were considered to have reached the primary endpoint. The percentage of
18 patients reaching the primary endpoint was determined using Kaplan-Meier
19 estimates. Factors associated with the occurrence of the primary endpoint were
20 analyzed with univariate and multivariable Cox proportional hazards models.
21 The following variables were tested: baseline age, gender, transmission group,
22 AIDS status, the CD4 cell nadir, the pre-ART CD4 cell count, the baseline CD4
23 cell count, pre-ART plasma HIV-1 RNA, baseline PMBC HIV DNA, the duration
24 of ART at treatment interruption, and the use of dual- or triple-drug therapy.
25 Akaike's criteria were used to determine whether continuous variables were

1
2
3 1 best modelled as continuous variables, or after log transformation, or as three-
4
5 2 class categorical variables based on the terciles. Based on the results of these
6
7
8 3 analyses, the baseline CD4 cell count and the baseline HIV DNA level,
9
10 4 expressed in \log_{10} values, were modelled as three-class variables, and the CD4
11
12 5 cell nadir and the pre-ART CD4 cell count expressed in \log_2 were modelled as
13
14
15 6 continuous variables in the final model. Variables with p values below 0.05 in
16
17 7 univariate analysis were entered in the Cox multivariable model by using a
18
19
20 8 backward procedure.
21
22 9 Changes in lipodystrophy and metabolic parameters were tested for
23
24 10 significance with Wilcoxon's paired test. The SPSS software package version
25
26
27 11 15.0 for Windows (SPSS Inc., Illinois, Chicago) and the SAS software package
28
29 12 version 9.1 (SAS institute, Cary, NC, USA) were used for all analyses.
30
31
32 13

1 **Results**

3 ***Study population***

4 One hundred and sixteen patients (37% women) were enrolled at 21 sites
5 between January 2003 and March 2004. Baseline characteristics of the study
6 population are shown in Table 1. Follow-up during treatment interruption lasted
7 a total of 270.2 person-years. Three patients had had AIDS-defining events
8 prior to enrolment (pulmonary tuberculosis, esophageal candidiasis, cutaneous
9 Kaposi's sarcoma). The interrupted treatments comprised a protease inhibitor-
10 based regimen in 16% of cases, a non-nucleoside analogue regimen in 35% of
11 cases, a triple nucleoside analogue regimen in 34% of cases (Trizivir in 17% of
12 cases), a dual nucleoside analogue regimen in 14% of cases, and a non
13 nucleoside analogue plus a protease inhibitor in 1% of cases. The median (IQR)
14 CD4 cell count at inclusion was 806/mm³ (676 – 940) and the median CD4
15 nadir was 381/mm³ (341 – 490). Only 11% of the patients had a CD4 nadir
16 below 300/mm³. The median pre-ART plasma HIV RNA level was 4.2 log₁₀
17 copies/mL (3.8 – 4.5). Ninety-one percent of the patients had a plasma HIV
18 RNA level below 400 copies at inclusion. The median baseline HIV DNA value
19 was 2.3 log copies per million PBMCs (1.9 – 2.8).

21 ***Patient disposition***

22 Four patients were lost to follow-up, at months 17, 24 (2 patients) and 27. Four
23 women left the study because they became pregnant, at months 2, 21, 24 and
24 32. One patient died, of a ruptured cerebral aneurysm at month 21.

1

2 ***Dynamics of CD4+ T cell count, plasma HIV-RNA and PBMC***

3 ***HIV-DNA values after treatment interruption***

4 Changes in the median CD4 cell count after treatment interruption are shown in
5 Figure 1a. The CD4 cell curve comprised three distinct slopes: a first steep
6 decline (-127.2 ± 21.4 cells) during the first month, then a slower decline (-15.4
7 ± 2.2 cells/month) between month 1 and month 12, and a very slow decline ($-$
8 3.1 ± 0.8 cells/month) between month 12 and month 36. The median CD4 cell
9 counts at months 12, 24 and 36 were respectively $497/\text{mm}^3$ (376 – 626),
10 $479/\text{mm}^3$ (394 - 628) and $455/\text{mm}^3$ (356 - 587).

11 Changes in plasma HIV RNA load after treatment interruption are shown in
12 Figure 1b. After a rapid increase from 2.6 to 4.0 \log_{10} copies per ml during the
13 first month, the median plasma HIV RNA level stabilized. Median plasma HIV
14 RNA values at months 12, 24 and 36 were respectively 4.1 \log_{10} copies/mL (3.6
15 – 4.4), 4.1 \log_{10} copies/mL (3.7 – 4.5) and 4.3 \log_{10} copies/mL (3.7 – 4.6).
16 Respectively 5%, 6% and 2% of patients still had plasma HIV RNA values
17 below 400 copies/mL at months 12, 24 and 36 of treatment interruption. Median
18 PBMC HIV DNA load in these patients at baseline and month 6 were
19 respectively 1.0 (1.0 – 1.4) and 2.2 (1.8 – 2.4) \log_{10} per million PBMCs.

20 Changes in HIV DNA load during the first 18 months of the study are shown in
21 Figure 1c. The HIV DNA level increased from 2.3 (1.9 -2.8) at baseline to 3.1
22 (2.7 – 3.4) at 18 months.

24 ***Incidence of the primary endpoint***

1
2
3 1 Within 36 months after treatment interruption, 23 patients (27.0%) reached a
4
5 2 CD4 cell count below $300/\text{mm}^3$ and 20 of them (22.8%) resumed therapy.
6
7
8 3 Another nine patients (12.4%) resumed therapy because of CDC stage B or C
9
10 4 events, and 20 patients (20.0%) resumed therapy for other reasons (mainly at
11
12 5 the patient's request).

13
14 6 The nine CDC stage B/C events consisted of severe thrombocytopenia (2
15
16 7 cases), oral hairy leukoplakia (3 patients), oral candidiasis (2 patients) and
17
18 8 cutaneous Kaposi's sarcoma (2 patients). One of the two patients who
19
20 9 developed cutaneous Kaposi's sarcoma during treatment interruption had
21
22 10 already had a first episode prior to enrolment in the study. The CD4 cell counts
23
24 11 of these two patients at the onset of Kaposi's sarcoma during treatment
25
26 12 interruption were $648/\text{mm}^3$ and $364/\text{mm}^3$. No AIDS-defining events occurred
27
28 13 during the first 12 months of treatment interruption.

29
30 14 In the intent-to-treat analysis the proportions of patients who did not meet the
31
32 15 criteria for ART resumption at months 12, 24 and 36 were respectively 97.3%
33
34 16 (95% CI: 92.5 – 99.1), 86.5% (78.5 – 91.8), and 63.9% (52.9 – 73.6). The
35
36 17 proportions of patient who had not resumed ART at months 12, 24 and 36
37
38 18 (observational data) were respectively 92.2% (85.8 – 95.8), 75.3% (66.5 –
39
40 19 82.4), and 55.9% (46.1 – 65.3).
41
42
43
44
45
46
47
48
49
50

21 ***Factors predictive of reaching the primary endpoint***

51
52 22 The results of univariate and multivariable Cox proportional hazards models are
53
54 23 shown in Table 2. In univariate analyses, HIV DNA load above $2.56 \log_{10}$ per
55
56 24 million PBMCs, a low CD4 nadir, the pre-ART CD4 cell count, the baseline CD4
57
58
59
60

1 cell count, and AIDS status at baseline were significantly associated with the
2 time taken to reach the primary endpoint.

3 In multivariable analyses, HIV DNA load above the upper tercile of 2.56
4 $\log_{10}/10^6$ PBMCs (hazards ratio, HR=2.15), a low CD4 nadir (HR=4.59 per 50%
5 reduction in the CD4 nadir) and pre-existing AIDS status (HR=5.74) were all
6 predictive of a shorter time to the first of two CD4 cell counts below $300/\text{mm}^3$,
7 15 days apart, or to a CDC stage B or C event. The Kaplan-Meier curves
8 representing the time to the primary endpoint are shown in Figure 2 according
9 to HIV DNA load (above or below 2.56 \log_{10} per million PBMCs) adjusted for the
10 CD4 nadir.

12 ***Severe non AIDS-defining morbidity***

13 Four cardiovascular events occurred during follow-up, including two cases of
14 coronary artery disease (CD4 cell counts at diagnosis: 337 and 448), one stroke
15 (CD4=346), and one ruptured cerebral aneurysm (CD4=559); the incidence of
16 cardiovascular events was 1.5/100 person-years (95% CI: 0.6 – 3.2).

17 No cases of renal or hepatic failure and no non AIDS-related malignancies
18 occurred during follow-up.

20 ***Changes in lipodystrophy and metabolic parameters***

21 Changes in anthropometric measurements, lipodystrophy scores and metabolic
22 parameters between day 0 and the last follow-up visit during treatment
23 interruption are shown in Table 3. No significant change in anthropometric
24 values occurred, but the proportion of patients with either lipodystrophy or

1 lipohypertrophy fell significantly (42% versus 23%, $p < 0.001$; and 43% versus
2 16%, $p < 0.001$, respectively).

3 The median changes in the total serum cholesterol level, the serum HDL
4 cholesterol level, and the serum LDL cholesterol level from baseline to the last
5 follow-up visit off treatment were respectively -0.35 mmol/l (IQR -0.99, 0.08) (p
6 < 0.001), -0.11 mmol/l (-0.33, 0.05) ($p < 0.001$) and -0.25 mmol/l (-0.78, 0.26) (p
7 = 0.001). Serum glucose and triglycerides levels did not change significantly
8 during treatment interruption.

9 10 ***Treatment resumption***

11 Of the 47 patients who resumed therapy during the 36 months of follow-up, 31
12 patients (66%) were prescribed a regimen different from that received prior to
13 enrolment. The median CD4 cell count was 345/mm³ (256 – 446) at resumption,
14 and 475/mm³ (388 – 587) six months later. The median plasma HIV RNA level
15 was 26470 copies/mL (4790 – 81430) at resumption, and 39 patients (81%) had
16 plasma HIV RNA levels below 400 copies/mL six months later. Genotypic
17 mutations conferring resistance to NRTIs and NNRTIs were identified in all 8
18 patients (19%) who had plasma HIV RNA levels above 400 copies/mL six
19 months after resumption. The baseline profile was not available in three of
20 these patients. Only one of the remaining five patients acquired resistance to a
21 new drug (3TC). At treatment interruption, 6 of these 8 patients had plasma HIV
22 RNA values below 400 copies/mL. The ART regimens were subsequently
23 optimized, and plasma HIV RNA became undetectable (< 400 copies/mL) after a
24 further 6 months in 7 of the 8 patients.

25 No B or C events were diagnosed after treatment resumption.

1 Discussion

2 This study indicates that treatment interruption in selected patients with a high
3 CD4 cell nadir is associated with a low rate of HIV disease progression after 36
4 months. Only 36% of the patients met the criteria for treatment resumption
5 during the 36 months of follow-up, namely a CD4 cell count below 300/mm³ or a
6 CDC stage B or C event. Only two CDC grade C clinical events (two cases of
7 cutaneous Kaposi's sarcoma) occurred during the 36 months of follow-up. It
8 should be noted that patients with AIDS were eligible for this study. One of the
9 two patients who developed Kaposi's sarcoma during follow-up, when their CD4
10 cell counts were above 350/mm³, had already had an episode of Kaposi's
11 sarcoma prior to inclusion. Both these events occurred after one year off
12 treatment, and the incidence was 0.7/100 P-Y. This is within the range of
13 incidence rates observed elsewhere in antiretroviral-naive patients with CD4 cell
14 counts >350/mm³ [Guiguet et al., 2008; Phillips et al., 2007]. Guiguet et al.
15 reported an incidence rate of 0.8/100 P-Y in such patients [Guiguet et al., 2008].
16 Our findings are in keeping with those of other studies of treatment interruption
17 in similar populations [Ananworanich et al., 2006; Boschi et al., 2004; Maggiolo
18 et al., 2008; Mata et al., 2005; Mussini et al., 2005; Pogany et al., 2007; Skiest
19 et al., 2004; Skiest et al., 2007; Wit et al., 2005]. Among the 284 patients
20 included in the treatment interruption arm of the Staccato trial [Ananworanich et
21 al., 2006], only minor HIV-related signs and symptoms were observed, with no
22 AIDS-defining events or AIDS-related deaths. In a recent trial [Maggiolo et al.,
23 2008], 329 patients with a CD4 cell nadir above 200/mm³ and a CD4 cell count
24 above 700/mm³ at inclusion were randomized to continue their ongoing
25 antiretroviral treatment or to discontinue it until their CD4 cell count fell to

1 350/mm³. During 689 P-Y of follow-up, only one AIDS-defining event occurred
2 in the STI arm and none in the control arm, while eight cases of pneumonia
3 occurred in the STI arm, compared to one in the control arm. However, recent
4 large studies have shown a higher rate of clinical events in patients undergoing
5 CD4-guided treatment interruption than in patients who continue on treatment
6 [Danel et al., 2006; El-Sadr et al., 2006]. These studies did not exclude patients
7 with AIDS or HIV-related disorders, and included patients with different degrees
8 of immune impairment and low CD4 cell nadirs. One study in which there was a
9 high rate of events (mainly bacterial infections) during treatment interruption
10 involved patients in Africa, where the background rate of opportunistic infections
11 may be higher [Danel et al., 2006].

12 Among the patients enrolled in the SMART trial, opportunistic infections and
13 deaths were significantly more frequent in the “drug conservation” arm, in which
14 ART initiation or resumption was deferred until the CD4 cell count fell to
15 250/mm³), than in the continuous ART arm. Even in the subgroup of patients
16 whose last CD4 cell count was above 350/mm³, the incidence of AIDS-related
17 events in the “drug conservation” arm was 1.28 per 100 person-years [Lundgren
18 et al., 2008], a rate similar to that found in the present study (0.7/100 P-Y; IQR
19 0.2 – 2.0). However, clinical events were infrequent in our study, in which the
20 patients had started first-line antiretroviral therapy with CD4 cell counts above
21 350/mm³. This further suggests that lengthy treatment interruption is less risky
22 in AIDS-free patients with a high CD4 cell nadir.

23 The SMART study highlighted an increased risk of cardiovascular events during
24 treatment interruption [El-Sadr et al., 2006]. A slight increase in cardiovascular
25 events was observed in the “drug conservation” arm of the SMART study, with

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 an incidence of 1.3/100 P-Y, compared to 1.5/100 P-Y in this study. As HIV
2 replication induces immune activation, plasma HIV RNA rebound during
3 treatment interruption might lead to inflammation and endothelial dysfunction,
4 thereby increasing the risk of cardiovascular events [Blum et al., 2005; Solages
5 et al., 2006; Tebas et al., 2008].

6 As also observed elsewhere [Hatano et al., 2000], a significant improvement of
7 lipodystrophy and lipid abnormalities was observed during treatment
8 interruption. However, changes in lipodystrophy were assessed with an
9 unvalidated clinical score, and no significant change in anthropometric values
10 was observed during treatment interruption.

11 As reported previously by others, this study showed a rapid initial CD4 cell
12 decline after ART interruption, followed by a more gradual loss during the first
13 months (accompanied by a rapid increase in plasma HIV RNA), and then by
14 CD4 cell count and plasma HIV RNA load stabilization [Boschi et al., 2004;
15 Fagard et al., 2005; Maggiolo et al., 2004; Skiect et al., 2007; Tarwater et al.,
16 2003; Tebas et al., 2002; Thiebaut et al., 2005; Wit et al., 2005]. The present
17 study showed an increase in HIV DNA levels after treatment interruption,
18 indicative of gradual replenishment of cellular reservoirs.

19 In Cox proportional hazards multivariable analyses, baseline factors predictive
20 of outcome 36 months after treatment interruption were PBMC HIV DNA load
21 above the upper tercile ($2.56 \log_{10}$ copies/ml), the CD4 nadir, and AIDS status.

22 It is commonly reported that the higher the CD4 cell nadir, the smaller the fall in
23 the CD4 cell count and the longer the duration of treatment interruption [Boschi
24 et al., 2004; Fernandez Guerrero et al., 2005; Giuntini et al., 2005; Maggiolo et
25 al., 2004; Mata et al., 2005; Mussini et al., 2005; Poulton et al., 2003; Skiect et

1 al., 2004; Skiest et al., 2007; Tarwater et al., 2003; Thiebaut et al., 2005]. As
2 also found in some other studies, the pre-ART plasma HIV RNA level was not
3 predictive of the duration of treatment interruption in this study [Maggiolo et al.,
4 2004; Tebas et al., 2002]. Interestingly, the PBMC HIV DNA levels above 2.56
5 \log_{10} copies/ 10^6 PBMCs were an independent predictor of a shorter time to
6 treatment resumption. PBMC HIV DNA load was also found to be a significant
7 predictor of treatment resumption in a pilot study involving 62 patients who
8 stopped their ongoing antiretroviral treatment [Sarmati et al., 2009]. The HIV-
9 DNA level provides an estimate of the proportion of circulating HIV-infected
10 cells and reflects the size of the viral reservoir. Central and transitional memory
11 CD4⁺ T cells contribute to the HIV reservoir, the size of which correlates with
12 the nadir CD4 cell count [Chomont et al., 2009]. PBMC HIV-1 DNA load shortly
13 after infection is predictive of disease progression, independently of the plasma
14 HIV-RNA level and the CD4 cell count [Goujard et al., 2006; Rouzioux et al.,
15 2005]. The HIV reservoir is established soon after infection, and the degree to
16 which it is diminished by ART, whether initiated during the acute or chronic
17 phase of infection, is variable [Ngo-Giang-Huong et al., 2001; Strain et al.,
18 2003]. While all patients in this study had low or undetectable plasma HIV-RNA
19 at inclusion, their HIV-DNA levels varied over a broad range and were therefore
20 informative. These results suggest that the larger the viral reservoir at the time
21 of treatment interruption, the less time the patient can remain off treatment. In
22 other words, a high level of HIV-DNA was indicative of a risk of progression in
23 case of treatment interruption, whatever the prior duration of ART.
24 Following the results of the SMART trial, treatment interruptions are not
25 currently recommended in HIV-infected patient management guidelines

1 [Hammer et al., 2008; Yeni, 2008]. In current practice, the number of patients
2 who stop taking ART, for whatever reason, has fallen since the results of the
3 SMART trial were first reported in January 2006, but still represented 5.7% of
4 patients monitored in 2007 [Yeni, 2008]. Thus, it remains important to estimate
5 the risk of AIDS and severe non-AIDS morbidity/mortality during treatment
6 interruption, and to identify factors associated with the duration of interruption.
7 The results of this study indicate that patients with a history of AIDS-defining
8 events should not interrupt their treatment. In addition to a low CD4 nadir, a
9 high PBMC HIV DNA level at time of treatment interruption is a strong predictor
10 of more rapid treatment resumption, contrary to the pre-therapy plasma HIV-1
11 RNA level.

1
2
3 **1 Acknowledgments**
4

5
6 **2 Sponsorship:**
7

8 This work was supported by Agence Nationale de Recherches sur le SIDA
9
10 (ANRS)
11

12
13 **5 The ANRS 116 study group:**
14

15 Hôpital Avicenne, Bobigny (M. Bentata); Hôpital Saint Antoine, Paris (P-M
16
17 Girard, J-L Meynard); Hôpital Pitié Salpêtrière, Paris (C. Katlama, R. Tubiana,
18
19 A. Simon); Hôpital Bicêtre (J-F Delfraissy, C. Gougard); Hôpital Henri Mondor,
20
21 Creteil (Y. Levy); Hôpital Tenon, Paris (G. Pialoux); Hôpital Necker, Paris (J-P
22
23 Viard); Hôpital Saint Jacques, Besançon (B. Hoen); Hôpital Bichat, Paris (P.
24
25 Yeni, R. Landman); Hôpital Sainte Marguerite, Marseille (JA. Gastaut, I. Poizot-
26
27 Martin); Hôpital Pellegrin, Bordeaux (JM Ragnaut); Hôpital Saint Jacques,
28
29 Bordeaux (P. Morlat); Hôpital Brabois, Nancy (T. May); CHU de Caen (C.
30
31 Bazin); CHU Strasbourg (JM. Lang); Hôpital Purpan, Toulouse (P. Massip);
32
33 Hôpital de l'Archet, Nice (JP. Cassuto); Hôpital Edouard Herriot, Lyon; Hôpital
34
35 Bretonneau, Tours (P. Choutet).
36
37
38
39
40

41 **17 ANRS 116 SALTO Scientific committee:**
42

43 C. Piketty, L. Weiss, D. Costagliola, C. Rouzioux, M. Burgard, C. Goujard, J-P
44
45 Viard, Y. Levy, G. Pialoux, J-L Meynard, S. Bachir Cherif, L. Assoumou, and
46
47 MJ. Commoy
48
49

50
51 **21 ANRS 116 SALTO DSMB:**
52

53 JL. Pellegrin, C. Fagard, L. Morand-Joubert, and J. Gilquin
54
55
56
57
58
59
60

References

- Ananworanich J, Gayet-Ageron A, Le Braz M, Prasithsirikul W, Chetchotisakd P, Kiertiburanakul S, Munsakul W, Raksakulkarn P, Tansuphasawasdikul S, Sirivichayakul S, Cavassini M, Karrer U, Genne D, Nuesch R, Vernazza P, Bernasconi E, Leduc D, Satchell C, Yerly S, Perrin L, Hill A, Perneger T, Phanuphak P, Furrer H, Cooper D, Ruxrungtham K, Hirschel B. 2006. CD4-guided scheduled treatment interruptions compared with continuous therapy for patients infected with HIV-1: results of the Staccato randomised trial. *Lancet* 368(9534):459-465.
- Avettand-Fenoel V, Mahlaoui N, Chaix ML, Milliancourt C, Burgard M, Cavazzana-Calvo M, Rouzioux C, Blanche S. 2007. Failure of bone marrow transplantation to eradicate HIV reservoir despite efficient HAART. *Aids* 21(6):776-777.
- Blum A, Hadas V, Burke M, Yust I, Kessler A. 2005. Viral load of the human immunodeficiency virus could be an independent risk factor for endothelial dysfunction. *Clin Cardiol* 28(3):149-153.
- Boschi A, Tinelli C, Ortolani P, Moscatelli G, Morigi G, Arlotti M. 2004. CD4+ cell-count-guided treatment interruptions in chronic HIV-infected patients with good response to highly active antiretroviral therapy. *Aids* 18(18):2381-2389.
- Chomont N, El-Far M, Ancuta P, Trautmann L, Procopio FA, Yassine-Diab B, Boucher G, Boulassel MR, Ghattas G, Brenchley JM, Schacker TW, Hill BJ, Douek DC, Routy JP, Haddad EK, Sekaly RP. 2009. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med* 15(8):893-900.
- Danel C, Moh R, Minga A, Anzian A, Ba-Gomis O, Kanga C, Nzunetu G, Gabillard D, Rouet F, Sorho S, Chaix ML, Eholie S, Menan H, Sauvageot D, Bissagnene E, Salamon R, Anglaret X. 2006. CD4-guided structured antiretroviral treatment interruption strategy in HIV-infected adults in west Africa (Trivacan ANRS 1269 trial): a randomised trial. *Lancet* 367(9527):1981-1989.
- El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino RC, Babiker A, Burman W, Clumeck N, Cohen CJ, Cohn D, Cooper D, Darbyshire J, Emery S, Fatkenheuer G, Gazzard B, Grund B, Hoy J, Klingman K, Losso M, Markowitz N, Neuhaus J, Phillips A, Rappoport C. 2006. CD4+ count-guided interruption of antiretroviral treatment. *N Engl J Med* 355(22):2283-2296.
- Fagard C, Bandelier CY, Ananworanich J, Le Braz M, Gunthard H, Perneger T, Garcia F, Hirschel B. 2005. Biphasic decline of CD4 cell count during scheduled treatment interruptions. *Aids* 19(4):439-441.
- Fagard C, Oxenius A, Gunthard H, Garcia F, Le Braz M, Mestre G, Battegay M, Furrer H, Vernazza P, Bernasconi E, Telenti A, Weber R, Leduc D, Yerly S, Price D, Dawson SJ, Klimkait T, Perneger TV, McLean A, Clotet B, Gatell JM, Perrin L, Plana M, Phillips R, Hirschel B. 2003. A prospective trial of structured treatment interruptions in human immunodeficiency virus infection. *Arch Intern Med* 163(10):1220-1226.
- Fernandez Guerrero ML, Rivas P, Molina M, Garcia R, De Gorgolas M. 2005. Long-term follow-up of asymptomatic HIV-infected patients who discontinued antiretroviral therapy. *Clin Infect Dis* 41(3):390-394.
- Giuntini R, Martinelli C, Ambu S, Bartolozzi D, Farese A, Boddi V, Leoncini F. 2005. Prolonged treatment interruption in chronic HIV infection: a new strategy? *Aids* 19(2):209-211.
- Goujard C, Bonarek M, Meyer L, Bonnet F, Chaix ML, Deveau C, Sinet M, Galimand J, Delfraissy JF, Venet A, Rouzioux C, Morlat P. 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(5):709-715.

- 1
2
3 1 Guiguet M, Porter K, Phillips A, Costagliola D, Babiker A. 2008. Clinical Progression
4 2 Rates by CD4 Cell Category Before and After the Initiation of Combination
5 3 Antiretroviral Therapy (cART). *The Open AIDS Journal* 2:3-9.
6 4 Hammer SM, Eron JJ, Jr., Reiss P, Schooley RT, Thompson MA, Walmsley S, Cahn P,
7 5 Fischl MA, Gatell JM, Hirsch MS, Jacobsen DM, Montaner JS, Richman DD,
8 6 Yeni PG, Volberding PA. 2008. Antiretroviral treatment of adult HIV infection:
9 7 2008 recommendations of the International AIDS Society-USA panel. *Jama*
10 8 300(5):555-570.
11 9 Hatano H, Miller KD, Yoder CP, Yanovski JA, Sebring NG, Jones EC, Davey RT, Jr.
12 10 2000. Metabolic and anthropometric consequences of interruption of highly
13 11 active antiretroviral therapy. *Aids* 14(13):1935-1942.
14 12 Hogg RS, Heath KV, Yip B, Craib KJ, O'Shaughnessy MV, Schechter MT, Montaner
15 13 JS. 1998. Improved survival among HIV-infected individuals following initiation
16 14 of antiretroviral therapy. *Jama* 279(6):450-454.
17 15 Hogg RS, Yip B, Chan KJ, Wood E, Craib KJ, O'Shaughnessy MV, Montaner JS. 2001.
18 16 Rates of disease progression by baseline CD4 cell count and viral load after
19 17 initiating triple-drug therapy. *Jama* 286(20):2568-2577.
20 18 Krolewiecki AJ, Zala C, Vanzulli C, Perez H, Iannella Mdel C, Bouzas MB, Gun A,
21 19 Valiente J, Cassetti I, Cahn P. 2006. Safe treatment interruptions in patients
22 20 with nadir CD4 counts of more than 350 cells/microL: a randomized trial. *J*
23 21 *Acquir Immune Defic Syndr* 41(4):425-429.
24 22 Lee LM, Karon JM, Selik R, Neal JJ, Fleming PL. 2001. Survival after AIDS diagnosis
25 23 in adolescents and adults during the treatment era, United States, 1984-1997.
26 24 *Jama* 285(10):1308-1315.
27 25 Li TS, Tubiana R, Katlama C, Calvez V, Ait Mohand H, Autran B. 1998. Long lasting
28 26 recovery in CD4 T-cell function and viral-load reduction after highly active
29 27 antiretroviral therapy in advanced HIV-1 disease. *Lancet* 351:1682-1686.
30 28 Lundgren JD, Babiker A, El-Sadr W, Emery S, Grund B, Neaton JD, Neuhaus J,
31 29 Phillips AN. 2008. Inferior clinical outcome of the CD4+ cell count-guided
32 30 antiretroviral treatment interruption strategy in the SMART study: role of CD4+
33 31 Cell counts and HIV RNA levels during follow-up. *J Infect Dis* 197(8):1145-
34 32 1155.
35 33 Maggiolo F, Airoidi M, Callegaro A, Martinelli C, Dolara A, Bini T, Gregis G, Quinzan G,
36 34 Ripamonti D, Ravasio V, Suter F. 2008. CD4 cell-guided scheduled treatment
37 35 interruptions in HIV-infected patients with sustained immunologic response to
38 36 HAART. *Aids* 24:24.
39 37 Maggiolo F, Ripamonti D, Gregis G, Quinzan G, Callegaro A, Suter F. 2004. Effect of
40 38 prolonged discontinuation of successful antiretroviral therapy on CD4 T cells: a
41 39 controlled, prospective trial. *Aids* 18(3):439-446.
42 40 Mata RC, Viciano P, de Alarcon A, Lopez-Cortes LF, Gomez-Vera J, Trastoy M,
43 41 Cisneros JM. 2005. Discontinuation of antiretroviral therapy in patients with
44 42 chronic HIV infection: clinical, virologic, and immunologic consequences. *AIDS*
45 43 *Patient Care STDS* 19(9):550-562.
46 44 Mocroft A, Katlama C, Johnson AM, Pradier C, Antunes F, Mulcahy F, Chiesi A,
47 45 Phillips AN, Kirk O, Lundgren JD. 2000. AIDS across Europe, 1994-98: the
48 46 EuroSIDA study. *Lancet* 356(9226):291-296.
49 47 Mouroux M, Descamps D, Izopet J, Yvon A, Delaunoy C, Matheron S, Coutellier A,
50 48 Valantin MA, Bonmarchand M, Agut H, Massip P, Costagliola D, Katlama C,
51 49 Brun-Vezinet F, Calvez V. 2001. Low-rate emergence of thymidine analogue
52 50 mutations and multi-drug resistance mutations in the HIV-1 reverse
53 51 transcriptase gene in therapy-naive patients receiving stavudine plus
54 52 lamivudine combination therapy. *Antivir Ther* 6(3):179-183.
55 53 Murphy EL, Collier AC, Kalish LA, Assmann SF, Para MF, Flanigan TP, Kumar PN,
56 54 Mintz L, Wallach FR, Nemo GJ. 2001. Highly active antiretroviral therapy

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- decreases mortality and morbidity in patients with advanced HIV disease. *Ann Intern Med* 135(1):17-26.
- Mussini C, Bedini A, Borghi V, Guaraldi G, Esposito R, Barchi E, Enilia R, Cozzi-Lepri A, Philips AN, Ortolani P, Bratt G, Eriksson LE, Sighinolfi L, Cossarizza A, d'Arminio Monforte A, De Luca A, Di Giambenedetto S, Antinori A. 2005. CD4 cell-monitored treatment interruption in patients with a CD4 cell count > 500 x 10⁶ cells/l. *Aids* 19(3):287-294.
- Ngo-Giang-Huong N, Deveau C, Da Silva I, Pellegrin I, Venet A, Harzic M, Sinet M, Delfraissy JF, Meyer L, Goujard C, Rouzioux C. 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(6):665-673.
- Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. *New England Journal of Medicine* 338(13):853-860.
- Palella FJ, Jr., Deloria-Knoll M, Chmiel JS, Moorman AC, Wood KC, Greenberg AE, Holmberg SD. 2003. Survival benefit of initiating antiretroviral therapy in HIV-infected persons in different CD4+ cell strata. *Ann Intern Med* 138(8):620-626.
- Parish MA, Tarwater P, Lu M, Raines C, Gallant JE. Prolonged treatment interruption after immunologic response to HAART; 2002; Barcelona, Spain. p abstract ThOrB1439.
- Pellegrin I, Thiebaut R, Blanco P, Viillard JF, Schrive MH, Merel P, Chene G, Fleury H, Moreau JF, Pellegrin JL. 2005. Can highly active antiretroviral therapy be interrupted in patients with sustained moderate HIV RNA and > 400 CD4+ cells/microl? Impact on immunovirological parameters. *J Med Virol* 77(2):164-172.
- Phillips AN, Gazzard B, Gilson R, Easterbrook P, Johnson M, Walsh J, Leen C, Fisher M, Orkin C, Anderson J, Pillay D, Delpech V, Sabin C, Schwenk A, Dunn D, Gompels M, Hill T, Porter K, Babiker A. 2007. Rate of AIDS diseases or death in HIV-infected antiretroviral therapy-naïve individuals with high CD4 cell count. *Aids* 21(13):1717-1721.
- Pogany K, van Valkengoed IG, Prins JM, Nieuwkerk PT, van der Ende I, Kauffmann RH, Kroon FP, Verbon A, Nievaard MF, Lange JM, Brinkman K. 2007. Effects of active treatment discontinuation in patients with a CD4+ T-cell nadir greater than 350 cells/mm³: 48-week Treatment Interruption in Early Starters Netherlands Study (TRIESTAN). *J Acquir Immune Defic Syndr* 44(4):395-400.
- Poulton MB, Sabin CA, Fisher M. 2003. Immunological changes during treatment interruptions: risk factors and clinical sequelae. *Aids* 17(1):126-128.
- Rouzioux C, Hubert JB, Burgard M, Deveau C, Goujard C, Bary M, Sereni D, Viard JP, Delfraissy JF, Meyer L. 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(1):46-55.
- Ruiz L, Martinez-Picado J, Romeu J, Paredes R, Zayat MK, Marfil S, Negro E, Sirera G, Tural C, Clotet B. 2000. Structured treatment interruption in chronically HIV-1 infected patients after long-term viral suppression. *Aids* 14(4):397-403.
- Sarmati L, Andreoni C, Nicastrì E, Tommasi C, Buonomini A, D'Ettorre G, Corpolongo A, Dori L, Montano M, Volpi A, Narciso P, Vullo V, Andreoni M. 2009. Prognostic factors of long-term CD4+count-guided interruption of antiretroviral treatment. *J Med Virol* 81(3):481-487.
- Skiest DJ, Morrow P, Allen B, McKinsey J, Crosby C, Foster B, Hardy RD. 2004. It is safe to stop antiretroviral therapy in patients with preantiretroviral CD4 cell counts >250 cells/microl. *J Acquir Immune Defic Syndr* 37(3):1351-1357.
- Skiest DJ, Su Z, Havlir DV, Robertson KR, Coombs RW, Cain P, Peterson T, Krambrink A, Jahed N, McMahan D, Margolis DM. 2007. Interruption of antiretroviral treatment in HIV-infected patients with preserved immune function

- 1 is associated with a low rate of clinical progression: a prospective study by
2 AIDS Clinical Trials Group 5170. *J Infect Dis* 195(10):1426-1436.
- 3 Solages A, Vita JA, Thornton DJ, Murray J, Heeren T, Craven DE, Horsburgh CR, Jr.
4 2006. Endothelial function in HIV-infected persons. *Clin Infect Dis* 42(9):1325-
5 1332.
- 6 Strain MC, Gunthard HF, Havlir DV, Ignacio CC, Smith DM, Leigh-Brown AJ,
7 Macaranas TR, Lam RY, Daly OA, Fischer M, Opravil M, Levine H, Bachelier L,
8 Spina CA, Richman DD, Wong JK. 2003. Heterogeneous clearance rates of
9 long-lived lymphocytes infected with HIV: intrinsic stability predicts lifelong
10 persistence. *Proc Natl Acad Sci U S A* 100(8):4819-4824.
- 11 Tarwater PM, Parish M, Gallant JE. 2003. Prolonged treatment interruption after
12 immunologic response to highly active antiretroviral therapy. *Clin Infect Dis*
13 37(11):1541-1548.
- 14 Tebas P, Henry K, Mondy K, Deeks S, Valdez H, Cohen C, Powderly WG. 2002. Effect
15 of prolonged discontinuation of successful antiretroviral therapy on CD4+ T cell
16 decline in human immunodeficiency virus-infected patients: implications for
17 intermittent therapeutic strategies. *J Infect Dis* 186(6):851-854.
- 18 Tebas P, Henry WK, Matining R, Weng-Cherng D, Schmitz J, Valdez H, Jahed N,
19 Myers L, Powderly WG, Katzenstein D. 2008. Metabolic and immune activation
20 effects of treatment interruption in chronic HIV-1 infection: implications for
21 cardiovascular risk. *PLoS ONE* 3(4):e2021.
- 22 Thiebaut R, Pellegrin I, Chene G, Viallard JF, Fleury H, Moreau JF, Pellegrin JL,
23 Blanco P. 2005. Immunological markers after long-term treatment interruption in
24 chronically HIV-1 infected patients with CD4 cell count above 400 x 10⁶ cells/l.
25 *Aids* 19(1):53-61.
- 26 Wit FW, Blanckenberg DH, Brinkman K, Prins JM, van der Ende ME, Schneider MM,
27 Mulder JW, de Wolf F, Lange JM. 2005. Safety of long-term interruption of
28 successful antiretroviral therapy: the ATHENA cohort study. *Aids* 19(3):345-
29 348.
- 30 Yeni P. 2008. *Prise en charge médicale des personnes infectées par le VIH.*
31 *Recommandations du groupe d'experts: Médecine-Sciences Flammarion.*

Table 1: Characteristics of the study population

N	116
Men, n (%)	73 (63%)
Age (years), median (IQR)	39 (25 – 80)
Transmission group, n (%)	
Homo/bisexual men	57 (49%)
Heterosexuals	46 (40%)
Intravenous drug users	9 (8%)
AIDS status, n (%)	3 (3%)
CD4 nadir (cells/mm ³)	
- median (IQR)	381 (341 – 490)
- CD4 nadir < 300/mm ³	13 (11%)
CD4 cell count/mm ³ at inclusion, median (IQR)	806 (676 – 940)
Pre-ART plasma HIV-RNA (log ₁₀ copies/mL), median (IQR)	4.2 (3.8 – 4.5)
Plasma HIV-RNA < 400 copies/mL at inclusion, n (%)	106 (91%)
HIV DNA (log ₁₀ copies/10 ⁶ PBMCs) at inclusion	
- median (IQR)	2.3 (1.9 – 2.8)
- HIV-DNA > 2.56 log	38 (33%)
Duration of ART (years), median (IQR)	5.3 (4.2 – 6.1)
Triple nucleoside-based regimen, n (%)	39 (34%)
Double nucleoside-based regimen, n (%)	17 (14%)
Non nucleoside-based regimen, n (%)	41 (35%)
Protease inhibitor-based regimen, n (%)	18 (16%)
Non nucleoside/protease inhibitor combination	1 (1%)

4

Table 2: Risk factors of reaching the criteria for treatment resumption in univariate analysis and multivariable Cox proportional hazards models

		Univariate analysis			Multivariable analysis	
Variables	Number of (patients, events)	Hazard Ratio (CI 95)	p	AIC	Hazard Ratio (CI 95)	p
Total	(N=116, n=32)					
Age (year)		1.00 (0.96 – 1.03)	0.779			
Gender	Women (N=43, n=12)	1 (reference)				
	Men (N=73, n=20)	0.99 (0.48 – 2.08)	0.966			
Homo/bisexual men	No (N=59, n=16)	1				
	Yes (N=57, n=16)	1.04 (0.52 – 2.09)	0.905			
Duration of ART		0.96 (0.76 – 1.21)	0.709			
Type of ART	cART (N=99, n=31)	1				
	dual therapy (N=17, n=1)	0.17 (0.02 – 1.27)	0.085			
Prior AIDS status		6.32 (1.85 – 21.64)	0.003		5.74 (1.60 – 20.56)	0.007
Pre-ART CD4 (log2) [§]		7.14 (1.43 – 33.33)	0.017	270.4		
Pre-ART CD4 cells/mm ³			0.116	272.8		
	>519 (N=39, n=8)	1				
	405-519 (N=40, n=10)	1.35 (0.53 – 3.43)	0.524			
	<405 (N=37, n=14)	2.40 (1.00 – 5.72)	0.049			
Pre-ART VL (log10) [#]		2.04 (0.85 – 4.90)	0.109	271.7		
Pre-ART VL copies/ml			0.324	272.4		
	<10000 (N=41, n=9)	1				
	10000-27000 (N=36, n=9)	1.35 (0.53 – 3.40)	0.529			
	>27000 (N=36, n=14)	1.89 (0.82 – 4.36)	0.138			
CD4 at inclusion (log2) [§]		3.03 (0.93 – 10.10)	0.065	273.6		
CD4 at inclusion cells/mm ³			0.035	270.3		
	>885 (N=35, n=7)	1				
	700-885 (N=39, n=8)	1.22 (0.44 – 3.37)	0.700			
	<700 (N=42, n=17)	2.77 (1.15 – 6.71)	0.024			
CD4 nadir (log2) [§]		6.25 (1.78 – 25.00)	0.004	268.8	4.59 (1.22 – 17.24)	0.024
CD4 nadir cells/mm ³			0.169	273.0		
	>448 (N=38, n=7)	1				
	361-448 (N=40, n=12)	2.10 (0.82 – 5.35)	0.120			
	<361 (N=38, n=13)	2.35 (0.94 – 5.89)	0.069			
HIV DNA (log10) copies/million PBMCs		1.94 (1.13 – 3.35)	0.017	251.9		
HIV DNA (log10)			0.031	250.8		
	<2.17 (N=39, n=7)	1				
	2.17-2.56 (N=36, n=6)	0.96 (0.32 – 2.86)	0.944			
	>2.56 (N=38, n=17)	2.60 (1.08 – 6.27)	0.034			
HIV DNA (log10) > 2.56	(N=38, n=17)	2.65 (1.28 – 5.45)	0.008		2.15 (1.02 – 4.53)	0.044

[§] Hazard Ratio per two-fold decrease in the CD4 cell count; [#] Hazard Ratio per ten-fold increase in plasma HIV-RNA; N=number of patients; n=number of events during follow-up; AIC=Akaike's information criterion: used to select models (the best model has the lowest AIC).

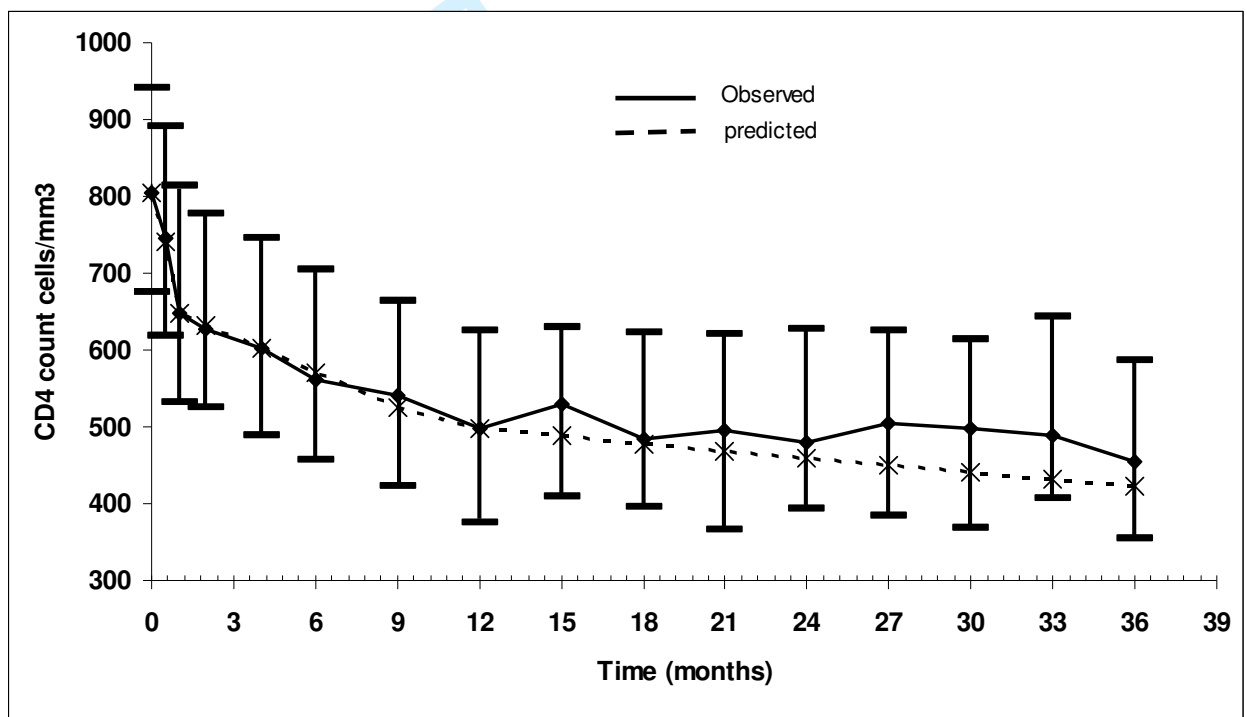
Table 3: Change in anthropometric measures, the lipodystrophy score and metabolic parameters during treatment interruption

	Baseline	Last values during interruption	p
Hip circumference (cm), median (IQR)	90.8 (86.0 – 90.8)	91.0 (86.8 – 97.0)	0.010
Waist (cm)	83.0 (76.0 – 90.0)	83.0 (76.0 – 91.3)	0.251
Waist/hip ratio	0.92 (0.87 – 0.99)	0.91 (0.87 – 0.97)	0.203
Thigh circumference (cm)	47.0 (44.0 – 50.0)	47.0 (44.0 – 51.0)	0.258
Body mass index (kg/m ²)	22.9 (20.7 – 24.5)	22.8 (20.7 – 25.1)	0.468
Lipodystrophy score, median (IQR)	1.0 (0.0 – 4.0)	0.0 (0.0 – 1.0)	<0.001
- atrophy ≥1, n (%)	49 (42%)	25 (23%)	<0.001
- hypertrophy ≥1, n (%)	50 (43%)	18 (16%)	<0.001
Total fasting cholesterol serum level (mmol/l), median (IQR)	5.10 (4.38 – 5.74)	4.58 (4.11 – 5.22)	<0.001
Fasting HDL cholesterol serum level (mmol/l), median (IQR)	1.22 (1.02 – 1.49)	1.13 (0.95 – 1.36)	<0.001
Fasting LDL cholesterol serum level (mmol/l), median (IQR)	3.08 (2.48 – 3.72)	2.82 (2.36 – 3.33)	0.001
Fasting triglyceride serum level (mmol/l), median (IQR)	1.18 (0.80 – 1.86)	1.08 (0.78 – 1.60)	0.076
Fasting glucose serum level (mmol/l), median (IQR)	5.00 (4.54 – 5.40)	4.94 (4.43 – 5.50)	0.928

Figure 1: Changes in the CD4 cell count (a) and plasma HIV-RNA (b) during treatment interruption and in PBMC HIV DNA during the first 18 months of treatment interruption (c)

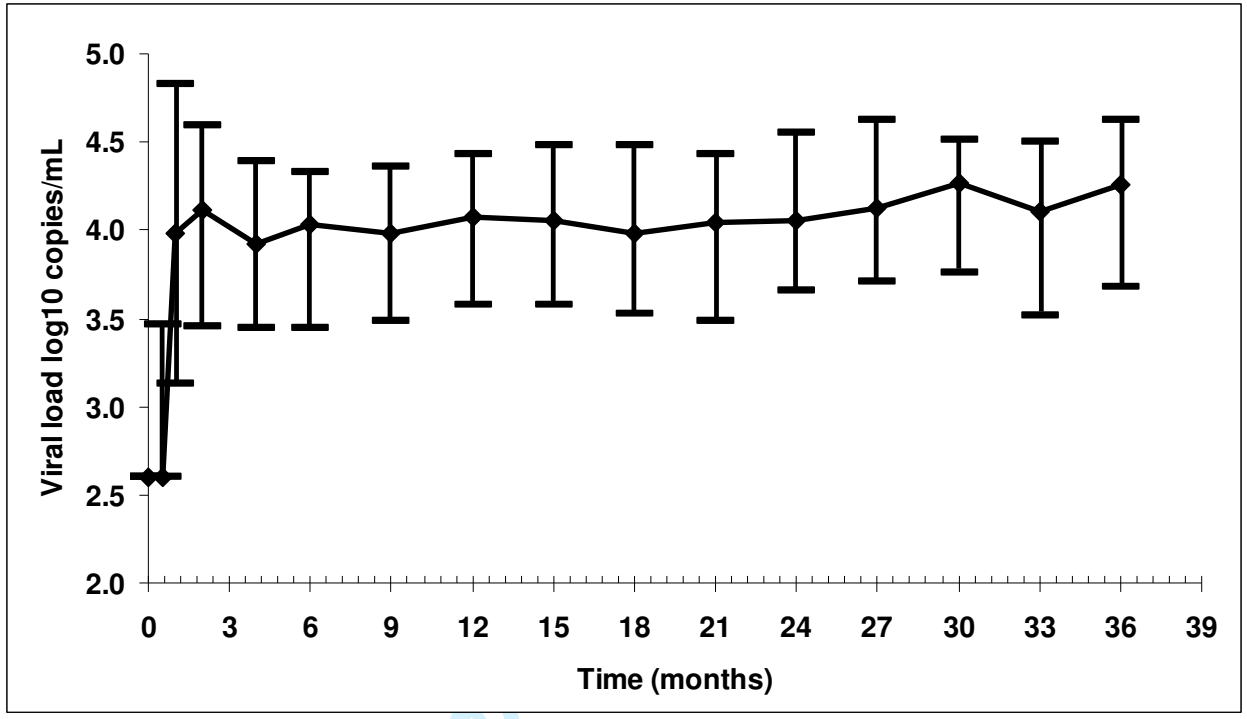
Data points represent median values and whiskers represent interquartile ranges in (a) and (b). The dotted curve in (a) represents predicted changes in the CD4 cell count predicted by mixed linear models. The box plots in (c) represent median and interquartile ranges and whiskers indicate minimum and maximum values.

a



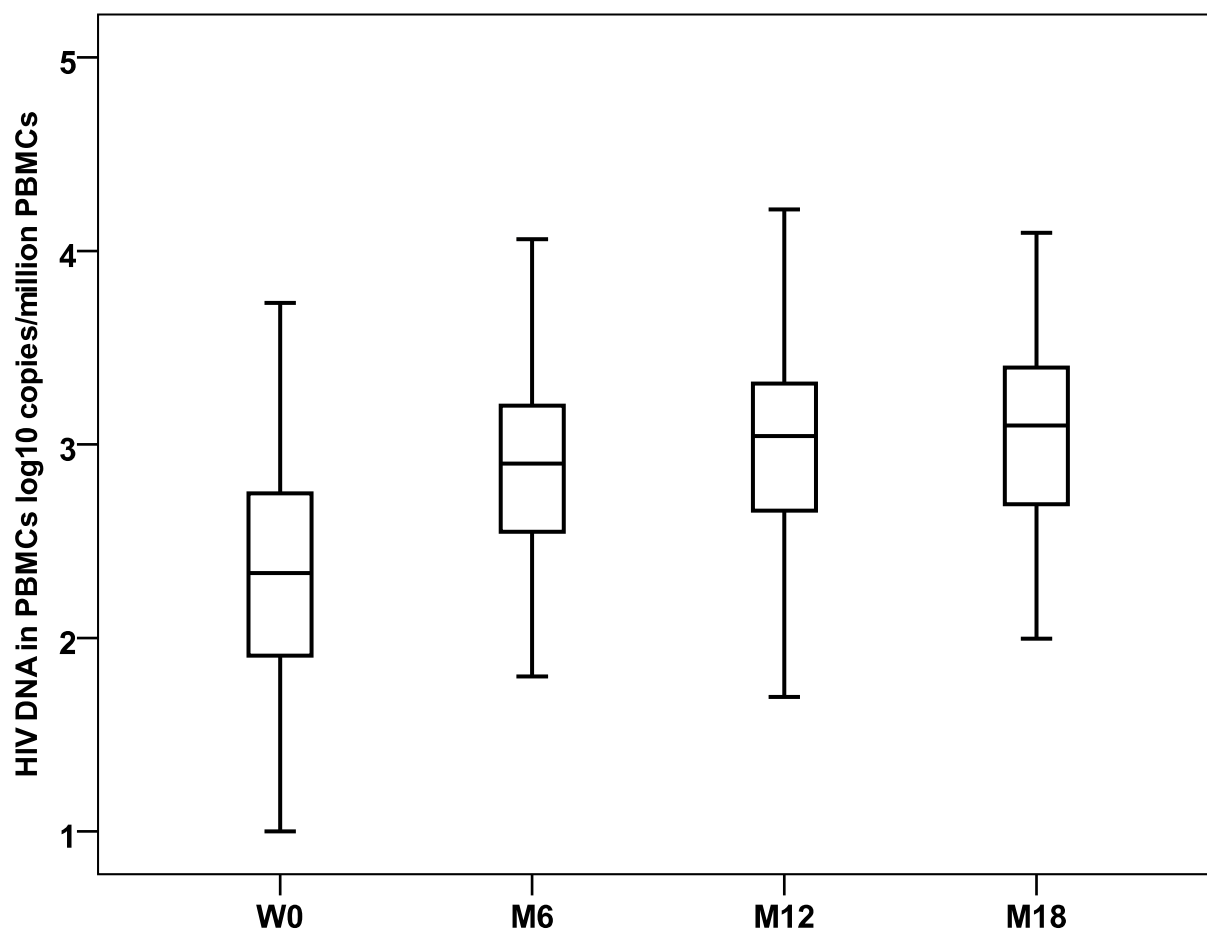
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

b



Peer Review

c



iew

Figure 2: Kaplan-Meier curve of time to the endpoint according to the HIV DNA level adjusted for the CD4 cell nadir

The curves represent the time taken to reach the criteria for treatment resumption in patients with baseline HIV DNA load below and above 2.56 log copies/10⁶ PBMCs (upper tercile).

