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First-line cART regimen impacts the course of CD8⁺ T-cell counts in HIV-infected patients that achieve sustained undetectable viral load.

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Abstract

The aim of the study was to investigate the impact of first-line combined antiretroviral therapy (cART) regimen on the course of CD8⁺ T-cell counts in human immunodeficiency virus (HIV)-infected patients.

A retrospective observational study conducted on the French DAT'AIDS Cohort of HIV-infected patients.

We selected 605 patients initiating a first-line cART between 2002 and 2009, and which achieved a sustained undetectable HIV plasma viral load (pVL) for at least 12 months without cART modification. The evolution of CD8⁺ T-cell counts according to cART regimen was assessed.

CD8⁺ T-cell counts were assessed in 572 patients treated with 2NRTIs+1PI/r (n= 297), 2NRTIs+1NNRTI (n= 207) and 3NRTIs (n= 68). In multivariate analysis, after 12 months of follow-up, the 3NRTIs regimen was associated with a significantly smaller decrease of CD8⁺ T-cell count compared with NNRTI-containing regimens (−10.2 cells/μL in 3NRTIs vs −105.1 cells/μL; *P*=0.02) but not compared with PI-containing regimens (10.2 vs −60.9 cells/μL; *P*=0.21). After 24 months, the 3NRTIs regimen was associated with a smaller decrease of CD8⁺ T-cell count and % compared with PI/r- and NNRTI-containing regimens (0.2 in 3NRTIs vs −9.9 with PI/r-regimens, *P*=0.001, and vs −11.1 with NNRTI-regimens, *p*<0.0001). A focus analysis on 11 patients treated with an INSTI-containing cART regimen during the study period showed after 12 months of follow-up, a median decrease of CD8⁺ T-cell count of −155 [inter quartile range: −302; −22] cells/μL.

Our data highlight the fact that cART regimens have differential effects on CD8 pool down regulation.

Abbreviations: cART = combined antiretroviral therapy, HIV = human immunodeficiency virus, INSTI = integrase strand transfer inhibitor, NNRTI = non-nucleotide reverse transcriptase inhibitor, NRTI = nucleotide reverse transcriptase inhibitor, PI/r = Ritonavir-boosted protease inhibitor, pVL = plasma viral load.

Keywords: CD4:CD8 ratio, CD8, first-line cart, non nucleotide reverse transcriptase inhibitors

1. Introduction

HIV infection leads to perturbations of peripheral T-cell homeostasis with a gradual depletion of CD4⁺ T-cells combined with an expansion of the CD8⁺ T-cell compartment. After

initiating combined antiretroviral therapy (cART), most HIV1-infected patients achieve sustained undetectable HIV plasma viral load (HIV-pVL) and restoration of the CD4⁺ T-cell compartment,^[1] with an extent that depends on the level of CD4⁺ T-cell counts at the time of cART initiation.^[2–6] In contrast, most

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patients fail to normalize CD8⁺ T-cell count despite long term cART, and this resilience of high CD8⁺ T-cell counts was recently linked to increased non-AIDS-related mortality.^[7] Previous studies have reported that the nature of antiretroviral drugs chosen for cART regimen affected CD4 recovery,^[8,9] while the impact of cART choice on the dynamics of CD8⁺ T-cells remains elusive.

The aim of this study was to determine the impact of cART regimen on the course of CD8⁺ T-cell counts in HIV-infected patients who achieved sustained undetectable HIV-pVL on first-line cART.

2. Methods

2.1. Patient & Data collection

“The Dat’AIDS Cohort” is a multicenter cohort of 26959 HIV infected patients followed in fifteen major French HIV care centers using the NADIS[®] electronic medical record (Fedialis Medica, Marly le Roi, France).^[10,11] In this cohort, patients’ data are recorded during medical visits, and quality control is performed by automated checks during data capture, regular controls by clinical research associates, annual assessments and *ad hoc* processes prior to analysis.^[10,11]

For this study, we selected HIV-1 infected and treatment-naïve patients who initiated a first-line triple cART between Jan 2002 and Dec 2009 and who maintained an undetectable HIV plasma viral load (HIV-pVL) for at least 12 months without modification of cART regimen. Among them, we selected patients treated with a backbone of 2NRTIs combined with a ritonavir-boosted protease inhibitor (PI/r), or a non-nucleoside reverse transcriptase inhibitor (NNRTI), or a third NRTI. In addition, we analyzed separately patients treated with Integrase Strand Transfer Inhibitor (INSTI)-containing regimens, whatever the drug combination. All of these latter patients received raltegravir.

2.2. Outcome variables

Data on T-cell immunophenotyping (CD4⁺, CD8⁺ T-cell counts and CD4:CD8 ratio) were collected at the time of cART initiation (baseline), at the time of the first undetectable HIV-pVL (D0), and then after 12 (M12) and 24 months (M24) of follow-up with a sustained undetectable HIV-pVL without modification of cART regimen.

For T-cell immunophenotyping, a standardized procedure was performed on each site, briefly, fresh EDTA-whole blood (100 μ L) was incubated with combinations of fluorochrome-conjugated monoclonal antibodies specific for CD3, CD4 and CD8 (Beckman Coulter) and analyzed using a FC500 cytometer (Beckman Coulter). Normal range of CD4⁺ and CD8⁺ T-cell counts were established at 500 to 1200 cells/ μ L and 300 to 830 cells/ μ L, respectively according to Reichert et al.^[12] Plasma HIV-RNA was quantified by successive standardized assays including Roche Cobas HIV-1 monitor, Roche Cobas Ampliprep/Cobas Taqman HIV-1 v.2 test, and Abbott RealTime HIV-1. Since the threshold value of HIV-RNA was not the same through the study period depending on the techniques available at each site (<20 copies/mL, <50 copies/mL or <40 copies/mL), the corresponding threshold for each period was considered.

2.3. Covariates

At baseline were collected the following potential confounders: sex, age, duration of HIV exposure since diagnosis, transmission

risk group, CDC stage, cytomegalovirus (Anti-cytomegalovirus IgG positive) and hepatitis C (hepatitis C virus (HCV)-RNA positive) co-infections, CD4 nadir, HIV-pVL at baseline, duration of treatment and delay to HIV suppression on cART.

2.4. Ethic statement

All subjects provided written informed consent for the use of their medical records on NADIS. This electronic medical record was approved by the French *Commission Nationale Informatique et Liberté* (Registration number: 2001/762876/nadiscnil.doc). This study was carried out in compliance with the International guidelines for human research protection as Declaration of Helsinki and International Conference on Harmonization in Good Clinical Practice (ICH-GCP).

2.5. Statistical Analysis

All analyses were performed on SPSS Advanced Statistics 20 (IBM Corp) and SAS 9.4. Patients’ characteristics were described at baseline according to CD8⁺ T-cell counts and cART regimen. Categories of patients according to CD8⁺ T-cell counts (low < 300 cells/ μ L; normal 300–830 cells/ μ L and high > 830 cells/ μ L) were defined after Reichert et al.^[12] Median absolute counts and percentage of CD4⁺, CD8⁺ T-cells and CD4:CD8 ratio as well as their variations (Delta, Δ) were compared between baseline and D0, and then between D0 and M12, D0 and M24, according to cART regimen (2NRTIs+PI/r vs 2NRTIs+1NNRTI vs 3NRTIs). Factors associated with CD8⁺ T-cell count and CD4:CD8 ratio were identified at each time in bivariate analysis using Kruskal–Wallis tests for qualitative variables, and Chi-2 test or simple linear regression for quantitative variables. Evolution of CD8⁺ T-cell count and CD4:CD8 ratio at M12 and M24 by cART regimen were then studied using multiple linear regression models including the potential confounders known. The value at D0 of each outcome tested was forced in the models. Missing data were considered as such and thus, were not included in the analyses, were not replaced or extrapolated.

3. Results

Among the 5688 HIV-infected patients who initiated a first-line triple antiretroviral treatment during the study period, 2074 had cART regimen unchanged for at least 12 months, of whom 830 patients had an undetectable HIV-pVL at all assessments, and 605 patients had data on HIV-pVL, CD4⁺ and CD8⁺ T-cell counts available at the time of cART initiation (Supplementary Figure 1: flowchart, <http://links.lww.com/MD/B358>). Hence, 605 patients composed the study cohort, which had a median time of cART exposure of 32.3 (23.8–43.7) months.

3.1. Patients’ characteristics at baseline according to CD8⁺ T-cell count

At baseline, CD8⁺ T-cell counts were high (> 830 cells/ μ L) in 268 (44.3%) patients and low (< 300 cells/ μ L) in 38 (6%) patients. After cART initiation, they were respectively 47.7% and 3.1% at D0 (viral suppression), 39.8% and 3.7% after 12 months, 43.7% and 4% after 24 months under cART and the difference for each categories was not significant. Table 1 shows the characteristics of patients distributed by ranges of CD8⁺ T-cell count at the time of cART initiation. According to this classification, patients did not differ in age, sex, HIV transmission risk group, HCV

Table 1
Patients' characteristics according to CD8⁺ T-cell count at baseline.

	CD8 (cells/ μ L)			P
	< 300 (n=38)	300–830 (n=299)	> 830 (n=268)	
Males	26 (68.4%)	213 (71.2%)	202 (75.4%)	0.442
Age, y	45.5 (38.5; 54.2)	42.0 (36.0; 51.0)	45.0 (38.0; 51.0)	0.065
Transmission risk group				0.196
Heterosexual	19 (50.0%)	137 (45.8%)	126 (47.0%)	
MSM	11 (28.9%)	128 (42.8%)	103 (38.4%)	
IV drug user	2 (5.3%)	7 (2.3%)	16 (6.0%)	
Others	3 (7.9%)	8 (2.7%)	7 (2.6%)	
Unknown	3 (7.9%)	19 (6.4%)	16 (6.0%)	
Follow-up of HIV infection, y	0.3 (0.1; 3.5)	0.9 (0.1; 3.1)	1.6 (0.3; 5.5)	<0.001
CDC stage C	19 (50.0%)	41 (13.7%)	28 (10.5%)	<0.001
CMV IgG+	27 (79.4%)	223 (89.2%)	194 (88.6%)	0.244
HCV-RNA+	3 (7.9%)	15 (5.1%)	16 (6.1%)	0.629
CD4 nadir, cells/ μ L	30 (12; 103)	210 (121; 269)	239 (177; 303)	<0.001
HIV-pVL, log cp/mL	5.2 (4.4; 5.6)	4.7 (4.3; 5.3)	4.7 (4.2; 5.2)	0.016
CD4 ⁺ T-cells:				
(cells/ μ L)	46 (12; 108)	236 (133; 287)	279 (215; 350)	<0.001
(%)	10.2 (4.0; 20.7)	19.7 (12.7; 24.0)	14.4 (11.0; 18.9)	<0.001
CD8 ⁺ T-cells:				
(cells/ μ L)	217 (135; 277)	616 (477; 722)	1169 (990; 1497)	<0.001
(%)	48.5 (30.7; 57.0)	52.6 (46.0; 62.0)	66.0 (59.8; 73.3)	<0.001
CD4:CD8 ratio	0.20 (0.07; 0.58)	0.38 (0.21; 0.51)	0.22 (0.15; 0.29)	<0.001
CD4:CD8 ratio \geq 1	5 (13%)	7 (2.3%)	2 (0.7%)	0.001

Data are n (%) or median (IQR).

cART = combined antiretroviral therapy, CDC=centers for disease control and prevention (CDC) classification system, CMV=cytomegalovirus, HCV=hepatitis C virus, HIV-pVL=HIV plasma viral load, IQR=inter quartile range, IV=intravenous, MSM=men who have sex with men.

co-infection or prevalence of cytomegalovirus-positive serology. However, patients with CD8⁺ T-cell count < 300 cells/ μ L had significantly higher HIV-pVL, lower CD4⁺ T-cell values and lower CD4 nadir. In contrast, median duration of HIV follow-up was significantly higher in patients with CD8⁺ T-cell count > 830 cells/ μ L. Median CD4:CD8 ratio was significantly higher in patients with CD8⁺ T-cell counts within the normal range, but the fraction of patients with CD4:CD8 ratio \geq 1 was significantly higher in patients with CD8⁺ T-cell counts < 300 cells/ μ L.

3.2. Patients' characteristics at baseline according to cART regimen

Overall, the impact of cART regimen on CD8⁺ T-cell counts was assessed on patients receiving 1 of these 3 cART regimens: 2NRTIs+1PI/r (n= 297), 2NRTIs+1NNRTI (n= 207) and 3NRTIs (n= 68). Characteristics of patients by cART regimens are reported in Table 2 and detailed antiretroviral regimens for each group are described in supplementary Table 1, <http://links.lww.com/MD/B358>. Eleven patients receiving an INSTI-containing cART regimen were analyzed separately. Twenty-two patients receiving other cART combinations were excluded.

At baseline, patients receiving 3NRTIs differed significantly from those receiving 2NRTIs+1PI/r or 2NRTIs+1NNRTI by their longer median duration of HIV infection follow-up, shorter delay to HIV suppression after cART initiation, lower HIV-pVL, higher CD4 nadir and median CD4:CD8 ratio. Median CD4⁺ and CD8⁺ T-cell counts, and CD4:CD8 ratio significantly differed between the 3 groups but the proportion of patients with a CD4:CD8 ratio \geq 1 was similar at baseline. At the time of the first undetectable HIV-pVL (D0), median CD4⁺ T-cell count was significantly higher in the 2NRTIs+1NNRTI group, while median CD8⁺ T-cell count was no different between groups either in absolute value or in percentage. Median CD4:CD8 ratio was

similar between the groups, but the proportion of patients with a CD4:CD8 ratio \geq 1 was significantly lower in the 3NRTIs group.

3.3. Evolution of CD8⁺ T-cell counts according to cART regimen after viral suppression

3.3.1. At M12. Twelve months after the first undetectable HIV-pVL, CD8⁺ T-cell counts were available for 505 patients (2NRTIs +1PI/r group: n= 262; 2NRTIs+1NNRTI group: n= 183; 3NRTIs group: n=60). Median CD8⁺ T-cell counts were similar in all groups after 12 months in absolute value and percentage, as were CD4⁺ T-cell counts (Fig. 1A and B). No significant differences were observed between the groups regarding the distribution of patients by CD8⁺ T-cell count (< 300; 300–830 or > 830 cells/ μ L, Fig. 2A–C), or the median CD4:CD8 ratio (Fig. 1C).

Regarding the variations between D0 and M12, patients receiving 3NRTIs displayed the smaller decrease of absolute CD8⁺ T-cell count (median variation (Δ CD8): –15 [inter quartile range (IQR): –173; 172] vs –43 [–212; 123] cells/ μ L in the 2NRTIs+1PI/r group and –128 [–275; 53] cells/ μ L in the 2NRTIs +1NNRTI group; $P= 0.011$). Median variation of CD4:CD8 ratio (Δ CD4:CD8) was significantly smaller in the 3NRTIs group (0.12 [0.04; 0.26]) compared to 2NRTIs+1NNRTI (0.16 [0.09; 0.31]; $P=0.05$), but this difference was not significant when compared to 2NRTIs+1PI/r (0.14 [0.07; 0.26]).

In multivariate analysis (Table 3), the 3NRTIs regimen was associated with a smaller decrease of CD8⁺ T-cell counts (Δ CD8) compared to 2NRTIs+1NNRTI regimens ($P=0.02$). No such significant difference was found between 3NRTIs and 2NRTIs +1PI/r regimens ($P=0.21$). Regarding Δ CD8%, the 3NRTIs regimen was associated with a smaller decrease of CD8% compared to PI/r- and NNRTI-containing regimens ($P=0.007$ and $P=0.001$, respectively). No significant difference of the variations of CD4:CD8 ratios were observed between the groups at M12 (Fig. 1C).

Table 2**Patients' characteristics by the cART regimen.**

	2NRTIs+1PI/r n=297	2NRTIs+1NNRTI n=207	3NRTIs n=68	P
Male	211 (71.0%)	153 (73.9%)	53 (77.9%)	0.472
Age, y	43 (36; 50)	43 (36; 52)	42 (39; 51)	0.791
Transmission risk group:				
Heterosexual	145 (48.8%)	93 (44.9%)	33 (48.5%)	0.329
Homo/bisexual	115 (38.7%)	86 (41.5%)	23 (33.8%)	
IV drug use	13 (4.4%)	6 (2.9%)	6 (8.8%)	
Other	6 (2.2%)	8 (3.9%)	2 (2.9%)	
Unknown	18 (6.1%)	14 (6.8%)	4 (5.9%)	
CDC stage:				
A	201 (67.7%)	152 (73.4%)	49 (72.1%)	0.549
B	45 (15.2%)	29 (14.0%)	10 (14.7%)	
C	51 (17.2%)	25 (12.1%)	9 (13.2%)	
HCV-RNA +	18 (6.1%)	10 (4.8%)	6 (8.8%)	0.438
Anti-CMV IgG+	223 (75.1%)	150 (72.5%)	54 (79.4%)	0.505
CD4 Nadir (cells/ μ L)	209 (92; 275)	226 (163; 296)	239 (155; 274)	0.012
Duration of HIV follow-up, y	0.8 (0.1; 3.4)	1.1 (0.2; 4.3)	1.9 (0.2; 6.5)	0.014
Duration of cART, mo	31.2 (23.2; 41.0)	32.2 (24.1; 41.1)	48.5 (30.4; 64.1)	<0.001
Delay to HIV suppression, mo	3.2 (2.0; 5.4)	3.7 (2.0; 5.5)	2.0 (1.2; 4.4)	0.001
Pre-cART HIV-pVL (Log cp/mL)	4.9 (4.3; 5.4)	4.8 (4.4; 5.3)	4.4 (4.1; 4.8)	<0.001
Pre-cART CD4 T-cells:				
cells/ μ L	238 (105; 311)	261 (173; 324)	257 (191; 300)	0.015
%	16.0 (8.5; 22.3)	16.5 (12.0; 20.7)	17.0 (13.0; 21.5)	0.430
<200	117 (39.4%)	61 (29.5%)	21 (30.9%)	0.233
200–350	133 (44.8%)	116 (56.0%)	37 (54.4%)	
350–500	38 (12.8%)	26 (12.6%)	8 (11.8%)	
>500	9 (3.0%)	4 (1.9%)	2 (2.9%)	
Pre-cART CD8 ⁺ T-cells:				
cells/ μ L	748 (505; 1070)	856 (590; 1204)	733 (557; 1035)	0.014
%	59.0 (49.1; 70.0)	61.0 (52.0; 68.0)	59.7 (49.9; 67.5)	0.747
<300	23 (7.7%)	8 (3.9%)	4 (5.9%)	0.034
300–830	157 (52.9%)	90 (43.5%)	36 (52.9%)	
>830	117 (39.4%)	109 (52.7%)	28 (41.2%)	
Pre-cART CD4:CD8 ratio	0.26 (0.12; 0.41)	0.28 (0.18; 0.38)	0.32 (0.20; 0.45)	0.027
Pre-cART CD4:CD8 \geq 1	8 (1.9%)	1 (0.5%)	2 (3%)	0.103
DO- CD4 ⁺ T-cells:				
cells/ μ L	354 (193; 484)	376 (285; 484)	338 (236; 412)	0.033
(%)	22.0 (13.0; 29.1)	23.0 (18.0; 28.8)	20.0 (16.3; 25.3)	0.074
DO- CD8 ⁺ T-cells:				
cells/ μ L	826 (561; 1158)	820 (622; 1133)	779 (574; 1005)	0.332
%	52.1 (43.0; 63.0)	52.0 (42.1; 61.0)	53.8 (46.7; 62.2)	0.665
DO-CD4:CD8 ratio	0.40 (0.22; 0.63)	0.44 (0.29; 0.62)	0.44 (0.28; 0.62)	0.147
DO-CD4:CD8 \geq 1	12.7%	8.2%	5.8%	0.05

Data are n (%) or median (IQR). IQR= inter quartile range.

DO correspond to the time of the first undetectable HIV plasma viral load.

cART = combined antiretroviral therapy, CMV = cytomegalovirus, HCV = hepatitis C Virus, HIV-pVL = HIV plasma viral load, IV = intravenous, NNRTI = non-nucleoside reverse transcriptase inhibitors, NRTI = nucleoside reverse transcriptase inhibitors, PI/r = ritonavir-boosted protease inhibitors.

3.3.2. At M24. In total 240 patients achieved 24 months of follow-up without modification of cART regimens, of which 117, 85 and 38 patients received 2NRTIs+1PI/r, 2NRTIs+1NNRTI, and 3NRTIs regimens, respectively.

Median CD4⁺ and CD8⁺ T-cell counts were similar between the groups at M24 (Figs. 1A and Fig. 2B). Patients receiving 3NRTIs had significantly higher CD8% (48.4 [44.1; 58.8] % vs 43.0 [36.6; 52.7] % in 2NRTIs+1PI/r group and 42 [34.5; 50.3] % in the 2NRTIs+1NNRTI group; $P=0.003$). Median CD4:CD8 ratio was significantly lower in patients receiving 3NRTIs ($P=0.028$, Fig. 1C) but the proportion of patients with a CD4:CD8 ratio \geq 1 did not differ significantly between the groups (Fig. 1D).

Median Δ CD8% was significantly smaller in patients receiving 3NRTIs (−2.0 [−8.4; 5.3] %) compared to those receiving 2NRTIs+1PI/r and 2NRTIs+1NNRTI (−8.4 [−16.2; −3.9] % and 10.5 [−16.1; −6] %, respectively; $P<0.001$) and median Δ CD4:CD8 was significantly smaller in patients receiving 3NRTIs (0.1 [0.1; 0.3] %) compared to 2NRTIs+1PI/r and 2NRTIs+1NNRTI (0.3 [0.2; 0.4] % and 0.3 [0.2; 0.5] %; $P<0.01$).

In multivariate analysis (Table 3, bottom), the 3NRTIs regimen was associated with a smaller decrease of CD8% compared to PI/r- and NNRTI-containing regimens ($P=0.001$ and $P<0.0001$, respectively), and to a smaller increase of CD4:CD8 ratio ($P=0.10$ and $P=0.01$, respectively, Table 3).

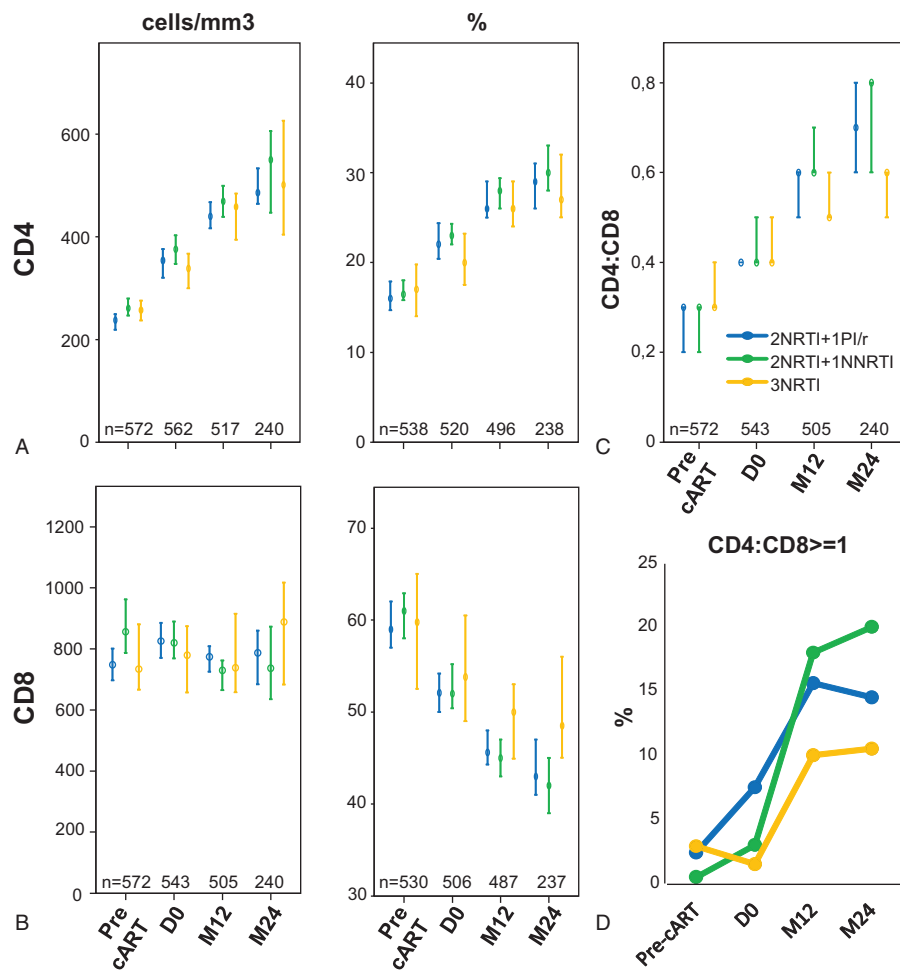


Figure 1. Comparative analysis of the impact of first-line cART regimen on T-cell compartments and CD4:CD8 ratio in HIV-infected patients. Graphs illustrate median [IQR] CD4⁺ (A) and CD8⁺ (B) T-cell counts (cells/ μ L) and percentages (%), and CD4:CD8 ratio (C) prior to cART initiation (Pre-cART), at the first undetectable HIV-pVL (D0) and after 12 (M12) or 24 (M24) months of HIV suppression, in patients receiving 2NRTIs+1PI/r, 2NRTIs+1NNRTI, or 3NRTIs as first-line cART. (D) Graph shows the fraction of patients (%) achieving normalization of CD4:CD8 ratio (≥ 1) according to first-line cART regimens. cART = combined antiretroviral therapy, HIV = human immunodeficiency virus, n=number of observations, INSTI = integrase strand transfer inhibitor, NNRTI = non-nucleotide reverse transcriptase inhibitor, NRTI = nucleotide reverse transcriptase inhibitor, PI/r = Ritonavir-boosted protease inhibitor, pVL = plasma viral load.

3.4. Focus on patients receiving an Integrase Strand Transfer Inhibitor (INSTI)-containing regimen in first-line cART

Eleven patients of the study cohort received an INSTI-containing cART regimen. Most of patients were males (82%) with a median age of 43 [38; 53] year old and median time of HIV infection follow-up of 1.9 [1.2; 7.2] years. INSTI was combined to 2NRTIs for 8 patients and to 2NRTIs+1NNRTI in 3 cases. At baseline, median CD4⁺ and CD8⁺ T-cell counts were 340 [266; 397] cells/ μ L and 646 [520; 1161] cells/ μ L, respectively, and 36.4% of patients had CD8⁺ T-cell count > 830cells/ μ L. Median CD4:CD8 ratio was 0.48[0.27; 0.69] and <1 in 90% of patients at baseline. At D0, median CD4⁺ and CD8⁺ T-cell counts had increased to reach 484 [366; 577] cells/ μ L and 835 [531; 1130] cells/ μ L, respectively. The proportion of patients with a CD8⁺ T-cell count > 830cells/ μ L dropped to 18.2%. Median CD4:CD8 ratio was 0.5 [0.41; 0.78], and CD4:CD8 ratio was < 1 in 82% of cases. All patients had their INSTI-containing first-line cART regimen unchanged after 12 months; they were only 2 patients after 24 months. After 12 months of viral suppression, median CD4⁺ T-cell count was 541[409; 797] cells/ μ L with a median Δ CD4 of 133 [-26; 206] cells/ μ L, whereas median CD8⁺ T-cell

count decreased to 683 [539; 797] cells/ μ L with a median variation of -155 [-302; -22] cells/ μ L. The proportion of patients with CD8⁺ T-cell count > 830cells/ μ L was 20% (Fig. 2D). Conversely, median CD4:CD8 ratio improved to 0.7 [0.63; 1.02] and was ≥ 1 in 36.4% of patients after 12 months of viral suppression.

4. Discussion

This study performed on a large cohort of HIV-1-infected patients achieving sustained HIV suppression for at least 12 months without modification of cART regimen showed that: (i) the proportion of patients presenting with a high CD8⁺ T-cell count remained stable despite a sustained HIV suppression for 24 months; (ii) compared to 2NRTIs+1PI/r and 2NRTIs+1NNRTI regimens, the combination of 3NRTIs was associated with the smaller decrease of CD8⁺ T-cell counts after 24 months of viral suppression.

Our data are consistent with recent observations reporting the persistence of elevated CD8⁺ T-cell counts after 10 years of cART.^[7] In our cohort, 44% of patients presented with high CD8⁺ T-cell counts (> 830cells/ μ L) at the time of cART

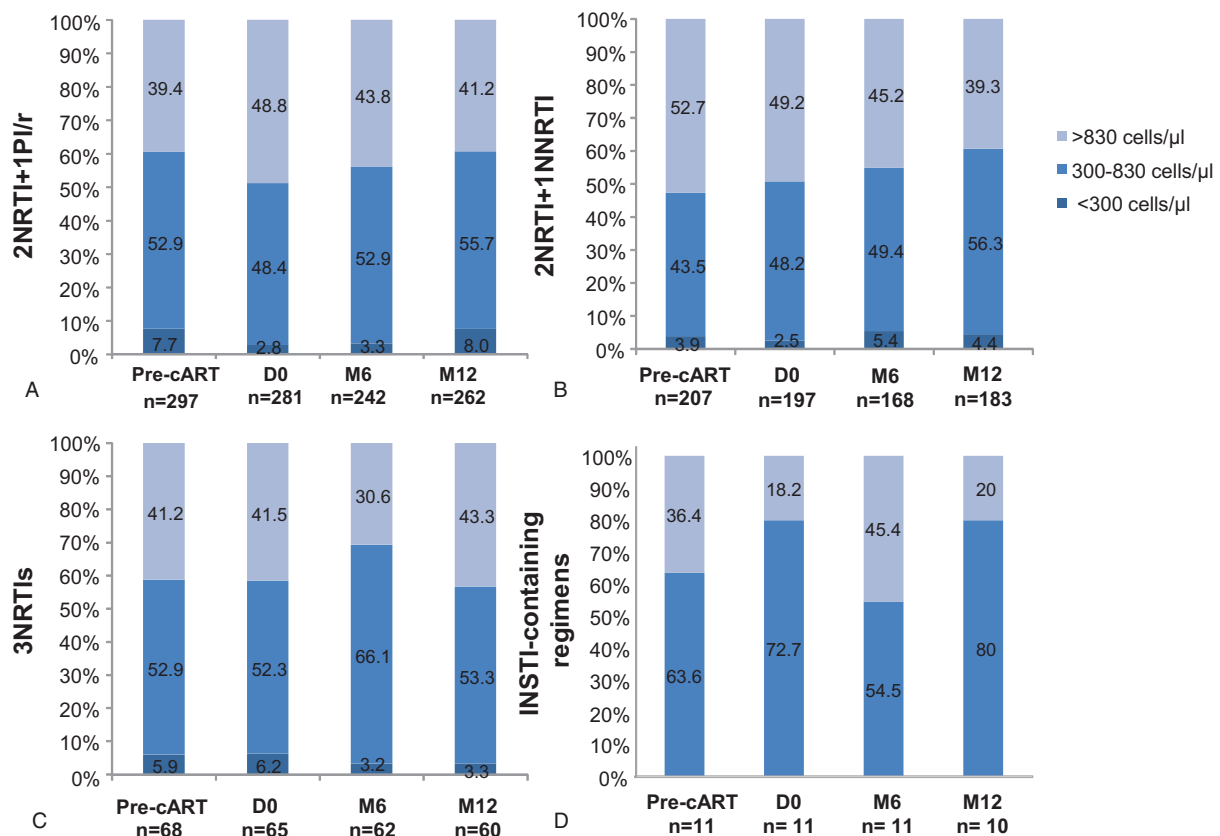


Figure 2. Distribution of patients by ranges of CD8⁺ T-cell counts according to first-line cART regimen. Stacked bars represent the proportions of patients with high (>830 cells/μL), normal (300–830 cells/μL), and low (<300 cells/μL) CD8⁺ T-cell counts among patients receiving 2NRTI+1PI/r (A), 2NRTI+1NNRTI (B), 3NRTIs (C), or in patients receiving an INSTI-containing regimen (D). cART = combined antiretroviral therapy, HIV = human immunodeficiency virus, n = number of observations, INSTI = integrase strand transfer inhibitor, NNRTI = non-nucleotide reverse transcriptase inhibitor, NRTI = nucleotide reverse transcriptase inhibitor.

initiation. These patients had significantly longer HIV follow-up, higher CD4 nadir and higher CD4⁺ T-cell counts, but their HIV-pVL did not differ from patients with normal or low CD8⁺ T-cell counts. Hence, a high HIV-pVL does not seem to be a pivotal factor for CD8 hyperlymphocytosis. The association between the duration of HIV follow-up and CD8⁺ T-cell count was no longer significant after 6 months of HIV suppression regardless of cART regimens (data not shown). However, other factors such viral coinfections may affect the down regulation of the CD8 compartment upon viral suppression. Indeed, we have previously shown that HCV coinfection is associated with high CD8⁺ T-cell counts in long-term HIV suppressors on cART.^[13] Nevertheless, in this cohort the number of HCV coinfecting patients was too small for being a confounder.

The persistence of high CD8⁺ T-cell counts despite a durable control of HIV replication might be explained by a premature immune senescence of CD8⁺ T-cells, as described for naive HIV-infected patients.^[14] Indeed, senescent CD8⁺ T-cells accumulate over time, which is likely to counter the normalization of CD4:CD8 ratio.^[15] Unfortunately, immune senescence could not be evaluated in our study. However, evidences of the association between expanded CD8 immunosenescent and/or activated subpopulations and accelerated progression of HIV disease keep on accumulating.^[16,17,18–21]

Up to date, the impact of cART regimen on immune recovery during clinical trials and observational cohorts was mostly evaluated in terms of CD4⁺ T-cell recovery, while data on CD8⁺

T-cells are scarce. Risk of non-AIDS related events and death was reported to correlate to high CD8⁺ T-cell counts (>1500 cells/μL) in patients achieving sustained undetectable HIV-pVL.^[7] Although CD4:CD8 ratio was shown to be a reliable predictor of the evolution of HIV-disease,^[22–24] its improvement does not always correlate with recovery of the CD4 compartment. Altogether, these data and ours stress the need to consider not only the evolution of CD4:CD8 ratio but also CD8⁺ T-cell count, in absolute value and percentage, for the evaluation of immune restoration under cART.

Interestingly, we did not observe a significant difference of the variation of the CD8 compartment between patients receiving PI/r and NNRTI-containing regimens. The small number of patients receiving an INSTI-containing cART regimen in our cohort did not allow us to compare this group to other. However, after 12 months of viral suppression, this subpopulation of patients receiving INSTI displayed the most important decrease of CD8⁺ T-cell counts observed in this study and, moreover, the highest CD8⁺ T-cell count at baseline (>830 cells/mL), the biggest drop after 12 months of HIV suppression. A recent study performed in 39 naive patients receiving a combination of raltegravir and TDF/FTC showed that CD4 recovery was associated with an early and transient increase of CD8⁺ T-cell count, but was not different from baseline after 12 months of follow-up.^[25]

In our study, 38 patients displayed low CD8⁺ T-cell counts (<300 cells/μL) at cART initiation, which has previously been associated with bad prognosis.^[7] According to Helleberg's study,

Table 3
Evolution at M12 and M24 of CD8 according to the cART regimen. Multivariate linear regression.

	2NRTI+1PI/r N = 250	2NRTI+1NNRTIs N = 177	3NRTIs N = 58
At M12			
Δ CD8-M12-D0 Cells/mm ³ (SE) [†]	-60.9 (17.1)	-105.1 (20.3) [*]	-10.2 (36.1)
% (SE) [‡]	-6.8 (0.5) ^{**}	-7.6 (0.7) ^{**}	-2.7 (1.4)
ΔCD4:CD8ratioM12-D0 (SE) [§]	0.19 (0.01)	0.22 (0.02)	0.15 (0.03)
At M24			
Δ CD8-M24-D0 Cells/mm ³ (SE)	-99.7 (31.5)	-121.0 (36.2)	-15.3 (55.8)
% (SE) [¶]	-9.9 (1.1) ^{***}	-11.1 (1.3) ^{***}	0.2 (2.3)
ΔCD4:CD8 ratio M24-D0 (SE) [#]	0.28 (0.02)	0.33 (0.03) ^{**}	0.20 (0.04)

NNRTI=non-nucleoside reverse transcriptase inhibitors, NRTI=nucleoside reverse transcriptase inhibitors, PI/r=ritonavir-boosted protease inhibitors, SE = standard error.

[†] Model adjusted for confounders: duration of HIV at treatment initiation, CD4 nadir, pre-cART HIV-pVL, CD8 at D0.

[‡] Model adjusted for confounders: duration of HIV at treatment initiation, duration of treatment, delay to HIV suppression, CD8% at D0.

[§] Model adjusted for confounders: CD4 nadir, pre-cART HIV-pVL, CD4/CD8 ratio at D0.

^{||} Model adjusted for confounders: CD4 nadir, duration of HIV at treatment initiation, duration of treatment, CD8 at D0.

[¶] Model adjusted for confounders: duration of treatment, CD8% at D0.

[#] Model adjusted for confounders: CD4 nadir, duration of treatment, delay to HIV suppression, CD4/CD8 ratio at D0.

^{*} P-value <0.05.

^{**} P-value <0.01.

^{***} P-value <0.001; ref = 3NRTIs.

patients that display a CD8⁺ T-cell count < 500 cells/μL a year after cART initiation, are at higher risk of mortality due to AIDS-related conditions. In our study, patients with low CD8⁺ T-cell count had higher HIV-pVL, lower CD4⁺ T-cell values and lower CD4 nadir, which was also observed after 10 years of cART treatment in Helleberg's study. During the study period, the proportion of these patients remained stable, whatever the cART regimens. Unfortunately, this retrospective study did not allow us to evaluate morbidities and mortality rate.

One limitation of this study is that, despite the large number of patients of the Dat'AIDS cohort, the fraction of patients receiving an unchanged first-line cART regimen that could be analyzed after 24 months of viral suppression is rather small. Nevertheless, it should be considered that the rate of cART regimen modification and interruption is particularly high during the first year of cART, as observed in our study and other contemporary studies.^[26] Secondly, due to the period of patient selection, the potential impact of last generation antiretroviral drugs currently recommended for cART initiation, like INSTI, was investigated in a very small number of patients. Finally, regarding the retrospective design of the study, only the potential confounders available in our database could be used in the multiple linear regression models. Thus, we cannot rule out the role of others factors than cART regimen, such as comorbidities, to explain the differences observed between the different drug combinations.

In conclusion, our study showed that the number of CD8⁺ T-cells remains high despite a sustained control of HIV replication on first-line cART, and that cART regimen has an impact on the course of CD8⁺ T-cells. NRTI-only regimens in naive patients may be associated with poor immune restoration, as reflected by a poor down regulation of the CD8 compartment, while patients receiving INSTI-containing regimens displayed encouraging

results regarding CD8 down regulation. Hence, the immune balance leading to CD4:CD8 recovery does not only depend on the time of cART initiation but might also be conditioned by the effect of drug regimen on the evolution of CD8⁺ T-cell count.

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References

- 1] Battegay M, Nuesch R, Hirschel B, et al. Immunological recovery and antiretroviral therapy in HIV-1 infection. *Lancet Infect Dis* 2006; 6:280-7.
- 2] Kaufmann GR, Perrin L, Pantaleo G, et al. CD4 T-lymphocyte recovery in individuals with advanced HIV-1 infection receiving potent antiretroviral therapy for 4 years: the Swiss HIV Cohort Study. *Arch Intern Med* 2003;163:2187-95.
- 3] Moore RD, Keruly JC. CD4+ cell count 6 years after commencement of highly active antiretroviral therapy in persons with sustained virologic suppression. *Clin Infect Dis* 2007;44:441-6.

- [4] Gras L, Kesselring AM, Griffin JT, et al. CD4 cell counts of 800 cells/mm³ or greater after 7 years of highly active antiretroviral therapy are feasible in most patients starting with 350 cells/mm³ or greater. *J Acquir Immune Defic Syndr* 2007;45:183–92.
- [5] Mocroft A, Phillips AN, Gatell J, et al. Normalisation of CD4 counts in patients with HIV-1 infection and maximum virological suppression who are taking combination antiretroviral therapy: an observational cohort study. *Lancet* 2007;370:407–13.
- [6] Le Moing V, Thiebaut R, Chene G, et al. Long-term evolution of CD4 count in patients with a plasma HIV RNA persistently <500 copies/mL during treatment with antiretroviral drugs. *HIV Med* 2007;8:156–63.
- [7] Helleberg M, Kronborg G, Ullum H, et al. Course and clinical significance of CD8+ T-cell counts in a large cohort of HIV-infected individuals. *J Infect Dis* 2015;211:1726–34.
- [8] Mocroft A, Phillips AN, Ledergerber B, et al. Relationship between antiretrovirals used as part of a cART regimen and CD4 cell count increases in patients with suppressed viremia. *AIDS* 2006;20:1141–50.
- [9] Wolbers M, Battegay M, Hirschel B, et al. CD4+ T-cell count increase in HIV-1-infected patients with suppressed viral load within 1 year after start of antiretroviral therapy. *Antivir Ther* 2007;12:889–97.
- [10] Pugliese P, Cuzin L, Cabie A, et al. A large French prospective cohort of HIV-infected patients: the Nadis Cohort. *HIV Med* 2009;10:504–11.
- [11] Pugliese P, Cuzin L, Enel P, et al. [NADIS 2000, development of an electronic medical record for patients infected by HIV, HBV and HCV]. *Presse Med* 2003;32:299–303.
- [12] Reichert T, DeBruyere M, Deneys V, et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopathol* 1991;60:190–208.
- [13] Zaegel-Faucher O, Bregigeon S, Cano CE, et al. Impact of hepatitis C virus coinfection on T-cell dynamics in long-term HIV-suppressors under combined antiretroviral therapy. *AIDS* 2015;29:1505–10.
- [14] Tassiopoulos K, Landay A, Collier AC, et al. CD28-negative CD4+ and CD8+ T cells in antiretroviral therapy-naïve HIV-infected adults enrolled in adult clinical trials group studies. *J Infect Dis* 2012;205:1730–8.
- [15] Effros RB, Dagarag M, Spaulding C, et al. The role of CD8+ T-cell replicative senescence in human aging. *Immunol Rev* 2005;205:147–57.
- [16] Giorgi JV, Detels R. T-cell subset alterations in HIV-infected homosexual men: NIAID Multicenter AIDS cohort study. *Clin Immunol Immunopathol* 1989;52:10–8.
- [17] Sousa AE, Carneiro J, Meier-Schellersheim M, et al. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol* 2002;169:3400–6.
- [18] Deeks SG, Kitchen CM, Liu L, et al. Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load. *Blood* 2004;104:942–7.
- [19] Liu Z, Cumberland WG, Hultin LE, et al. CD8+ T-lymphocyte activation in HIV-1 disease reflects an aspect of pathogenesis distinct from viral burden and immunodeficiency. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;18:332–40.
- [20] Effros RB, Allsopp R, Chiu CP, et al. Shortened telomeres in the expanded CD28-CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. *AIDS* 1996;10:F17–22.
- [21] Unemori P, Leslie KS, Hunt PW, et al. Immunosenescence is associated with presence of Kaposi's sarcoma in antiretroviral treated HIV infection. *AIDS* 2013;27:1735–42.
- [22] Margolick JB, Gange SJ, Detels R, et al. Impact of inversion of the CD4/CD8 ratio on the natural history of HIV-1 infection. *J Acquir Immune Defic Syndr* 2006;42:620–6.
- [23] Buggert M, Frederiksen J, Noyan K, et al. Multiparametric bioinformatics distinguish the CD4/CD8 ratio as a suitable laboratory predictor of combined T cell pathogenesis in HIV infection. *J Immunol* 2014;192:2099–108.
- [24] Sainz T, Serrano-Villar S, Diaz L, et al. The CD4/CD8 ratio as a marker T-cell activation, senescence and activation/exhaustion in treated HIV-infected children and young adults. *AIDS* 2013;27:1513–6.
- [25] Funderburg NT, Andrade A, Chan ES, et al. Dynamics of immune reconstitution and activation markers in HIV+ treatment-naïve patients treated with raltegravir, tenofovir disoproxil fumarate and emtricitabine. *PLoS One* 2013;8:e83514.
- [26] Abgrall S, Ingle SM, May MT, et al. Durability of first ART regimen and risk factors for modification, interruption or death in HIV-positive patients starting ART in Europe and North America 2002–2009. *AIDS* 2013;27:803–13.