# Original article

# HIV-1 control after transient antiretroviral treatment initiated in primary infection: role of patient characteristics and effect of therapy

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**Background: The occurrence of viral control after interruption of an antiretroviral treatment (ART) initiated during primary HIV-1 infection (PHI) is rare and the frequency and predictive factors of such a control are unknown.**
**Methods: Within the French ANRS PRIMO Cohort, 164 patients interrupted ART initiated during PHI. We compared patients whose viral load (VL) remained undetectable (<50 copies/ml) or low (50–500 copies/ml) 1 year after ART interruption to those who evidenced a rapid viral rebound.**
**Results: After ART interruption, VL remained undetectable for a median time of 4.5 years in 14 patients ('post-ART controllers') and low in another 14 patients for a median time of 1.5 years. Post-ART controllers also maintained higher CD4+ T-cell counts compared to other patients. Female gender, a high CD4+ T-cell count and low VL during PHI, and a high CD4+ T-cell count and low HIV DNA levels at interruption, were associated with post-ART HIV control. Treatment characteristics did not differ between controllers and non-controllers. Post-ART controllers had lower specific CD8+ T-cell frequencies and CD8+ T-cell activation on ART and after ART interruption than non-controllers.**
**Conclusions: Few patients maintain very low VL after interruption of treatment initiated during PHI. Early patient characteristics were the main factors of viral control, although early initiation of ART and the effect of ART on reservoir might contribute to control.**

## Introduction

The value of transient combined antiretroviral treatment (ART) during primary HIV-1 infection (PHI) is controversial, particularly with respect to its effect on the subsequent disease course [1–3]. Viral load usually rebounds after treatment interruption, even when ART is initiated during the acute phase of infection, and most studies suggest that transient ART initiated during PHI does not influence the viral set point or lowers it slightly and transiently [4–12]. This suggests that viral reservoirs persist despite ART [4,5,12]. Actually, the decay of reservoir measured by HIV DNA occurs slowly on ART, with HIV DNA usually remaining detectable in patients reaching undetectable levels of HIV RNA, and with ART interruption accompanied by HIV DNA rebound [4,12]. However, recent results from the randomized SPARTAC study suggest that transient ART during 48 weeks of ART could be associated with a persistent virological benefit after ART interruption and a significant delay in time to CD4+ T-cell decrease or long-term ART initiation [11]. Moreover, some authors have reported that viral control may be achieved in patients treated during primary infection following ART interruption [13–16].

The question of the effect of early ART on viral control after ART interruption has not been answered yet.

The precise frequency of viral control after treatment interruption and predictive factors remain to be defined. We therefore studied patients from the ANRS PRIMO Cohort, in whom viral replication was controlled after the end of early transient combined ART, and compared them to patients in whom viral load rebounded. We also examined the phenotypic and functional characteristics of CD8+ T-cells.

## Methods

### Patients

Between 1996 and 2009 the ongoing multicentre French ANRS PRIMO Cohort (CO6) enrolled 1,089 patients with PHI. The study protocol was approved by the Paris Cochin Ethics Committee (Paris, France) and all patients gave written informed consent before enrolment. PHI was diagnosed on the basis of a negative or incomplete western blot with detectable HIV-1 RNA or an interval of <3 months between a negative and a positive ELISA [17]. The date of infection was estimated as the date of symptom onset minus 14 days, or, in asymptomatic patients, the date of the incomplete western blot minus 1 month or the midpoint between a negative and a positive ELISA result. Acute seroconversion was defined as the absence of antibody or the presence of only 1 antibody (usually anti-p24), whereas recent seroconversion was defined by the presence of ≥2 antibodies [3,17]. PHI was defined as 'symptomatic' if ≥1 symptom associated with the acute HIV syndrome was present (fever, enlarged lymph node, pharyngitis or skin rash mucositis). All patients were treatment-naive at enrolment and the course of treatment was left to the physician, according to French guidelines. Among the 493 patients who were treated within the first 3 months following the estimated date of infection and who had a virological response (HIV RNA<50 copies/ml on ART), we selected the 164 patients who subsequently interrupted their treatment and were followed for ≥12 months.

### Procedure

CD4+ T-cell counts and plasma HIV RNA levels (detection threshold 50 copies/ml) were routinely determined at each site and all data were collected. Cell-associated HIV DNA levels were determined on frozen samples in a centralized laboratory at baseline and in the last sample available before ART interruption. Total HIV DNA was extracted from peripheral blood mononuclear cells (PBMC) and quantified by real-time PCR with a detection limit of 10 copies/$10^6$ PBMC [18]. Resistance genotypic tests and viral subtyping were performed at enrolment and the results were interpreted with the ANRS algorithm. Coreceptor usage (X4 or R5X4) was also determined after V3 loop sequencing at enrolment [19].

### HIV-specific CD8+ T-cell responses and activation

Patients were invited to participate in an immunological substudy on a voluntary basis. Approximately 25% of patients agreed to participate and blood samples were then collected at baseline, 6 and 12 months, and every 3 years thereafter. Additional samples were collected according to events occurring in the spontaneous course of infection or to therapeutic changes. Interferon (IFN)-γ secretion by HIV-specific CD8+ T-cells was quantified *ex vivo* with an ELISPOT assay using appropriate stimuli [20]. We used a set of 124 peptides (Neosystem, Strasbourg, France) corresponding to known optimal cytotoxic T-lymphocyte epitopes derived from the HIV-1 Env, Gag, Pol, and Nef proteins (National Institutes of Health HIV Molecular Immunology Database). For each subject, optimal peptides were tested depending on the results of HLA typing (mean SD 46 ±18 peptides were tested per subject; the numbers of epitopes did not differ between patient groups [$P$=0.04]). IFN-γ spot-forming cells (SFCs) were counted with a KS-ELISPOT system (Carl Zeiss Vision, Le Pecq, France) and expressed as SFCs/$10^6$ PBMC after subtracting the background of control unstimulated cells. Wells were considered positive if they contained ≥50 SFCs/$10^6$ PBMC and exhibited ≥2× the background level. Cell phenotyping was performed with the following antibodies: CD8-ECD or -PC5 (clone B9.11), CD3-PC5 (UCHT1), HLA-DR-ECD (Immu-357) and CD38-FITC (T16; Beckman Coulter, Fullerton, CA, USA) [21]. Data from these patients in the ANRS PRIMO Cohort were compared to those of spontaneous chronic HIV controllers (HIC) studied by our group and reported elsewhere [22].

### Definitions and statistical analysis

Patients whose HIV RNA levels remained undetectable (<50 copies/ml) for ≥12 months after ART interruption were referred to as 'post-ART controllers'. They were compared to 'post-ART low viraemic patients' in whom HIV RNA values fluctuated between 50 and 500 copies/ml after ART interruption, and with 'post-ART non-controllers' who experienced an increase of HIV RNA>500 copies/ml within the 12 months following ART interruption. The duration of viral control in post-ART controllers was calculated as the interval between the first HIV RNA value <50 copies/ml after treatment interruption and the first of two consecutive HIV RNA values ≥50 copies/ml, corresponding to the end of the control period. In low viraemic post-ART patients, the duration of viral control was estimated as the interval between the first RNA value <500 copies/ml after treatment interruption and the first of two consecutive HIV RNA values >500 copies/ml. Time to loss of viral control and percentages of patients in whom virological response was still undetectable (<50 copies/ml) at different times since ART interruption were estimated through

**Table 1.** Characteristics of 164 patients in the ANRS PRIMO Cohort who interrupted their treatment initiated during primary HIV-1 infection

| Characteristics | Post-ART controllers ($n$=14)[a] | Post-ART low viraemic patients ($n$=14)[b] | Post-ART non-controllers ($n$=136) | $P$-value[c] |
|---|---|---|---|---|
| Median age (IQR) | 33 (29–42) | 34 (31–44) | 36 (30–42) | 0.83 |
| Women, % | 36 | 43 | 14 | 0.005 |
| Symptomatic primary infection, %[d] | 89 | 93 | 90 | 0.75 |
| Caucasian, % | 93 | 86 | 87 | 1.00 |
| **At enrolment** | | | | |
| Median CD4$^+$ T-cell count, cells/mm$^3$ (IQR) | 582 (489–775) | 583 (451–651) | 485 (343–617) | 0.049 |
| Median CD4$^+$ T-cell percentage (IQR) | 33 (27–36) | 28 (24–32) | 24 (18–30) | 0.003 |
| Median CD4$^+$/CD8$^+$ T-cell ratio (IQR) | 0.79 (0.58–0.98) | 0.55 (0.36–0.71) | 0.47 (0.26–0.75) | 0.04 |
| Median HIV-1 RNA, $\log_{10}$ copies/ml (IQR) | 4.8 (3.5–5.3) | 5.0 (4.3–5.6) | 5.4 (4.8–6.9) | 0.04 |
| Median HIV-1 DNA, $\log_{10}$ copies/10$^6$ PBMC (IQR) | 3.1 (2.8–3.4) | 3.1 (2.8–3.5) | 3.4 (2.9–3.7) | 0.09 |
| Acute seroconverters, % | 36 | 36 | 37 | 1.00 |
| **Treatment characteristics** | | | | |
| Median delay between PHI and treatment, weeks (IQR) | 3.1 (2.4–5.7) | 3.3 (3.0–5.0) | 3.1 (2.1–4.6) | 0.74 |
| Median treatment duration, months (IQR) | 20.1 (12.5–37.1) | 13.2 (9.3–18.9) | 17.0 (11.2–25.1) | 0.27 |
| **Before treatment interruption** | | | | |
| Median CD4$^+$ T-cell count (IQR) | 972 (734–1,315) | 895 (660–1,003) | 744 (595–971) | 0.047 |
| Median CD4$^+$/CD8$^+$ T-cell ratio (IQR) | 1.4 (1.3–2.1) | 1.0 (0.72–1.3) | 1.1 (0.8–1.4) | 0.02 |
| Median HIV-1 DNA, $\log_{10}$ copies/10$^6$ PBMC (IQR) | 2.1 (1.7–2.7) | 2.0 (1.7–2.4) | 2.5 (2.0–3.0) | 0.03 |
| Median control duration after interruption, years[e] | 4.5 (3.5–7.1) | 1.5 (1.3–1.6) | – | 0.001 |

[a]HIV RNA<50 copies/ml. [b]HIV RNA 50–500 copies/ml. [c]$P$-values for comparison of the three groups. [d]Defined as ≥1 symptom related to the acute viral syndrome (fever, enlarged lymph nodes, pharyngitis, skin rash or mucositis). [e]Kaplan–Meier estimates (95% CI). ART, antiretroviral treatment; PBMC, peripheral blood mononuclear cells; PHI, primary HIV-1 infection.

Kaplan–Meier curves. Post-ART interruption CD4$^+$ T-cell dynamics and HIV RNA rebound were depicted graphically through Lowess curves. Characteristics of patients were compared by using the non-parametric Wilcoxon and Kruskal–Wallis tests for continuous variables. Percentages were compared by using the $\chi^2$ test and Fisher's exact test. Data were analysed with SAS software (SAS Institute Inc., Cary, NC, USA).

## Results

### Patient characteristics during primary HIV-1 infection

Among the 164 patients who started ART during the first 3 months after HIV infection, and who were virological responders when ART was interrupted, HIV RNA levels were spontaneously maintained <50 copies/ml for >12 months in 14 post-ART controllers, and remained low (50–500 copies/ml) for >12 months in another 14 patients. The other 136 patients experienced a larger viral increase after ART interruption.

The patients' characteristics during PHI are shown in Table 1. Demographic characteristics did not differ significantly between post-ART controllers, low viraemic patients and non-controllers, except that the percentage of women was significantly higher among post-ART controllers and low-viraemic patients. Median HIV RNA levels were significantly lower in post-ART controllers (4.8 $\log_{10}$ copies/ml) and low viraemic patients (5.0 $\log_{10}$ copies/ml) than in non-controllers (5.4 $\log_{10}$ copies/ml; $P$=0.04 between the three groups), and HIV DNA levels tended to be lower in post-ART controllers and low viraemic patients (3.1 $\log_{10}$ copies/10$^6$ PBMC) than in non-controllers (3.4 $\log_{10}$ copies/10$^6$ PBMC; $P$=0.09). Baseline viral characteristics (frequency of B and non-B subtypes, X4 tropism and genotypic resistance at enrolment) did not differ between post-ART controllers and other patients.

At baseline, post-ART controllers and low viraemic patients had significantly higher median CD4$^+$ T-cell counts and percentages (582 [33%] and 583 [28%] cells/mm$^3$, respectively) than non-controllers (485 [24%] cells/mm$^3$). HLA genotype was tested in 24 (85%) post-ART controllers and low viraemic patients and in 106 (80%) non-controller patients. The frequency of the protective B27 and B57 HLA alleles did not differ between post-ART non-controller patients (6.4% and 1.8%, respectively) and other patients with post-ART undetectable or low viral load: one post-ART controller and one low viraemic patient carried HLA-B27 (8.3%), and one low viraemic patient carried HLA-B57 (4.2%).

### Treatment

ART was initiated at a median time of 3.2 weeks after PHI. The proportion of patients diagnosed with acute infection was similar in post-ART controllers, low

viraemic and non-controller patients (36%, 36% and 37% respectively). All patients received a combination of two reverse transcriptase inhibitors plus either a protease inhibitor (mainly lopinavir/ritonavir or nelfinavir) or efavirenz (or, rarely, abacavir). The frequency of protease inhibitor (PI)-containing and boosted PI-containing regimens was similar among patients. These ART combinations conformed to French guidelines, which recommend PIs when treatment is initiated during PHI.

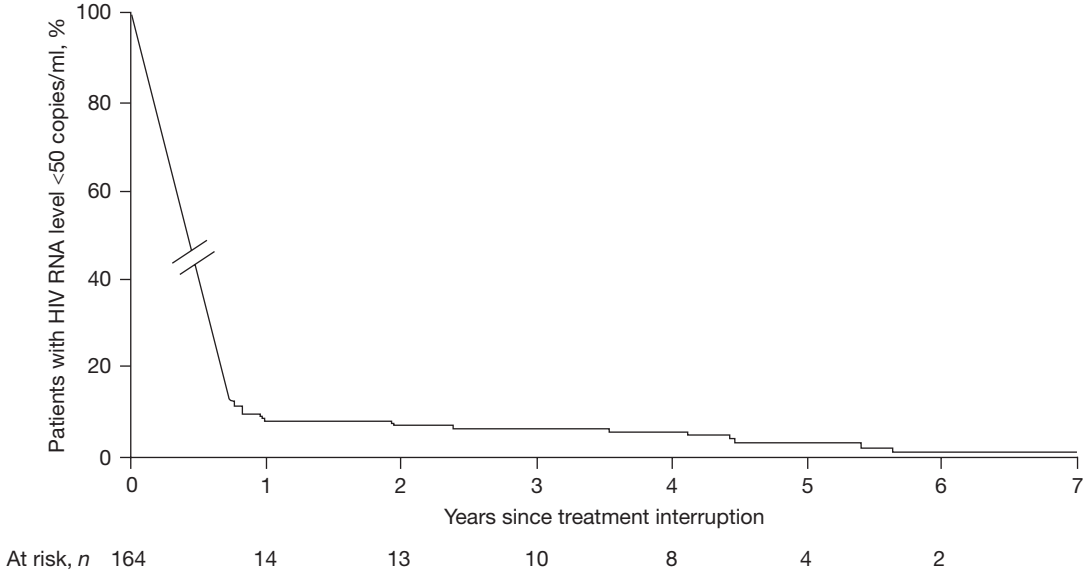
Treatment characteristics, such as time to treatment initiation, time to virological response and the treatment duration, did not differ between the three groups (Table 1). HIV DNA levels at ART interruption were significantly lower and CD4+ T-cell counts were higher in post-ART controllers and low viraemic patients than in non-controllers (2.1 and 2.0 $\log_{10}$ copies/$10^6$ PBMC versus 2.5 $\log_{10}$ copies/$10^6$ PBMC, and 972 and 895 cells/$mm^3$ versus 744 cells/$mm^3$), whereas the decrease in HIV DNA levels and the increase in CD4+ T-cell counts during ART did not differ significantly across the three groups. Post-ART controllers had highly favourable immunological status at ART interruption, as they all had CD4+ T-cell counts >600 cells/$mm^3$ (median 972 cells [range 668–2,074]) and CD4+/CD8+ T-cell ratios >0.75 (median 1.4 [range 0.77–2.83]), whereas the CD4+ T-cell values attained with a similar ART duration in low viraemic and non-controller patients were significantly lower.

### HIV control period

After ART interruption, viral control was observed immediately, with no transient viral rebound in 10 patients out of the 14 post-ART controllers. The four other patients experienced a small viral rebound (HIV RNA levels 2.1–3.1 $\log_{10}$ copies/ml) before the establishment of viral control in the 8.7 months following ART interruption. The probability of having an HIV RNA level <50 copies/ml was 11% 1 year after ART interruption, 8.5% at 2 years and 7.2% at 3 years (Figure 1). In these post-ART controllers, viral control lasted a median of 4.5 years (range 1.9–8.0). Control was lost in 10 patients, but their viral load remained low after loss of control (median HIV RNA 3.2 $\log_{10}$ copies/ml at 12 months [IQR 2.6–3.5]; Figure 2A). Interestingly, the four patients who maintained a tight control (<50 copies/ml) at their last follow-up visit had not experienced any viral rebound after ART interruption.

Despite similar characteristics during the primary infection and treatment period to those of the post-ART controllers, the 14 low viraemic post-ART patients had shorter periods of viral control (median 1.5 years [range 1.2–6.0]) and exhibited a very gradual increase in viral load as shown in Figure 2A.

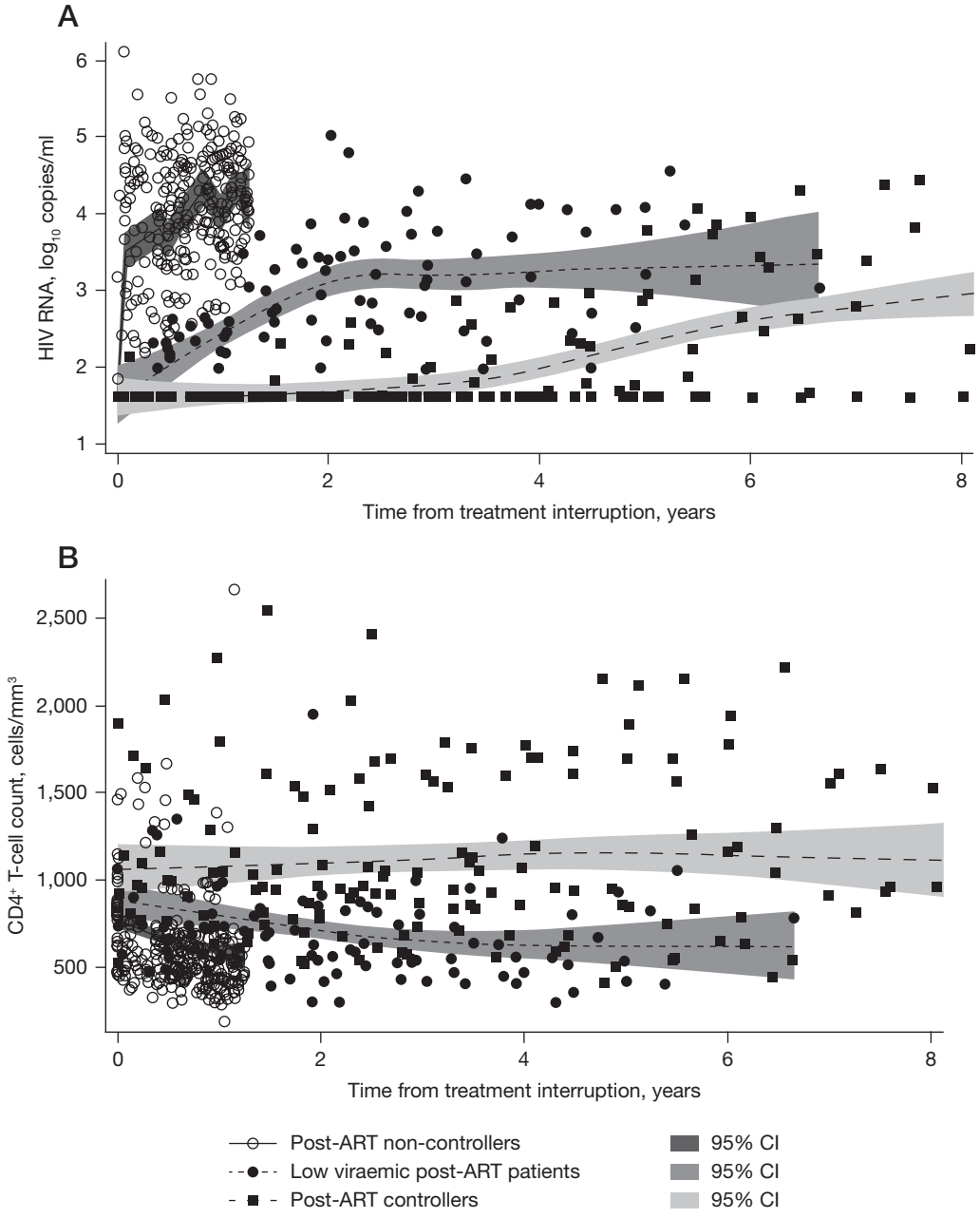
**Figure 1.** Outcome after ART interruption in 164 patients from the ANRS PRIMO Cohort who were treated early during primary HIV-1 infection and were virological responders at the time of ART interruption




Kaplan–Meier curve displaying time (years) to loss of virological control (expressed as the percentage of patients with HIV RNA levels <50 copies/ml) is shown. Numbers of patients at risk along time are given at the bottom of the graph. Data were censored either at the last value <50 copies/ml or at the last visit. ART, antiretroviral treatment.

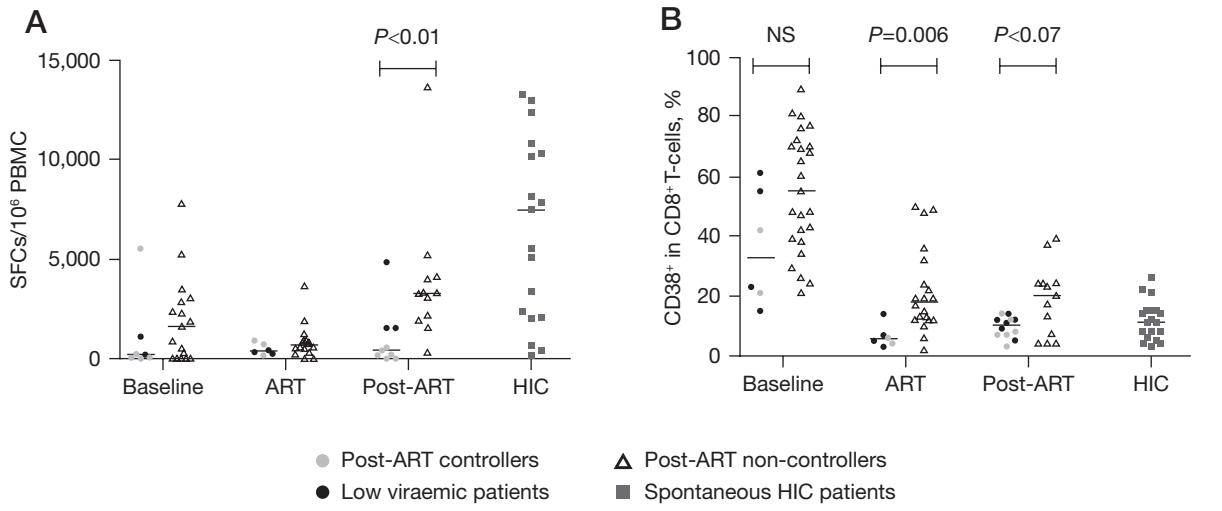
The kinetics of CD4+ T-cell counts after ART interruption is shown in Figure 2B. In post-ART controllers, CD4+ T-cell counts remained stable throughout the control period with a median of 1,000 cells/mm$^3$ at the last point of viral control (IQR 760–1,530 cells/mm$^3$), whereas CD4+ T-cell counts tended to decline in low viraemic patients (median 695 cells/mm$^3$ at the last point of viral control [IQR 575–797]).

**Figure 2.** Kinetics of HIV RNA rebound and CD4+ T-cell counts upon ART interruption in 164 patients treated early during primary infection in the ANRS PRIMO Cohort




**(A)** Plasma HIV RNA rebound and **(B)** CD4+ T-cell dynamics after treatment interruption. Kinetics results are displayed for post-antiretroviral treatment (ART) controller patients (<50 HIV RNA copies/ml 12 months after ART interruption), low viraemic patients (HIV RNA 50–500 copies/ml 12 months after ART interruption) and post-ART non-controllers (HIV RNA>500 copies/ml 12 months after ART interruption). Undetectable HIV RNA measurements (<50 copies/ml) are set to 1.7 $\log_{10}$ copies/ml. All data were collected; data from non-controllers are given only in the first year after ART interruption. Results are represented with Lowess curves for each patient group, with 95% CIs for all curves.

**Figure 3.** HIV-specific CD8+ T-cell frequencies and CD8+ T-cell activation in 38 patients treated early during primary infection in the ANRS PRIMO Cohort




**(A)** Frequencies of HIV-specific interferon-γ-secreting CD8+ T-cells in post-antiretroviral treatment (ART) controllers (<50 HIV RNA copies/ml 12 months after ART interruption), low viraemic patients (HIV RNA 50–500 copies/ml 12 months after ART interruption) and non-controllers (HIV RNA>500 copies/ml 12 months after ART interruption) after a transient ART initiated during primary infection in the ANRS PRIMO Cohort. Results for spontaneous HIV controller (HIC) patients from the ANRS Cohort are given as a reference [22]. **(B)** Percentage of CD8+ T-cells expressing CD38 in post-ART controllers, low viraemic and non-controller patients after transient ART initiated during primary infection. Results are given at baseline before ART initiation, during ART and after cessation of ART. Results for HIC patients are given as a reference [22]. NS, non-significant; PBMC, peripheral blood mononuclear cells; SFCs, spot-forming cells.

### HIV-specific CD8+ T-cell responses and activation

A total of 38 of the 164 patients enrolled in this study were included in an immunological substudy: 6 were post-ART controllers, 7 low viraemic patients and 25 non-controllers. Characteristics of these patients were similar among the groups in terms of baseline HIV RNA (median 4.9 $\log_{10}$ copies/ml in the post-ART controllers and low viraemic patients versus 5.3 $\log_{10}$ copies/ml in the non-controllers).

HIV-specific CD8+ T-cell frequencies were quantified by means of an IFN-γ ELISPOT assay (Figure 3A). In non-controller patients, these frequencies declined on therapy (from median 1,600 SFCs/$10^6$ PBMC [IQR 0–2,923] to 681 SFCs/$10^6$ PBMC [IQR 33–931]) and increased again after ART interruption (median 3,263 SFCs/$10^6$ PBMC [IQR 1,973–4,050]; Kruskall–Wallis $P$=0.004). By contrast, in post-ART controllers and low viraemic patients, HIV-specific CD8+ T-cell responses were remarkably low at baseline and remained low during ART and after ART interruption (median 205 SFCs/$10^6$ PBMC [IQR 68–1,100]), 379 SFCs/$10^6$ PBMC [IQR 231–776] and 425 SFCs/$10^6$ PBMC [IQR 84–1,542], respectively). These frequencies were much lower than those observed in a reference group of long-term spontaneous HIC previously studied by our group (median 7,456 SFCs/$10^6$ PBMC [IQR 2,060–10,760]; Figure 3A) [22].

Overall CD8+ T-cell activation, evaluated in terms of CD38 expression, was high at baseline, especially in the post-ART non-controller patients, although the difference did not reach statistical significance (Figure 3B). The median level of activation fell markedly during ART in all patients but remained significantly higher in post-ART non-controllers (18% [IQR 12–30]) than in others (5% [IQR 4–9]; $P$=0.006). After ART interruption, median CD8+/CD38+ T-cell percentages remained roughly at the same level in each group (8% in controllers and low viraemic patients and 20% in non-controllers, respectively; $P$=0.07). The expression of HLA-DR paralleled that of CD38 (data not shown). Similar changes in the activation level were observed when HIV-specific CD8+ T-cells were stained with MHC-class I pentamers: the activation level was high at baseline, decreased on ART, and increased again slightly after ART interruption.

These changes in HIV-specific CD8+ T-cell frequencies and CD8+ T-cell activation levels were associated with differences in viral load. At baseline, a correlation was observed between plasma HIV RNA levels and percentage of T-cells expressing CD8+/CD38+ (Spearman's $r$=0.4; $P$=0.03). During ART, no correlation could be assessed because ultrasensitive measurements of plasma HIV RNA levels were unavailable. However, cellular

HIV DNA levels tended to correlate with CD8+ T-cell activation levels (Spearman's $r$=0.4; $P$=0.08).

## Discussion

After transient ART that is initiated during PHI, HIV RNA levels remained undetectable, <50 copies/ml, for >1 year in 14 patients (median 4.5 years) and low (<500 copies/ml) in 14 other patients out of 164 patients from the ANRS PRIMO Cohort who interrupted their treatment. Compared with patients who experienced rapid viral rebound after ART interruption, post-ART controllers were more frequently female and had higher CD4+ T-cell counts, lower HIV RNA and a tendency for lower HIV DNA levels at the time of PHI. They also had higher CD4+ T-cell counts and lower HIV DNA levels at ART interruption than post-ART non-controllers. No viral characteristics (subtype, genotypic resistance or tropism) or HLA genotype were associated with post-ART control, and neither were the treatment regimen or treatment duration.

Patient characteristics at the time of PHI were shown to greatly determine both the viral set point and the CD4+ T-cell loss after ART interruption. Results from this study also confirm the highly predictive value of early patient characteristics, which were associated with the level and frequency of viral control after interruption of ART initiated during PHI [4,8,16,23].

Spontaneous control has been observed in a few untreated patients followed since PHI diagnosis [24]. The crucial question of whether patients who experienced viral control after ART interruption would have become spontaneously HIC is difficult to answer. A large body of evidence suggests that post-ART controller patients were not identical to long-term chronic HIC [25]. First, no overrepresentation of the protective HLA B27 and B57 alleles was observed in post-ART controllers and low viraemic patients, in contrast to data reported in HIC [22,24–26]. Second, although the baseline characteristics of patients who maintained undetectable or low viral load after ART interruption described here were more favourable than those of non-controllers, the patients that we previously described from the ANRS PRIMO Cohort as spontaneous controllers had even more favourable characteristics, with higher CD4+ T-cell counts and lower HIV RNA and DNA levels during primary infection [24]. Third, high frequencies of HIV-specific CD8+ T-cells are commonly found in HIC, contrasting with the very low frequencies of such cells observed in this study in post-ART controllers whatever the period when evaluations were performed after ART interruption [27–31]. This point also suggests that HIV-specific immune responses may not be responsible for post-ART viral control and that different mechanisms are thus involved in primary-infected post-ART controllers and HIC. Therefore, the likelihood that post-ART controllers described here would have become HIC without ART is low.

The question of the supplementary role of initial ART in the onset of viral control after ART interruption is still unclear. We did not find any association between viral control and the time between infection and treatment or the duration of treatment, as also reported by Volberding *et al.* [8] although the delay between PHI and treatment was short in our patients, contrary to those of the SPARTAC Study [11]. As mentioned above, baseline characteristics of post-ART controllers and low viraemic patients were already more favourable than those of non-controllers, as they had lower HIV RNA levels and higher CD4+ T-cell counts than those observed in non-controllers. Therapeutic response in terms of CD4+ T-cell increase and HIV DNA decrease on ART was similar between our patients. Therefore the differences in CD4+ T-cell counts and HIV DNA levels observed at ART interruption could be partly explained by differences at baseline. However, HIV DNA levels in post-ART controllers and low viraemic patients were significantly lower than in non-controllers at treatment interruption, whereas they only tended to be lower during PHI. Noteworthy, DNA levels observed at ART interruption in our patients who controlled HIV replication were very similar to those found in spontaneous HIC [24,25]. The treatment efficacy might therefore have played a role in the containment of reservoir and the establishment of viral control. These data are also in line with previous results in which all patients with low viral load after ART interruption exhibited low and stable HIV DNA levels off treatment [12,16,32]. This is also strengthened by the higher CD8+ T-cell activation levels observed during treatment in non-controllers. A higher viral burden in these patients could explain both the higher frequency of HIV-specific CD8+ T-cells and the higher level of T-cell activation which, in turn, could contribute to feeding the viral reservoir [33].

This could thus suggest that treatment had helped some patients, who would not have spontaneously controlled viral replication, to achieve a higher CD4+ T-cell count, a restrained activation and a lower reservoir associated with viral control, and this might be preferentially observed in patients with favourable initial parameters. Such patients maintained high CD4+ T-cell counts after ART interruption, which prevent disease progression. This could be particularly the case in patients in whom viral control was established without any viral rebound, since a sustained tight control was only observed in these patients. By contrast, post-ART low viraemic patients who had less favourable CD4+ T-cell parameters exhibited a gradual increase in HIV RNA with a shorter duration of viral containment. Actually, among the patients from the ANRS PRIMO

Cohort who started ART at distance of primary infection, we did not observe any viral control after ART interruption, further suggesting a possible beneficial role of early initiation of therapy. In addition, no cases of post-ART viral control have been reported in the chronic stage of HIV infection, with the exception of a bone marrow transplant recipient [34].

This observational study presents some limitations, and neither treatment initiation nor treatment interruption was randomized. However, the large number of patients recruited in the ANRS PRIMO Cohort and their long-term follow up offer the opportunity to describe specific situations occurring in rare patients who cannot be observed in clinical trials with lower sample size and shorter follow-up.

In conclusion, HIV control may occur in a small number of patients who interrupt ART treatment initiated during PHI. Early patient's characteristics are the main factors of post-ART viral control. We hypothesize that transient ART could also contribute to subsequent viral control by lowering the viral reservoir down to values observed in HIC. Results from ongoing studies with more potent early treatments may contribute to clarifying this hypothesis.

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## Disclosure statement

The authors declare no competing interests.

## Additional file

Additional file 1: A list of the PRIMO Cohort Scientific Committee can be found at http://www.intmedpress.com/uploads/documents/AVT-11-OA-2366_Goujard_Add_file_1.pdf

## References

1. D'Souza MP, Axten KL, Hecht FM, Altfeld M. Acute HIV-1 Infection: what's new? Where are we going? *J Infect Dis* 2010; **202 Suppl 2:**S267–S269.
2. Bell SK, Little SJ, Rosenberg ES. Clinical management of acute HIV infection: best practice remains unknown. *J Infect Dis* 2010; **202 Suppl 2:**S278–S288.
3. Cohen MS, Shaw GM, McMichael AJ, Haynes BF. Acute HIV-1 infection. *N Engl J Med* 2011; **364:**1943–1954.
4. Desquilbet L, Goujard C, Rouzioux C, *et al*. Does transient HAART during primary HIV-1 infection lower the virological set-point? *AIDS* 2004; **18:**2361–2369.
5. Streeck H, Jessen H, Alter G, *et al*. Immunological and virological impact of highly active antiretroviral therapy initiated during acute HIV-1 infection. *J Infect Dis* 2006; **194:**734–739.
6. Hecht FM, Wang L, Collier A, *et al*. A multicenter observational study of the potential benefits of initiating combination antiretroviral therapy during acute HIV infection. *J Infect Dis* 2006; **194:**725–733.
7. Lampe FC, Porter K, Kaldor J, *et al*. Effect of transient antiretroviral treatment during acute HIV infection: comparison of the Quest trial results with CASCADE natural history study. *Antivir Ther* 2007; **12:**189–193.
8. Volberding P, Demeter L, Bosch RJ, *et al*. Antiretroviral therapy in acute and recent HIV infection: a prospective multicenter stratified trial of intentionally interrupted treatment. *AIDS* 2009; **23:**1987–1995.
9. Koegl C, Wolf E, Hanhoff N, *et al*. Treatment during primary HIV infection does not lower viral set point but improves CD4 lymphocytes in an observational cohort. *Eur J Med Res* 2009; **14:**277–283.
10. Steingrover R, Fernandez Garcia E, van Valkengoed IGM, *et al*. Transient lowering of the viral set-point after temporary antiretroviral therapy of primary HIV type 1 infection. *AIDS Res Hum Retroviruses* 2010; **26:**379–387.
11. Fidler S, SPARTAC trial investigators. The effect of short-course antiretroviral therapy in primary HIV infection: final results from an international randomised controlled trial; SPARTAC. *6th IAS Conference on HIV Pathogenesis, Treatment and Prevention.* 17–20 July 2011 Rome, Italy. Abstract WELBX06.
12. von Wyl V, Gianella S, Fischer M, *et al*. Early antiretroviral therapy during primary HIV-1 infection results in a transient reduction of the viral setpoint upon treatment interruption. *PLoS ONE* 2011; **6:**e27463.
13. Lisziewicz J, Rosenberg E, Lieberman J, *et al*. Control of HIV despite the discontinuation of antiretroviral therapy. *N Engl J Med* 1999; **340:**1683–1684.
14. Rosenberg ES, Altfeld M, Poon SH, *et al*. Immune control of HIV-1 after early treatment of acute infection. *Nature* 2000; **407:**523–526.
15. Kaufmann DE, Lichterfeld M, Altfeld M, *et al*. Limited durability of viral control following treated acute HIV infection. *PLoS Med* 2004; **1:**e36.
16. Hocqueloux L, Prazuck T, Avetand-Fenoel V, *et al*. Long term immuno-virologic control following ART interruption in patients treated at the time of primary HIV-1 infection. *AIDS* 2010; **24:**1598–1601.
17. Ghosn J, Deveau C, Chaix ML, *et al*. Despite being highly diverse, immunovirological status strongly correlates with clinical symptoms during primary HIV-1 infection: a cross-sectional study based on 674 patients enrolled in the ANRS CO 06 PRIMO cohort. *J Antimicrob Chemother* 2010; **65:**741–748.
18. Avettand-Fènoël V, Boufassa F, Galimand J, Meyer L, Rouzioux C. HIV-1 DNA for the measurement of the HIV reservoir is predictive of disease progression in seroconverters whatever the mode of result expression is. *J Clin Virol* 2008; **42:**399–404.
19. Delobel P, Nugeyre MT, Cazabat M, *et al*. Population-based sequencing of the V3 region of env for predicting the coreceptor usage of human immunodeficiency virus type 1 quasispecies. *J Clin Microbiol* 2007; **45:**1572–1580.
20. Lacabaratz-Porret C, Urrutia A, Doisne JM, *et al*. Impact of antiretroviral therapy and changes in virus load on human immunodeficiency virus (HIV)-specific T cell responses in primary HIV infection. *J Infect Dis* 2003; **187:**748–757.
21. Lécuroux C, Girault I, Boutboul F, *et al*. Antiretroviral therapy initiation during primary HIV infection enhances both CD127 expression and the proliferative capacity of HIV-specific $CD8^+$ T cells. *AIDS* 2009; **23:**1649–1658.

22. Sáez-Cirión A, Sinet M, Shin SY, *et al*. Heterogeneity in HIV suppression by CD8 T cells from HIV controllers: association with Gag-specific CD8 T cell responses. *J Immunol* 2009; **182:**7828–7837.
23. Seng R, Goujard C, Desquilbet L, *et al*. Rapid CD4+ cell decrease after transient cART initiated during primary HIV infection (ANRS PRIMO and SEROCO cohorts). *J Acquir Immune Defic Syndr* 2008; **49:**251–258.
24. Goujard C, Chaix ML, Lambotte O, *et al*. Spontaneous control of viral replication during primary HIV infection: when is 'HIV controller' status established? *Clin Infect Dis* 2009; **49:**982–986.
25. Okulicz JF, Lambotte O. Epidemiology and clinical characteristics of elite controllers. *Curr Opin HIV AIDS* 2011; **6:**163–168.
26. Pereyra F, Addo MM, Kaufmann DE, *et al*. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. *J Infect Dis* 2008; **197:**563–571.
27. Migueles SA, Laborico AC, Shupert WL, *et al*. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat Immunol* 2002; **3:**1061–1068.
28. Sáez-Cirión A, Lacabaratz C, Lambotte O, *et al*. HIV controllers exhibit potent CD8 T cell capacity to suppress HIV infection *ex vivo* and peculiar cytotoxic T lymphocyte activation phenotype. *Proc Natl Acad Sci U S A* 2007; **104:**6776–6781.
29. Streeck H, Nixon DF. T cell immunity in acute HIV-1 infection. *J Infect Dis* 2010; **202 Suppl 2:**S302–S308.
30. McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes B. The immune response during acute HIV-1 infection: clues for vaccine development. *Nat Rev Immunol* 2010; **10:**11–23.
31. Betts MR, Nason MC, West SM, *et al*. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood* 2006; **107:**4781–4789.
32. Sáez-Cirión A, Hocqueloux L, Avettand-Fenoel V, *et al*. Long term HIV-1 control after interruption of a treatment initiated at the time of primary infection is associated to low cell associated HIV DNA levels: ANRS Visconti Study. *18th Conference on Retroviruses and Opportunistic Infections*. 27 February–2 March 2011, Boston, MA, USA. Abstract 515.
33. Casazza JP, Betts MR, Picker LJ, Koup RA. Decay kinetics of human immunodeficiency virus–specific CD8+ T cells in peripheral blood after initiation of highly active antiretroviral therapy. *J Virol* 2001; **75:**6508–6516.
34. Hütter G, Nowak D, Mossner M, *et al*. Long-term control of HIV by CCR5 delta32/ delta32 stem-cell transplantation. *N Engl J Med* 2009; **360:**692–698.

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