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# Predictors for CD4<sup>+</sup> T-cell count increase for treatment-naïve patients with sustained viral load suppression within 1 year after start of combination antiretroviral therapy

[AU: Are author names ok?]

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**Background:** CD4<sup>+</sup> T-cell recovery in patients with continuous suppression of plasma HIV-1 viral load (VL) is highly variable. This study aimed to identify predictive factors for long-term CD4<sup>+</sup> T-cell increase in treatment-naïve patients starting combination antiretroviral therapy (CART).

**Methods:** Treatment-naïve patients in the Swiss HIV Cohort Study reaching two VL measurements <50 copies/ml >3 months apart during the 1st year of CART were included (*n*=1,816 patients). We studied CD4<sup>+</sup> T-cell dynamics until the end of suppression or up to 5 years, subdivided into three periods: 1st year, years 2–3 and years 4–5 of suppression. Multiple median regression adjusted for repeated CD4<sup>+</sup> T-cell measurements was used to study the dependence of CD4<sup>+</sup> T-cell slopes on clinical covariates and drug classes.

**Results:** Median CD4<sup>+</sup> T-cell increases following VL suppression were 87, 52 and 19 cells/μl per year in the three periods. In the multiple regression model, median CD4<sup>+</sup> T-cell increases over all three periods were significantly higher for female gender, lower age, higher VL at CART start, CD4<sup>+</sup> T-cell <650 cells/μl at start of the period and low CD4<sup>+</sup> T-cell increase in the previous period. Patients on tenofovir showed significantly lower CD4<sup>+</sup> T-cell increases compared with stavudine.

**Conclusions:** In our observational study, long-term CD4<sup>+</sup> T-cell increase in drug-naïve patients with suppressed VL was higher in regimens without tenofovir. The clinical relevance of these findings must be confirmed in, ideally, clinical trials or large, collaborative cohort projects but could influence treatment of older patients and those starting CART at low CD4<sup>+</sup> T-cell levels.

## Introduction

The ultimate goal of combination antiretroviral therapy (CART) is the reconstitution of the immune system through optimal viral suppression and the achievement of a high CD4<sup>+</sup> T-cell count that optimally protects against opportunistic infections and HIV-related malignancies. In recent clinical trials between 70 and 85% of treatment-naïve patients initiating modern CART can now achieve a plasma HIV-1 viral load (VL) <50 copies/ml at 48 weeks or after even longer periods of follow up [1,2]. With these remarkable advances of

CART the determinants of optimal immune recovery, once viral suppression is reached, are becoming an important question for the management of a majority of HIV-infected patients starting CART.

In patients achieving sustained VL suppression, CD4<sup>+</sup> T-cell counts increase for at least 3–5 years after initiation of CART. The initial increase of CD4<sup>+</sup> T-cells in the first 3–6 months is usually rapid and is followed by a second phase of slower increase approaching stable CD4<sup>+</sup> T-cell counts at 4–6 years [3,4]. However, in a

recent study by the Swiss HIV Cohort Study (SHCS) [5], approximately one-third of the patients failed to reach CD4<sup>+</sup> T-cell counts above 500 cells/ $\mu$ l after 5 years of CART despite continuous suppression of VL values below 1,000 copies/ml. The purpose of this study was therefore to investigate in more detail predictive factors for the rate of CD4<sup>+</sup> T-cell change in CART-naïve patients reaching sustained VL suppression.

## Methods

### Patients

The SHCS ([www.shcs.ch](http://www.shcs.ch)) is a prospective cohort study with continuing enrolment of HIV-infected individuals aged  $\geq 16$  years. RNA VL and CD4<sup>+</sup> T-cell counts from registered individuals are electronically transferred from the centre laboratories at follow up or routine visits to the SHCS data centre.

For this study, we included treatment-naïve patients with HIV-1 virus starting CART who had CD4<sup>+</sup> T-cell and VL measurements at start of CART and  $\geq 2$  subsequent measurements. CART was defined as a single or boosted protease inhibitor (PI)-based regimen, non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen or triple nucleoside reverse transcriptase inhibitor (NRTI) regimen. Patients with concurrent immunosuppressive therapies (for example, chemotherapies for lymphoma and cancer or therapy against cytomegalovirus), pregnant patients and patients receiving hydroxyurea [6] at start of CART were excluded from the analysis; patients initiating any of these therapies or getting pregnant while on CART and patients who interrupted CART for  $>3$  months were censored at the start date of these events, respectively.

We studied all patients satisfying the above criteria who reached suppressed VL ( $<50$  copies/ml or below the limit of detection of the test) within 9 months after starting CART and had consecutive suppressed VL measurements at least 3 months apart. The suppression episode was defined as starting at the first suppressed VL measurement (time 0) and ending if a subsequent VL measurement exceeded 400 copies/ml. We studied CD4<sup>+</sup> T-cell count dynamics for these patients until the end of suppression or for a maximum of 5 years subdivided into three periods: 1st year, years 2–3 and years 4–5 of suppression.

### Statistical methods

**Modelling CD4<sup>+</sup> T-cell change.** For descriptive analyses, individual patients' CD4<sup>+</sup> T-cell count increases during the three periods were estimated by fitting a linear spline model with knots at 0, 1, 3 and 5 years after entry into suppression to the patient's CD4<sup>+</sup> T-cell measurements while under CART. This provides an estimate of the CD4<sup>+</sup> T-cell increase for a

period even if the patient started but has not yet completed that period or dropped out during the period, for example, because the VL is no longer suppressed. To assess whether further subdivision into periods of 1 year each was necessary, we applied a linear spline model to all patients simultaneously and tested whether additional knots at 2 and 4 years after entry into suppression significantly improved the fit. We used a median regression model and a cluster bootstrap to adjust for multiple CD4<sup>+</sup> T-cell measurements for one patient as described below for the multiple regression model.

**Covariates.** The following pre-specified covariates were selected as potential predictors for CD4<sup>+</sup> T-cell changes in each period: gender, age, prior AIDS, time from first positive HIV test to start of CART, coinfection with chronic hepatitis B or C, time from start of CART to start of suppression, VL at start of CART, CD4<sup>+</sup> T-cell count at start of the period, change in CD4<sup>+</sup> T-cell count per year in the previous period (for the first period, change in CD4<sup>+</sup> T-cell count from start of CART until start of suppression was used instead), type of CART (boosted PI, single PI, NNRTI or triple NRTI), nucleoside pair (any with tenofovir, any with stavudine [and no tenofovir], lamivudine/zidovudine or other) and use of cotrimoxazole at the start of the respective period. In cases where the date of the first positive HIV test was missing, it was input as the date of registration in the SHCS; patients with missing hepatitis B or C status were assumed to be free of hepatitis. A more detailed breakdown of CART into individual antiretrovirals was not considered, because sample size would have been too low for these comparisons. Patients were only included in the statistical model for a specific time period if they contributed  $\geq 2$  CD4<sup>+</sup> T-cell measurements to that period. No stepwise covariate selection was performed to avoid standard errors that are biased low [7].

**Regression analysis.** For each time period, the patient's repeated CD4<sup>+</sup> T-cell counts were modelled as depending on an intercept, the CD4<sup>+</sup> T-cell count at the start of the period and an interaction between the slope and time. The slope term was modelled as depending linearly on the covariates specified above. We used a median regression model [8] instead of the more conventional mean (least-squares) regression model to gain better interpretability and robustness in the results, that is, we modelled the median CD4<sup>+</sup> T-cell count as a function of the covariates specified above. A generalized estimating equation approach was applied to adjust for multiple measurements within patients [9,10]: a median regression was fit ignoring the dependence structure ('working independence'). Confidence intervals and standard errors, however, were calculated

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with a cluster bootstrap where patients (and not individual CD4<sup>+</sup> T-cell counts) were resampled accounting for the dependence structure within patients [11]. Bootstrap samples were stratified by the time from the start of suppression to the patient's last CD4<sup>+</sup> T-cell measurement under suppression (<1, 1–3, >3 years). A *P*-value for testing the significance of the average yearly effect of the variable over all three periods was derived by inverting the corresponding bootstrap confidence interval (CI).

**Sensitivity analyses.** Several alternative models were fit to assess the robustness of the results. First, we modelled the logarithm of the CD4<sup>+</sup> T-cell count (plus 100 cells/ $\mu$ l) instead, which is more symmetrically distributed than the original CD4<sup>+</sup> T-cell count. Second, we added other variables to the original model (race, weight, likely source of HIV infection [intravenous drug use versus other], year of CART initiation and CD4<sup>+</sup> T-cell percentage at the start of the period) to adjust for additional potential confounders. Third, instead of including the exposure to a drug at the beginning of a period into the model for the slope, we included the time-dependent cumulative exposure of the drug during that period which leads to a difference from the original model if patients switched treatment during a period. Continuous covariates were alternatively entered as natural cubic splines into the model to assess potential non-linearity. Fourth, to examine potential informative drop-out, we repeated the primary analysis but included interaction terms between CD4<sup>+</sup> T-cell slope and the following drop-out events during the respective period: death, loss to follow-up, viral rebound and censoring. Other interactions between covariates and drop-out patterns were not examined due to sample size restrictions. Fifth, we tested for interactions between CART and nucleoside pair and for the influence of the number of previous CART modifications in extended models including those additional terms. The combination of didanosine and tenofovir causes suboptimal CD4<sup>+</sup> T-cell increases [12–15]. Because of the small number of patients receiving this combination, we excluded all patients receiving both didanosine and tenofovir (sequentially or concurrently) in an additional sensitivity analysis.

In a second set of sensitivity analyses, we reanalysed the primary model, the model with splines and the model for the logarithm of the CD4<sup>+</sup> T-cell count using an alternative time scale with time 0 being start of CART instead of start of VL of suppression.

All reported CIs are two-sided 95% CIs and tests were performed at the two-sided 5% level. Analyses were performed with SAS 9.1 (SAS Institute, Cary, NC, USA) and R version 2.3.1 [16]. Median regression was performed with the contributed package *quantreg* for R.

## Results

A total of 2,860 treatment-naive patients satisfied the inclusion criteria. Of these, 1,816 (63%) had two suppressed VL measurements (<50 copies/ml or below the detection limit of the test)  $\geq 3$  months apart within the 1st year of starting CART. Patients reaching VL suppression were more likely to have a low VL and a CD4<sup>+</sup> T-cell count >200 cells/ $\mu$ l at start of CART, to be without AIDS and hepatitis C and to start on an NNRTI regimen than non-suppressed patients. The percentage of patients reaching VL suppression steadily increased from 52% in the years 1995–1998 to 74% in the years 2003–2005. The baseline characteristics of the 1,816 suppressed patients are displayed in Table 1.

By 23 May 2006, 340 (19%) of the patients had completed the observation period of 5 years of sustained VL suppression, 642 (35%) were under continuous VL suppression but had not yet completed the 5 years of follow up, 351 (19%) had experienced VL rebound, 418 (23%) were censored (370 of them due to a CART interruption for >90 days), 34 (2%) were lost to follow up and 31 (2%) died under VL suppression. There were 5,261 person-years of follow up under VL suppression; the median duration of VL suppression was 5.6 years (Kaplan–Meier estimate, CI: 4.8–6.3). The median number of different CART regimens per patient until the end of the observation period was 1 (upper quartile: 2; maximum: 7).

The median CD4<sup>+</sup> T-cell increase per year in the different periods was 87 cells/ $\mu$ l (interquartile range [IQR]: 8–180) in the 1st year, 52 cells/ $\mu$ l (IQR: 2–100) in years 2–3 and 19 cells/ $\mu$ l (IQR: –33–64) in years 4–5 of suppression. Similarly, CD4<sup>+</sup> T-cell percentage increased by 3.9% (IQR: 0.8–7.3), 2.0% (IQR: 0.3–3.6) and 1.0% (IQR: –0.4–2.5) per year in the different periods. A median regression for all patients modelling the CD4<sup>+</sup> T-cell count in the first 5 years under VL suppression with a linear spline function with knots after 1 and 3 years as a covariate yielded similar median increases as above; increases in the different periods were significantly different ( $P < 0.001$  for all differences). Additional knots at 2 or 4 years after entry into suppression, respectively, did not result in significant model improvements ( $P = 0.942$  and  $P = 0.314$ , respectively).

Figure 1 displays the unadjusted CD4<sup>+</sup> T-cell increases in the different periods according to CD4<sup>+</sup> T-cell count and selected CART regimen at entry into the period. The displayed *n*-values provide information about how many patients were included in each period and their CART regimens. Median CD4<sup>+</sup> T-cell increases were positive in all subgroups and all periods (except for the eight patients on boosted PI with tenofovir in years 4–5) with smaller increases in later

Table 1. Baseline characteristics of 1,816 ART-naïve patients reaching VL suppression within 9 months of starting CART with a second suppressed VL  $\geq 3$  months apart

Characteristic	Summary statistic
Gender: Female	522 (29)
Age at first CART, years	37 (32–44)
Race: White	1,494 (82)
Most likely source of infection	
Heterosexual intercourse	787 (43)
Homosexual intercourse	636 (35)
Intravenous drug use	222 (12)
Other	171 (9)
AIDS prior first CART	325 (18)
Time from first positive HIV test to start of CART*, years	1.3 (0.1–6.9)
[Au: OK?] Hepatitis B surface antigen prior to first CART	
Negative	1,380 (76)
Positive	93 (5)
Unknown	343 (19)
Anti-HCV antibody prior to first CART	
Negative	1,229 (68)
Positive	365 (20)
Unknown	222 (12)
Time from first CART to suppression, months	3.7 (2.3–5.7)
Viral load at first CART, log <sub>10</sub> copies/ml	4.7 (4.0–5.2)
CD4 <sup>+</sup> T-cell count at first CART, cells/ $\mu$ l	214 (100–344)
CD4 <sup>+</sup> T-cell count at entry into suppression, cells/ $\mu$ l	325 (197–486)
CD4 <sup>+</sup> T-cell percentage at first CART	16 (9–22)
CD4 <sup>+</sup> T-cell percentage at entry into suppression	21 (14–29)
First CART regimen	
Single PI	813 (45)
Boosted PI	376 (21)
NNRTI	584 (32)
Triple NRTI	43 (2)
Year of CART initiation	2000 (1998–2003)

Continuous variables are given as median (interquartile range [IQR]). Non-continuous variables are given as n (%). \*Median (IQR) is for patients with non-missing date of first positive HIV test only. The date was missing for 260 (14%) of the patients. ART, antiretroviral therapy; CART, combination ART; HCV, hepatitis C virus; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, viral load.

periods. CD4<sup>+</sup> T-cell slopes were lower and more variable if the CD4<sup>+</sup> T-cell count at entry into the phase was  $\geq 650$  cells/ $\mu$ l. Regimens with single or boosted PIs combined with stavudine showed the largest CD4<sup>+</sup> T-cell count increase in the first two periods where sample sizes are largest. With regard to the nucleoside pair, patients on stavudine generally showed the largest improvement in CD4<sup>+</sup> T-cell count whereas patients on tenofovir did worst. We observed this pattern for all CART regimens but most prominently for boosted PI.

In the multiple median regression model (Table 2), median CD4<sup>+</sup> T-cell increase over all three periods was

significantly higher for patients with female gender ( $P < 0.001$ ), lower age ( $P < 0.001$ ), higher VL at start of CART ( $P = 0.002$ ), CD4<sup>+</sup> T-cell count  $< 650$  cells/ $\mu$ l at start of the period ( $P = 0.010$ ) and low CD4<sup>+</sup> T-cell increase in the previous period (or increase from start of CART for the first period;  $P < 0.001$ ). Patients without hepatitis B ( $P = 0.031$ ) or with a CD4<sup>+</sup> T-cell  $> 200$  cells/ $\mu$ l ( $P = 0.017$ ) also had a significantly higher CD4<sup>+</sup> T-cell increases but this was not confirmed in all sensitivity analyses (see below).

Patients on tenofovir showed a significantly lower increase than patients on stavudine ( $P < 0.001$ ); the median CD4<sup>+</sup> T-cell change per year was between -39 and -44 cells/ $\mu$ l lower during the three periods. There was also a trend towards lower CD4<sup>+</sup> T-cell changes for patients on NNRTI-based CART (compared with boosted PI,  $P = 0.067$ ) and patients on lamivudine/zidovudine (compared with stavudine;  $P = 0.065$ ). Patients coinfecting with chronic hepatitis C and patients without AIDS had a significantly lower CD4<sup>+</sup> T-cell increase in some periods but not overall. Other covariates did not significantly influence CD4<sup>+</sup> T-cell change.

Sensitivity analyses which kept time 0 to be the time of entry into suppression were consistent with the results of the primary analysis. Modelling the logarithm of the CD4<sup>+</sup> T-cell count (instead of the crude CD4<sup>+</sup> T-cell count), adding additional covariates (as defined in *Methods*), including treatments as time-dependent covariates or including selected continuous covariates as natural splines into the model confirmed the significant factors found in the primary model. An exception was that the negative influence of hepatitis B and CD4<sup>+</sup> T-cell count  $< 200$  cells/ $\mu$ l at the start of the period was not significant in all sensitivity analyses (the respective  $P$ -values ranged from 0.028 to 0.118 and 0.017 to 0.350). The trend for lower CD4<sup>+</sup> T-cell counts for patients on an NNRTI (compared with boosted PI;  $P$ -values between  $< 0.001$  and 0.067) and lamivudine/zidovudine (compared with stavudine;  $P$ -values between 0.008 and 0.094) was confirmed in all sensitivity analyses.

Of all drop-out reasons – death ( $P = 0.223$ ), loss to follow up ( $P = 0.408$ ), viral rebound ( $P = 0.690$ ) and censoring (primarily due to CART interruption;  $P = 0.025$ ) – occurring in the respective period, only censoring significantly influenced CD4<sup>+</sup> T-cell slopes. CD4<sup>+</sup> T-cell slopes for patients who were subsequently censored were between 6 and 35 cells/ $\mu$ l per year higher in the three periods. Moreover, death was associated with between 36 and 55 cells/ $\mu$ l per year lower CD4<sup>+</sup> T-cell slopes in the three periods and was probably non-significant because of the low number of death events (31). Adding interactions between drop-out reasons and CD4<sup>+</sup> T-cell slope to the model

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only minimally affected estimates and *P*-values of the other covariates.

Interactions between nucleoside pair and CART regimen were not significant although the relevant tests have limited power (data not shown). Adding the number of previous CART modifications as a covariate did also not significantly improve the fit (*P*=0.372). When excluding all 70 patients who received both didanosine and tenofovir the median CD4<sup>+</sup> T-cell change for patients on tenofovir (compared with stavudine) remained significantly lower (*P*<0.001).

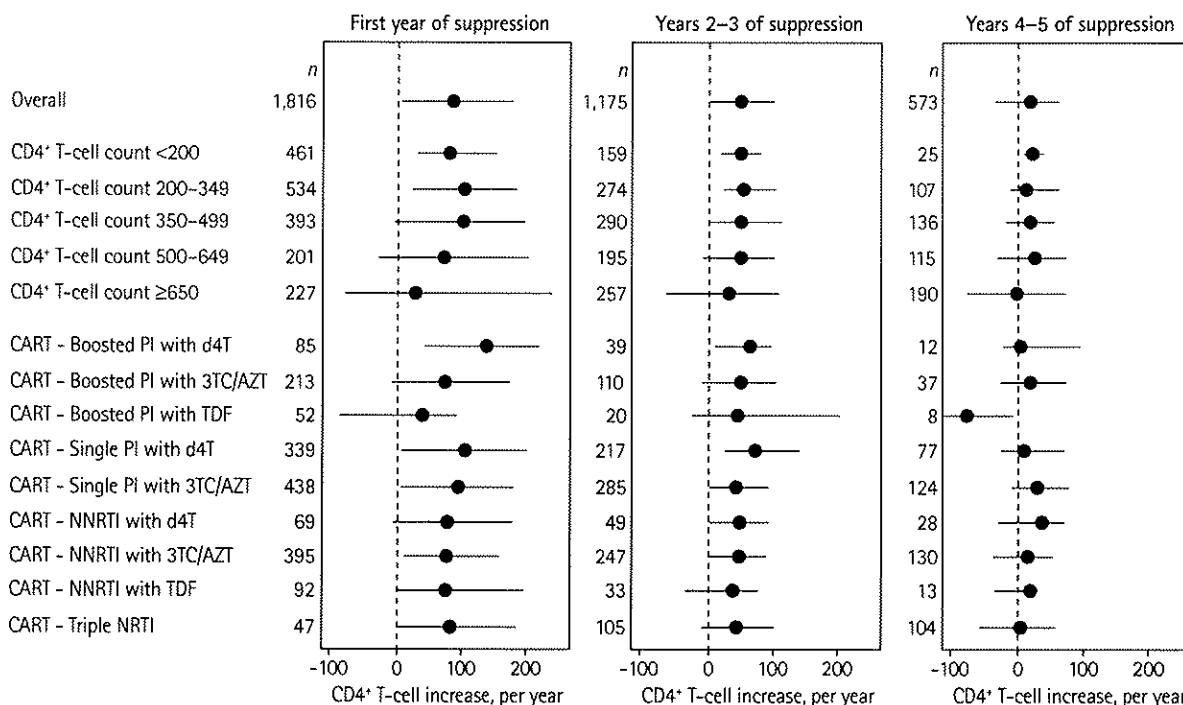
When start of CART (and not start of suppression) was defined as time 0 instead, crude median CD4<sup>+</sup> T-cell increases per year were 135 (IQR: 43–237) in the 1st year, 58 (IQR: 6–109) in years 2–3 and 22 (IQR: -24–73) cells/μl in years 4–5. The three sensitivity analyses which used start of CART as time 0 were also broadly consistent with the primary model: age, gender, VL load at start of CART, CD4<sup>+</sup> T-cell count and previous CD4<sup>+</sup> T-cell changes were confirmed as predictive factors for CD4<sup>+</sup> T-cell increases. However, patients without AIDS and patients with hepatitis C showed significantly lower CD4<sup>+</sup> T-cell increases in

these analyses (all *P*-values <0.02), whereas hepatitis B was no longer statistically significant overall. Both tenofovir and lamivudine/zidovudine (compared with stavudine) and NNRTI (compared with boosted PI) showed consistently significantly lower CD4<sup>+</sup> T-cell increases in all three analyses (except for a border-line significant *P*-value in one analysis for tenofovir; *P*=0.063), confirming the primary analysis. Closer examination of the treatment effects revealed that differences were most pronounced in the period 2–3 years after start of CART (data not shown).

### Discussion

In this analysis of the SHCS we investigated a subset of 1,816 (63%) from 2,860 treatment-naïve HIV-infected patients who initiated CART and reached sustained VL suppression (two measurements <50 copies/ml ≥3 months apart) within 12 months after the start of CART. In a multivariate median regression model we identified factors associated with a significantly higher CD4<sup>+</sup> T-cell slope over all three time periods, that is, younger age, female gender, higher VL at start of

Figure 1. Median and IQR of CD4<sup>+</sup> T-cell increases during years 1, 2–3, and 4–5 of viral load suppression by CD4<sup>+</sup> T-cell count and selected CART regimen at the start of the time period



Medians and interquartile ranges are shown. Regimens with stavudine or tenofovir also include one or more additional nucleosides. 3TC/AZT, lamivudine/zidovudine; CART, combination antiretroviral therapy; d4T, stavudine; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir.

Table 2. Adjusted model for median CD4<sup>+</sup> T-cell increase in the different periods

	CD4 <sup>+</sup> T-cell count increase in year 1 under suppression		CD4 <sup>+</sup> T-cell count increase per year in year 2-3 under suppression		CD4 <sup>+</sup> T-cell count increase per year in year 4-5 under suppression		Overall P-value
	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI	
	Intercept*	+126	+99--152	+75	+52--94	+46	
Female gender	+28	+13--50	+16	+5--30	+15	-2--35	<0.001
Age, per 10 years	-16	-24--9	-4	-8--2	-4	-10--3	<0.001
Prior AIDS	+21	+2--35	+5	-7--17	-11	-31--9	0.810
Time from positive HIV test, years <sup>†</sup>	0	-2--1	-1	-3--0	-1	-2--1	0.176
Hepatitis B surface antigen positive	-25	-56--4	+6	-16--24	-31	-77--9	0.031
Hepatitis C positive	-20	-37--1	+8	-4--27	-21	-47--2	0.156
Time from first CART to suppression, years	+5	+2--10	-2	-5--0	-1	-4--3	1.000
[Au: OK?] Viral load at first cART, per log <sub>10</sub> /ml	+15	+8--21	+3	-2--8	+4	-2--8	0.002
CD4 <sup>+</sup> T-cell count at start of period, cells/μl							
<200	-29	-48--9	-10	-26--5	-12	-48--12	0.017
200-350	0	-	0	-	0	-	-
350-500	+34	+11--59	-1	-17--12	-30	-53--14	0.206
500-649	+3	-43--38	+4	-16--20	-3	-29--19	1.000
≥650	-15	-67--32	-10	-32--15	-37	-71--12	0.010
CD4 <sup>+</sup> T-cell change per year during previous period, per 100 cells/μl increase <sup>‡</sup>	-50	-61--37	-16	-22--9	-40	-55--22	<0.001
CART regimen							
Boosted PI	0	-	0	-	0	-	-
Single PI	-12	-34--4	0	-15--17	-11	-38--15	0.296
NNRTI	-6	-23--16	-11	-25--8	-22	-49--6	0.067
Triple NRTI	-37	-93--24	-16	-34--7	-12	-45--22	0.097
Nucleoside pair							
Any with stavudine	0	-	0	-	0	-	-
Zidovudine and lamivudine	-9	-26--8	-18	-30--5	-3	-20--18	0.065
Any with tenofovir	-39	-78--16	-42	-67--16	-44	-185--7	<0.001
Other	+19	-19--61	-36	-55--10	+5	-20--43	0.531
On cotrimoxazole at entry into period	-22	-39--5	-9	-22--5	-10	-40--25	0.145

Samples sizes are as follows: year 1 under suppression - n=1,816 patients, 7,007 CD4<sup>+</sup> T-cell counts; years 2-3 under suppression - n=1,175 patients, 6,493 CD4<sup>+</sup> T-cell counts; years 4-5 under suppression - n=573 patients, 3,001 CD4<sup>+</sup> T-cell counts. \*Intercept corresponds to a male, 40-year old patient, who took 4 months to enter suppression, had a viral load of 5 log<sub>10</sub> copies/ml at start of combination antiretroviral therapy (CART) and an increase of 100 cells/μl per year in the previous period. <sup>†</sup>Input as the date of registration into the SHCS in cases where the date of first positive HIV test was missing. <sup>‡</sup>This refers to the change from start of CART to first VL suppression for the first period. CI, confidence interval; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

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CART, CD4<sup>+</sup> T-cell count <650 cells/μl at start of the period and small CD4<sup>+</sup> T-cell increase in the previous period. We demonstrated that patients on tenofovir showed a lower increase than patients on stavudine. Moreover, there were consistent trends for lower CD4<sup>+</sup> T-cell increases for patients on NNRTI (compared with boosted PI) or on lamivudine/zidovudine (compared with stavudine).

The rate of 63% of patients reaching confirmed virological suppression within 1 year after start of CART is similar to results from clinical trials [17] confirming that such rates can be achieved in less selected populations [18,19]. We used the time of entry

into suppression as the baseline for our analyses as from this time point onwards CD4<sup>+</sup> T-cell count changes are unconfounded with VL. However, our results were confirmed in sensitivity analyses where time 0 was the time of CART start. Because CD4<sup>+</sup> T-cell count increased more rapidly in the first 3-6 months after starting CART than in later phases [3,4] and the median time from start of CART to suppression was 3.7 months in this study, the estimates in our primary analyses are lower than reported CD4<sup>+</sup> T-cell increases from the start of CART in clinical trials [17] or observational cohorts restricted to suppressed patients [20-22].

Some other studies also analysed predictors of CD4<sup>+</sup> T-cell count increases in the subset of patients with suppressed VL [5,23–26] and there is a large literature on factors influencing immune reconstitution [27,28]. Some but not all factors identified in this study confirm results of earlier studies. Our study differs from other studies in also including antiretrovirals as covariates, in the statistical analysis technique and in limiting the patient population to treatment-naïve patients reaching complete suppression (VL <50 copies/ml).

The positive effect of younger age on immune reconstitution has been repeatedly documented in large cohort studies [3,5,23–26,29] and may be related to thymic function. Gender-related differences are more controversial. We found a consistent gender effect favouring females. A gender effect was also found in previous studies [24,30,31] while others did not confirm our findings [5,23,26,32].

Higher VL on starting CART has been shown to be related to higher increases in CD4<sup>+</sup> T-cell count in our study and a number of other studies [3,25,33]. Similarly, we confirmed findings from the Euro-SIDA study [23] of a lower CD4<sup>+</sup> T-cell increase in individuals with a high count of >650 cells/μl at the start of the period. In our primary analysis, patients with a CD4<sup>+</sup> T-cell count <200 cells/μl also showed a lower CD4<sup>+</sup> T-cell increase than patients with a CD4<sup>+</sup> T-cell count between 200 and 350 cells/μl, but this was not confirmed in all sensitivity analyses. Finally, we found that patients with a high CD4<sup>+</sup> T-cell increase in the previous period showed a lower increase in the subsequent period and vice versa. We believe that this is both a manifestation of regression to the mean and a ceiling effect for patients approaching the normal range of CD4<sup>+</sup> T-cell count.

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are arguably the most important coinfections of HIV [34]. However, the role of coinfections on CD4<sup>+</sup> T-cell recovery is a subject of debate [27,35] and results might also depend on how HBV and HCV are measured and what is its status. Our study was based on hepatitis B surface antigen and anti-HCV antigen, respectively, and we found a significant negative influence of hepatitis B and C on CD4<sup>+</sup> T-cell slopes in the primary analysis or sensitivity analyses, respectively, but results were not consistent over all analyses. Previous findings from the SHCS showed an association between a reduced increase in CD4<sup>+</sup> T-cells and coinfection with hepatitis C [36], but this effect might be constrained to the 1st year only [3]. Thus, our data might indicate a negative influence of coinfections on the pace of immune reconstitution. However, the proportion of patients with chronic active hepatitis B was low (5%) and there was limited power to test for this factor.

Current treatment guidelines [37] recommend an NNRTI regimen or a boosted PI regimen and there are no clinical trials supporting the superiority of either one in efficacy at any stage of the disease. We did observe a trend for higher CD4<sup>+</sup> T-cell increases in the first 5 years of suppression after boosted PI regimens compared with NNRTI-regimens. This is consistent with findings of a meta-analysis of treatment-naïve patients from randomized, controlled trials with 48 weeks of follow up [17].

We confirmed in this treatment-naïve patient population a trend towards lower CD4<sup>+</sup> T-cell increases for patients on the lamivudine/zidovudine nucleoside backbone compared with patients receiving stavudine and a significantly lower CD4<sup>+</sup> T-cell increase for patients on tenofovir [23]. Two clinical trials [38,39] comparing stavudine/lamivudine and stavudine/didanosine, respectively, with lamivudine/zidovudine also noted significantly higher CD4<sup>+</sup> T-cell increases in the first 48 weeks for patients on stavudine but this was not confirmed in other trials [30] for the stavudine/didanosine combination. In our study, stavudine was most frequently (in 66%) administered with lamivudine. There is evidence that the combination of didanosine and tenofovir causes suboptimal CD4<sup>+</sup> T-cell gains [12–15]. In our study, tenofovir was most frequently administered with lamivudine (72%), emtricitabine (11%) or didanosine (11%). Importantly, the suboptimal CD4<sup>+</sup> T-cell gains for patients on tenofovir persisted after exclusion of all patients receiving didanosine and tenofovir. Tenofovir might lead to an increased accumulation of adenosine by blocking purine nucleoside phosphorylase, thereby impairing CD4<sup>+</sup> T-cell responses in a subset of patients [15]. It is difficult to compare these results with clinical trials because reporting in trials is not restricted to virologically suppressed patients. In the trial by Gallant *et al.* the mean CD4<sup>+</sup> T-cell increases in patients on tenofovir was slightly lower compared with stavudine (263 cells/μl and 283 cells/μl at week 144, respectively) but no *P*-value is reported [40].

Finally, the present study has several important limitations. First, the ideal settings for the comparison of CART regimens are randomized clinical trials; in observational cohorts CART regimens are assigned by investigator's choice and there is a substantial risk of confounding. Moreover, in the last observation period (years 4–5 of VL suppression), the number of patients on some CART regimens was low. Although we have adjusted for a number of potential confounders and performed several sensitivity analyses, results from our study should be interpreted with caution and require confirmation by (ideally) clinical trials or large collaborative cohorts. Second, although disentangling immunological response from virological response by

analysing patients with suppressed VL only is a strength of the current study, this could introduce selection bias because the population of suppressed patients might differ from the full population. Third, it cannot be completely excluded that our effect estimates are adversely affected by non-ignorable drop-out primarily due to patients interrupting treatment. Fourth, although the rate of CD4<sup>+</sup> T-cell increase in suppressed patients is an important secondary endpoint for guiding clinical decisions, it must be balanced with other endpoints, for example, the rate of viral suppression. Fifth, the results of this study allow for a comparison of different prognostic factors on a population level but have limited use as a prognostic tool for individual patients. This is due to large individual variations in CD4<sup>+</sup> T-cell recovery as shown by large IQRs (Figure 1) and wide CIs (Table 3).

In conclusion, in this analysis of naive patients with persistent viral suppression younger age, female gender, higher VL at start of CART, CD4<sup>+</sup> T-cell count <650 cells/ $\mu$ l at start of the period and small CD4<sup>+</sup> T-cell increase in the previous period were associated with higher CD4<sup>+</sup> T-cell increase. Patients on NNRTIs and those on backbone regimens with lamivudine/zidovudine or tenofovir experienced lower CD4<sup>+</sup> T-cell increases. The clinical relevance of the observed differences remains to be elucidated in large collaborative cohort studies. The increasingly homogenous population of drug-naive patients with long-term complete viral suppression might be an important source for observational studies to better understand factors determining optimal immune reconstitution.

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