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ORIGINAL RESEARCH

Reference curves for CD4 T-cell count response to combination antiretroviral therapy in HIV-1-infected treatment-naïve patients

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*See Appendix for a list of the Standard Reference Distribution of CD4 Response to HAART Project Team for the Collaboration of Observational HIV Epidemiological Research in Europe (COHERE) in EuroCoord, who are the authors of this paper, and their affiliations.

Objectives
The aim of this work was to provide a reference for the CD4 T-cell count response in the early months after the initiation of combination antiretroviral therapy (cART) in HIV-1-infected patients.

Methods
All patients in the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) cohort who were aged ≥ 18 years and started cART for the first time between 1 January 2005 and 1 January 2010 and who had at least one available measurement of CD4 count and a viral load ≤ 50 HIV-1 RNA copies/mL at 6 months (± 3 months) after cART initiation were included in the study. Unadjusted and adjusted references curves and predictions were obtained using quantile regressions.

Results
A total of 28 992 patients were included in the study. The median CD4 T-cell count at treatment initiation was 249 [interquartile range (IQR) 150, 336] cells/μL. The median observed CD4 counts at 6, 9 and 12 months were 382 (IQR 256, 515), 402 (IQR 274, 543) and 420 (IQR 293, 565) cells/μL. The two main factors explaining the variation of CD4 count at 6 months were AIDS stage and CD4 count at cART initiation. A CD4 count increase of ≥ 100 cells/mL is generally required in order that patients stay 'on track' (i.e. with a CD4 count at the same percentile as when they started), with slightly higher gains required for those starting with CD4 counts in the higher percentiles. Individual predictions adjusted for factors influencing CD4 count were more precise.

Conclusions
Reference curves aid the evaluation of the immune response early after antiretroviral therapy initiation that leads to viral control.

Keywords: antiretroviral treatment monitoring, CD4 response, HIV monitoring, longitudinal data.

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Introduction

The improvement of antiretroviral treatments since the mid-1990s has led to a large decrease in the incidence of severe morbidities [1] in those living with HIV, with a substantial increase in life expectancy as a result [2]. For an individual starting combination antiretroviral therapy (cART) for the first time, the current goal of treatment is to ensure that the individual's viral load reaches undetectable levels as soon as possible after initiation of cART with his/her CD4 T-cell count increasing soon thereafter [3]. Hence, a good virological response to cART is usually defined as an undetectable viral load within the first 6 months after cART initiation. A good immunological response, however, is less clearly defined. CD4 counts in the HIV-negative population usually lie within the range of 500–1500 cells/μL [4]. Among those with HIV infection, a higher CD4 T-cell count is associated with a lower risk of clinical progression [5,6]; in particular, the longer an HIV-positive individual is able to maintain a CD4 count > 500 cells/μL, the closer is his/her life expectancy to that of the general population [7]. The risk of clinical progression appears to be more strongly associated with the absolute CD4 count at a given time rather than the rate of increase in the CD4 count [8,9].

Despite the difficulty of defining a good immune responder, it is important that clinicians are provided with a reference so that they are able to evaluate an individual's CD4 T-cell response in the early months after starting cART. This will allow them to make decisions about intervening in the management of the patient; for instance, decisions about the appropriate frequency of clinical monitoring. Our aim was to provide 'reference curves' for CD4 T-cell responses during the first 12 months of cART for patients with virological suppression, according to the characteristics of the patients at cART initiation. It is hoped that these curves will allow clinicians to determine how an individual's CD4 count response compares to those of other patients who started the same type of cART under the same conditions.

Methods

Patients

The Collaboration of Observational HIV Epidemiological Research Europe (COHERE) is a collaboration involving 39 cohorts from across Europe and is part of the EuroCoord network (www.EuroCoord.net). COHERE was

established in 2005 with the aim of conducting epidemiological research on prognosis and outcome in HIV-positive persons, which the individual contributing cohort studies cannot address themselves because of small sample sizes or heterogeneity of specific subgroups of HIV-positive persons. Local ethical committee and/or other regulatory approval was obtained as applicable according to local and/or national regulations in all participating cohorts. Each cohort study group submits data using the standardized HIV Collaboration Data Exchange Protocol (HICDEP) [10], including information on patient demographics, use of cART, CD4 counts, AIDS and deaths. Further details can be found at http://www.eurocoord.net/partners/founding_networks/cohere.aspx. Data were pooled in September 2011 within COHERE in EuroCoord (www.cohere.org and www.EuroCoord.net). Data on 27 cohorts across 35 European countries were provided for the present analysis. All persons aged ≥ 18 years who started cART for the first time between 1 January 2005 and 1 January 2010 and who had at least one available measurement of CD4 count (in cells/μL) and a viral load ≤ 50 HIV-1 RNA copies/mL 6 months (± 3 months) after cART initiation were included in the study. Patients were considered to have maintained viral suppression at month 6 when at least one HIV-1 RNA measurement was available within 6 months after cART initiation (including the measurement at month 6) and the HIV-1 RNA measurement at month 6 was < 50 copies/mL (previous measurements could be > 50 copies/mL). cART was defined as any regimen that contained at least three drugs, including a protease inhibitor (PI), a nonnucleoside reverse transcriptase inhibitor (NNRTI), an entry inhibitor or an integrase inhibitor, or that contained three nucleoside reverse transcriptase inhibitors (NRTIs), of which one was abacavir. Baseline was defined as the date of cART initiation. The follow-up was censored first at 9 months (i.e. 6 + 3 months) and then at 15 months (i.e. 12 + 3 months) because we were interested in the short-term response of CD4 count.

Table 1 Baseline characteristics

Characteristic	Analysed population (n = 28 992)
Gender, male [n (%)]	21 130 (72.9)
Age at cART initiation (years) [median (IQR)]	39 (33, 46)
Duration of follow-up after initiating cART (years) [median (IQR)]	2.3 (1.3, 3.5)
HIV transmission risk group [n (%)]	
Heterosexual	11 601 (40.0)
Men who have sex with men	12 547 (43.3)
Injecting drug use	1952 (6.7)
Other/unknown	2892 (10.0)
CDC stage C [n (%)]	
No	21 626 (74.6)
Yes	6144 (21.2)
Unknown	1222 (4.2)
HCV positive [n (%)]	2250 (7.8)
HIV-1 RNA [n (%)]	
< 50 copies/mL	2738 (9.4)
50–9999 copies/mL	4346 (15.0)
10 000–99 999 copies/mL	9730 (33.6)
≥ 100 000 copies/mL	8731 (30.1)
Unknown	3447 (11.9)
cART initiation year [n (%)]	
2005	5342 (18.4)
2006	5864 (20.2)
2007	6266 (21.6)
2008	6958 (24.0)
2009	4562 (15.7)
Number of CD4 measurements within 9 months [median (IQR)]	3 (2, 4)
CD4 count prior to cART initiation (cells/μL) [median (IQR)]	249 (150, 336)
0–199 cells/μL [n (%)]	9647 (35.6)
200–349 cells/μL [n (%)]	11 395 (42.1)
350–499 cells/μL [n (%)]	3769 (13.9)
≥ 500 cells/μL [n (%)]	2286 (8.4)
Time from baseline to 6-month HIV-1 RNA measurement (months) [median (IQR)]	5.9 (5.1, 6.7)
Duration of first-line regimen (months) [median (IQR)]	13.5 (5.5, 26.5)
First-line cART regimen [n (%)]	
PI/r-based	12 706 (43.8)
NNRTI-based	14 665 (50.6)
PI- and NNRTI-based	328 (1.1)
Unboosted PI-based	607 (2.1)
Other*	686 (2.4)

cART, combination antiretroviral therapy; IQR, interquartile range; CDC, Centers for Disease Control and Prevention; HCV, hepatitis C virus; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor.
*Regimens containing abacavir (n = 496), integrase inhibitors (n = 151), fusion inhibitors (n = 24) and other combinations (n = 15).

Strategy for statistical analyses

Any estimate of the effect of a given factor on CD4 T-cell dynamics is likely to be significant in this large study because of the statistical power conferred by the size of the data set. Therefore, factors used for stratified analyses or included in the regression model were defined *a priori*. Baseline factors known to be associated with CD4 T-cell change after cART initiation selected for the analyses were: CD4 T-cell count, AIDS clinical stage, age, HIV transmission group [especially injecting drug use (IDU)], sex, presence *vs.* absence of hepatitis C virus (HCV) coinfection, HIV-1 RNA level, year of cART initiation and type of cART. cART regimen was classified as: ritonavir-boosted PI-based, NNRTI-based, both PI- and NNRTI-based, unboosted PI-based, or another regimen that did not include either a PI or an NNRTI. Integrase or fusion inhibitor-based regimens were too infrequently used to constitute specific subgroups and were included in the previously described categories. The distribution of CD4 T-cell count at 6 (12) months was then described by using the closest measurement between 3 (9) and 9 (15) months.

Quantile regression

Quantile regression was performed on repeated measurements of CD4 count available for each individual until 9 months and then until 15 months. Quantiles of specific interest were the 5, 10, 25, 50th (i.e. median), 75, 90 and 95th percentiles. The models were adjusted for time with squared and cubic effects to allow enough flexibility to fit a nonlinear evolution of CD4 count over time. A set of models (one for each percentile of interest) was fitted without including any other covariates in the models after stratifying according to the baseline CD4 count (0−199, 200−349, 350−499 and ≥ 500 cells/μL). These models were used to draw reference curves for the overall distribution of CD4 T-cell responses among individuals in the study. A second set of models was then fitted after additionally including adjustment for the covariates listed above; this set of models can be used to make predictions for individual responses where individual characteristics are taken into account. The models presented were fitted using PROC QUANTREG in SAS v9. 3 (SAS Institute, Cary, NC, USA). Robustness analyses with median regression including a random intercept to take into account correlation of repeated measurements in patients yielded similar results [11], as did a nonparametric approach [12].

References curves can be generated for each individual using a web tool developed with SHINY, a web application framework for R (http://shiny.rstudio.com/).

Results

Study population

A total of 28 992 patients were included in the study. A description of the study population is provided in Table 1 (Fig. 1). Compared with included individuals (Table S1), those who were excluded were younger (median 38 *vs.* 39 years for included individuals), were less frequently male (69 *vs.* 73%, respectively), were less frequently men who have sex with men (MSM) (35 *vs.* 43%, respectively), and had more frequently started a PI/r-based cART regimen (51 *vs.* 44%, respectively) ($P < 10^{-4}$ for all comparisons). The difference in baseline CD4 count at cART initiation was marginal (Wilcoxon rank sum test $P = 0.066$). The median number of CD4 T-cell

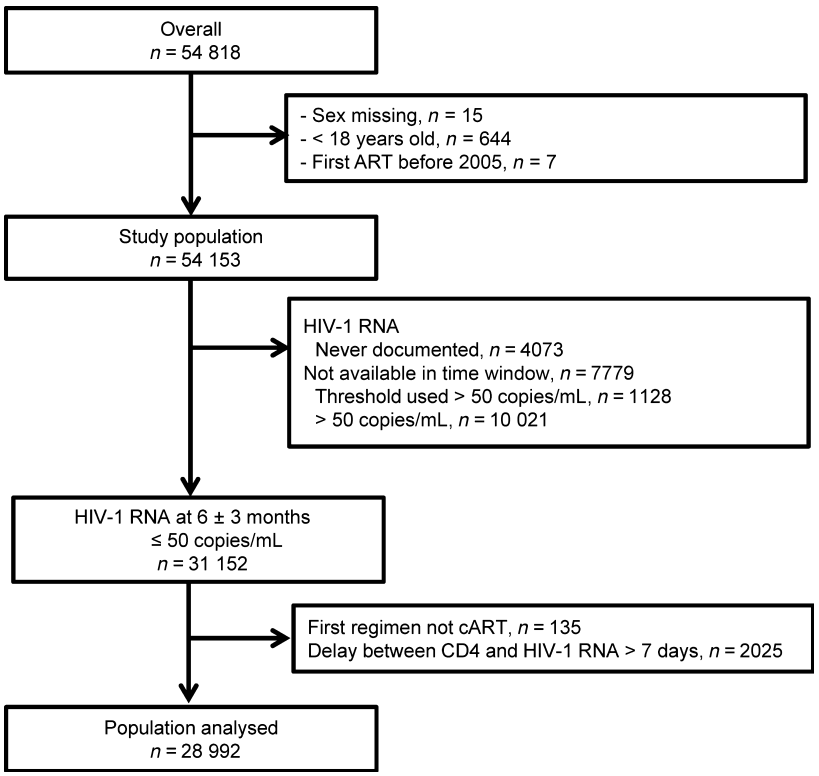

Fig. 1 Flow chart of patient selection. ART, antiretroviral therapy; cART, combination antiretroviral therapy.

measurements available per patient was 3 [interquartile range (IQR) 2, 4].

Global overview of the quantiles of CD4 T-cell changes

The median (IQR) observed CD4 count at treatment initiation was 249 (150, 336) cells/μL (Figs 2 and 3). The median (IQR) observed CD4 counts at 6, 9 and 12 months were 382 (256, 515), 402 (274, 543) and 420 (293, 565), respectively. Box plots of the CD4 T-cell response at month 6 according to various baseline characteristics are presented in Fig. 2. The two main factors explaining the variation of CD4 count at 6 months were AIDS stage at cART initiation and, as expected, the baseline CD4 count. A similar picture was seen at 12 months (Fig. S1).

Figure 3 shows the predicted percentiles of CD4 count after cART initiation for the whole population. The figure shows that a CD4 count increase of at least 100 cells/mL is generally required in order that patients stay 'on track' (i.e. at the same percentile as when they started), with slightly higher gains required to stay on track for those starting with CD4 counts in the higher percentiles (Table S2). For example, the median line demonstrates that patients who started cART at the median level of 251 cells/μL needed to have a CD4 count of 367 cells/μL at 6 months after cART initiation to remain on the median line. The gain required to stay on track was somewhat larger for those initiating cART at higher percentiles of the CD4 T-cell distribution. For instance, a patient starting cART with a CD4 count of 500 cells/μL (roughly equating to the 90th percentile) would need to have attained a CD4 count of 650 cells/μL by 6 months in order to remain on the same percentile line.

As examples, the trajectories of two specific patients are depicted in Fig. 3. The first one (purple line) represents an individual who was female and 30 years old who started cART (lopinavir/r + zidovudine/lamivudine) with a CD4 count of 390 cells/μL, representing the 82th percentile of baseline values. Her CD4 count had increased to only 438 cells/μL over the first 7.9 months. This increase was modest, but was more obviously seen to be a poor response when looked at relative to the population as a whole, representing a drop from the 82th percentile to the 61th percentile. In the second example (green line), cART (efavirenz + rilpivirine + tenofovir/emtricabine) was started at 112 cells/μL (the 18th percentile); by 7.3 months, this individual's CD4 count had

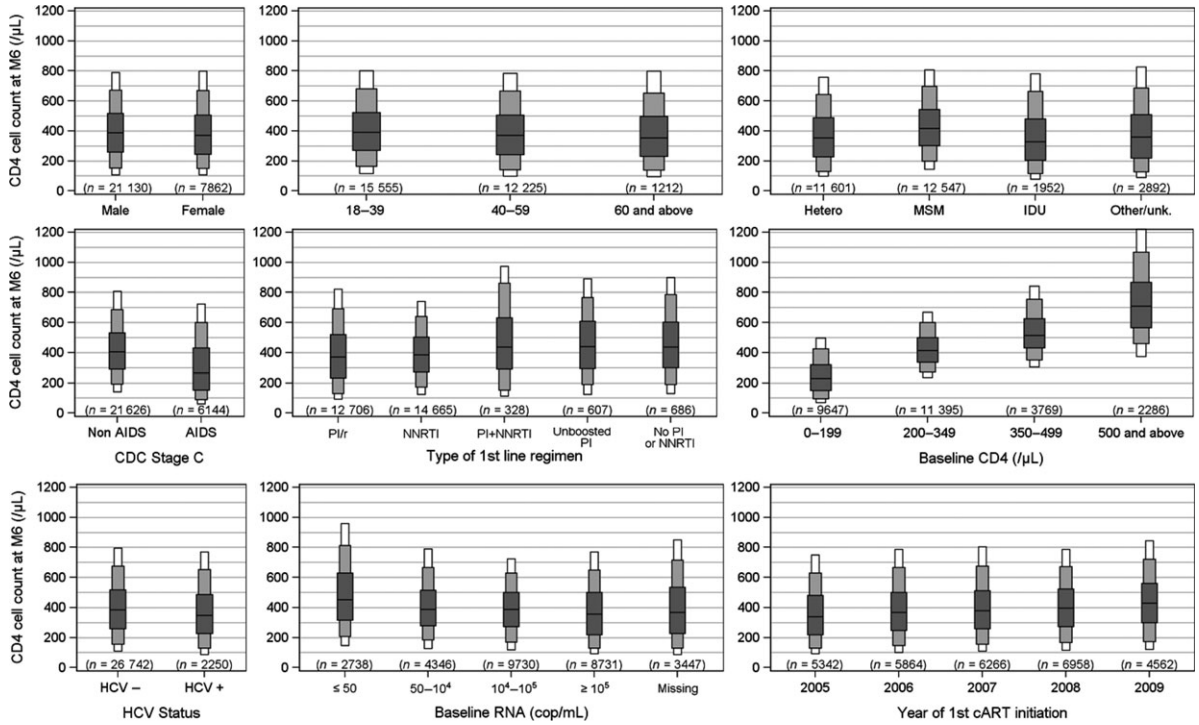

Fig. 2 Box plot of CD4 count at month 6 according to various baseline characteristics. Boxplots show, respectively, the 5, 10, 25, 50, 75, 90 and 95th percentiles. M6, month 6. cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; HCV, hepatitis C virus; IDU, injecting drug use; MSM, men who have sex with men; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; r, ritonavir.

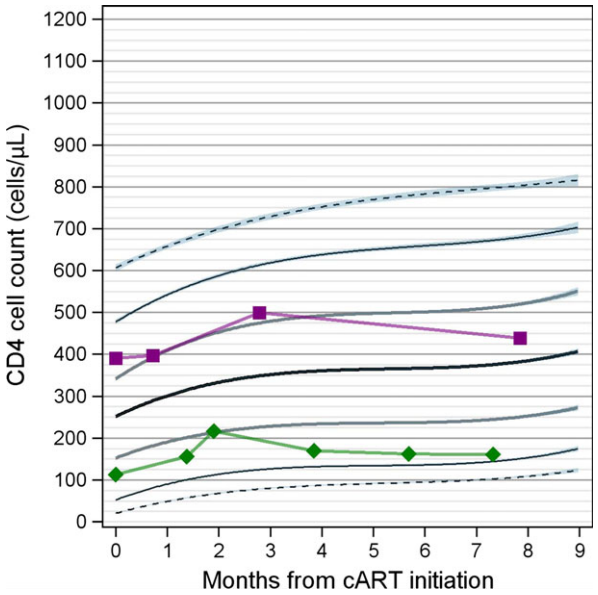

Fig. 3 Percentiles (5, 10, 25, 50, 75, 90 and 95th) of CD4 count over time from combination antiretroviral therapy (cART) initiation. Two individual trajectories are added for illustration: one (purple line) drops from the 82th percentile to the 61th percentile, and the other (green line) starts at the 18th percentile and has dropped to the 12th percentile at 7 months. cART, combination antiretroviral therapy.

reached 161 cells/μL, representing the 12th percentile, which could be considered a bad response. This figure illustrates how this type of representation could help clinicians when evaluating the CD4 count change after cART initiation.

Factors associated with CD4 count

Factors associated with CD4 count were evaluated for each percentile of interest using quantile regression (Table 2). For instance, the 25th percentile for women was 14 cells/μL higher than that for men and this difference was consistent over the other percentiles (14 and 11 cells/μL for the 50 and 75th percentiles, respectively). However, the impact of some baseline characteristics varied according to the percentile. AIDS clinical stage had the greatest impact for individuals with CD4 counts around the 25th (-111 cells/μL) and 50th (-115 cells/μL) percentiles, whereas the type of cART regimen had the greatest impact for those with counts in the highest ($\geq$ 75th) percentiles. For instance, those starting a regimen including both a PI and an NNRTI had CD4 counts 103 cells/μL higher at 6 months than patients who started a regimen including only boosted PI in addition to NRTIs.

Our predictions were then further refined using the measured baseline characteristics in a multivariable quantile regression. Examples are provided in Fig. 4 for the two patients whose data are presented in Fig. 3. The first patient, who started with a CD4 count of 390 cells/μL at the 82th percentile (solid purple line, left part), is classified as starting at the 44th percentile after adjustment for sex, age, transmission risk group, clinical stage, HIV RNA and cART regimen at baseline (dotted purple line, left part). She had attained a CD4 count of 438 cells/μL at 7.9 months after cART initiation, which corresponds to the 61th percentile in the crude analysis (solid purple line, right part). However, taking the individual's characteristics into account, the patient's attained CD4 count is found to lie on only the 22th percentile after adjustment (dotted purple line, right part). Therefore, this increase of only 48 cells/μL means that, in relative terms, the patient has switched from the 44th percentile to the 22th percentile; this is clearly a poor response. The second patient (solid and dotted green lines) presented with a not so bad response, as he started at the 62th adjusted percentile with a CD4 count of 112 cells/μL and dropped to the 30th adjusted percentile with a CD4 count of 161 cells/μL at 7 months, as compared to the crude percentiles which were 18th at baseline and 12th at 7 months.

Discussion

The CD4 T-cell reference curves presented here provide an indication of how the immunological response of an individual patient may compare to that of a large sample of HIV-infected patients who have achieved viral control with potent antiretroviral therapy. These reference curves can easily be drawn using the online tool we developed, available at http://shiny.isped.u-bordeaux.fr/CD4refcurves/.

The definition of immune nonresponse is highly variable in the literature [13–16]. One obvious explanation for the variability is that the latest absolute CD4 count is more closely associated with clinical prognosis than the slope of the CD4 T-cell increase [9]. Hence, there is no obvious threshold for a *good* immune response other than having the highest possible CD4 count. This is why we propose that individual trajectories are referred to the distribution of the whole population, rather than considering whether the absolute count is above or below an arbitrary threshold value.

Using our reference curve, an immune nonresponder may be defined as a person who, despite cART, was not able to maintain an immune response that allowed her/him to remain on the same percentile curve as at the start of cART. In the figures, we provide quartiles and extreme percentiles. Although there is no evidence for an additional

Table 2 Factors associated with the CD4 count according to percentile (25, 50 and 75th percentiles): multivariate quantile regressions of CD4 count

	25th percentile		50th percentile		75th percentile	
	Beta (95% CI)	*P*	Beta (95% CI)	*P*	Beta (95% CI)	*P*
Female (reference: male)	13 (11, 16)	$< 10^{-4}$	13 (11, 16)	$< 10^{-4}$	10 (6, 13)	$< 10^{-4}$
Age (per 10-year increase)	−8 (−9, −7)		−9 (−10, −8)		−9 (−10, −8)	$< 10^{-4}$
HIV transmission risk group (reference: MSM)						
Heterosexual	−59 (−61, −57)	$< 10^{-4}$	−60 (−62, −57)	$< 10^{-4}$	−62 (−65, −58)	$< 10^{-4}$
Injecting drug use	−67 (−72, −62)		−66 (−71, −61)		−55 (−62, −48)	
Other/unknown	−64 (−67, −60)		−61 (−65, −57)		−54 (−60, −49)	
CDC stage C (reference: A/B)						
Yes	−111 (−113, −109)	$< 10^{-4}$	−115 (−117, −112)	$< 10^{-4}$	−89 (−93, −86)	$< 10^{-4}$
Unknown	1 (−4, 6)		9 (4, 14)		11 (5, 18)	
HCV coinfection (reference: no)	−6 (−10, −3)	7.10^{-4}	−6 (−10, −3)	4.10^{-4}	−12 (−17, −7)	$< 10^{-4}$
HIV-1 RNA (reference: < 50 copies/mL)						
50–9999 copies/mL	−32 (−36, −28)	$< 10^{-4}$	−62 (−67, −58)	$< 10^{-4}$	−105 (−111, −98)	$< 10^{-4}$
10 000–99 999 copies/mL	−42 (−46, −38)		−72 (−76, −68)		−122 (−128, −116)	
≥ 100 000 copies/mL	−69 (−72, −65)		−91 (−96, −87)		−126 (−132, −120)	
Unknown	−62 (−67, −58)		−79 (−84, −74)		−100 (−107, −92)	
First-line cART regimen (reference: PI/r-based)						
NNRTI-based	8 (6, 10)	$< 10^{-4}$	−2 (−5, −0)	$< 10^{-4}$	−25 (−28, −22)	$< 10^{-4}$
PI- and NNRTI-based	40 (31, 50)		57 (47, 67)		103 (85, 121)	
Unboosted PI-based	32 (25, 40)		48 (39, 56)		56 (46, 66)	
Other	52 (45, 59)		51 (43, 59)		49 (39, 58)	
Year of cART initiation (reference: 2009)						
2005	−48 (−51, −45)	$< 10^{-4}$	−65 (−68, −62)	$< 10^{-4}$	−70 (−75, −66)	$< 10^{-4}$
2006	−36 (−39, −32)		−44 (−47, −41)		−45 (−50, −40)	
2007	−26 (−29, −23)		−38 (−41, −35)		−40 (−45, −36)	
2008	−21 (−25, −18)		−29 (−32, −25)		−31 (−36, −27)	

cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; CI, confidence interval; HCV, hepatitis C virus; PI, protease inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; MSM, men who have sex with men.

value of immune interventions [17] or changing cART regimens in such a situation, research on boosting the immune response is ongoing [18]. In addition, as long as the observed increase for a given patient means that s/he remains on the same percentile and provided that viral replication is controlled, there may be an argument for scheduling fewer visits in the subsequent months. With the availability of more potent antiretroviral drugs with fewer side effects, and a possible overall benefit of starting antiretroviral therapy as early as possible [19], patients will tend to start cART at higher CD4 cell counts. Thus, a patient starting at 200 cells/μL could be at the 30th percentile in 2004 but at the 20th percentile in 2014, whereas attainment of a count of 300 cells/μL at 6 months may place him/her on the 30th percentile in 2004 but only the 20th in 2014. Therefore, these reference curves need to be regularly updated to remain relevant.

We restricted the analysis to the first 9 months after cART initiation to retain homogeneity among the treatment regimens that were being used (physicians are unlikely to change regimens in the first few months unless there is toxicity) [20]. Furthermore, the increase in CD4 count over the first few months is more pronounced than at later times, partly as a result of the redistribution of T cells [21]. However, it is likely that adherence is at its highest in the first 9 months, and thus our reference curves are likely to identify those with suboptimal CD4 responses despite relatively good adherence to therapy. The extension of our findings to longer durations of cART exposure is under consideration. The results presented in this work were obtained from statistical analyses that did not account for the correlation of repeated measures performed for each patient. Although the method proposed by Geraci and Bottai [11] (LQMM R package) based on random effects yielded similar results for the median, we found inconsistent results for the other percentiles. However, we assume that the use of a correlation matrix would have a very slight impact on our estimations, considering the high number of patients compared with the limited number of observations for each patient.

Despite these limitations, this work has demonstrated the advantages of having a large sample size with a good representation of the HIV-infected population; this may be difficult to achieve in a single study/cohort. For instance, CD4 T-cell dynamics depend on the baseline level. In those starting cART with low CD4 counts, full immune restoration might be expected to be slow because of the profound immune suppression that has led to

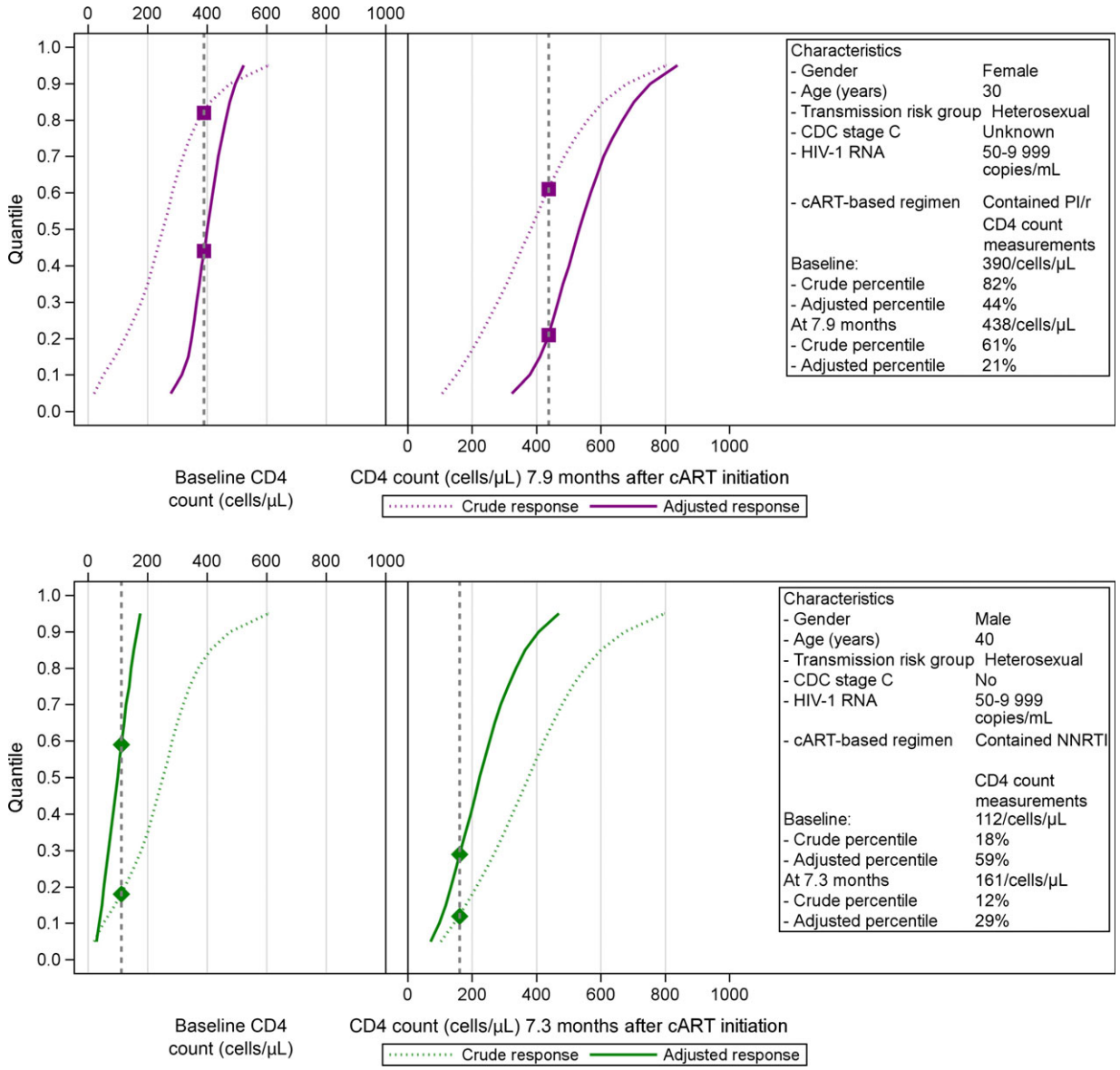

Fig. 4 Example of individual CD4 cell count responses (crude and adjusted) for two patients, data for whom are also presented in Fig. 3. Crude (dotted lines) and adjusted (solid lines) percentiles at baseline (left) and during follow-up (right) are provided according to the measured CD4 count (dashed line). cART, combination antiretroviral therapy; CDC, Centers for Disease Control and Prevention; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; r, ritonavir.

tissue damage in the thymus and lymph nodes [22,23]. However, immune responses are also regulated by homeostatic mechanisms that might result in a ceiling effect, such that those initiating cART with higher CD4 counts might experience less pronounced responses than those starting with lower counts [24]. Hence, looking at the literature, one can find studies where the CD4 T-cell increase was more pronounced in those with higher baseline values [9,25–28], whereas in other studies the CD4 T-cell increase was larger in those starting cART with lower CD4 T-cell values [29–32]. These differences could be attributable to the differences between study populations and thus indicate a need for large data sets supported by collaborations such as COHERE to derive reference curves.

In conclusion, we propose reference curves for the CD4 count that may be used as an additional tool for the clinician when evaluating responses to cART. A web tool is available at http://shiny.isped.u-bordeaux.fr/CD4refcurves/.

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Appendix

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Percentiles (5th, 10th, 25th, 50th, 75th, 90th, 95th) of CD4+ T cell count over time from cART initiation until 15 months

Table S1. Included vs non-included patients' baseline characteristics

Table S2. CD4 change at 6 months to maintain or accelerate the CD4+ T cell response according