CD4 Cell Counts of 800 Cells/mm^3 or Greater After 7 Years of Highly Active Antiretroviral Therapy Are Feasible in Most Patients Starting With 350 Cells/mm^3 or Greater

Luuk Gras, MSc, Anouk M. Kesselring, MSc, MD,* James T. Griffin, PhD,† Ard I. van Sighem, PhD,* Christophe Fraser, PhD,† Azra C. Ghani, PhD,‡ Frank Miedema, PhD,§ Peter Reiss, MD, PhD,‖ Joep M. A. Lange, MD, PhD,‖ and Frank de Wolf, MD, PhD*† on Behalf of the ATHENA, Netherlands National Observational Cohort Study*

Objective: CD4 cell count changes in therapy-naive patients were investigated during 7 years of highly active antiretroviral therapy (HAART) in an observational cohort.

Methods: Three endpoints were studied: (1) time to ≥800 CD4 cells/mm^3 in 5299 therapy-naive patients starting HAART, (2) CD4 cell count changes during 7 years of uninterrupted HAART in a subset of 544 patients, and (3) reaching a plateau in CD4 cell restoration after 5 years of HAART in 366 virologically suppressed patients.

Results: Among patients with <50, 50 to 200, 200 to 350, 350 to 500, and ≥500 CD4 cells/mm^3 at baseline, respectively, 20%, 26%, 46%, 73%, and 87% reached ≥800 CD4 cells/mm^3 within 7 years of starting HAART. Periods with HIV RNA levels >500 copies/mL and age ≥50 years were associated with lesser increases in CD4 cell counts between 6 months and 7 years. Having reached ≥800 CD4 cells/mm^3 at 5 years, age ≥50 years, and ≥1 HIV RNA measurement >1000 copies/mL between 5 and 7 years were associated with a plateau in CD4 cell restoration.

Conclusions: Restoration to CD4 cell counts ≥800 cells/mm^3 is feasible within 7 years of HAART in most HIV-infected patients starting with ≥350 cells/mm^3 and achieving sufficient suppression of viral replication. Particularly in patients ≥50 years of age, it may be beneficial to start earlier than current guidelines recommend.

Key Words: age, CD4 cell counts, HIV-1, immune restoration, long-term highly active antiretroviral therapy, viremia

(*J Acquir Immune Defic Syndr* 2007;45:183–192)

Received for publication September 1, 2006; accepted February 16, 2007.

From the *HIV Monitoring Foundation, Amsterdam, The Netherlands; †Department of Infectious Diseases Epidemiology, Imperial College School of Medicine, London, United Kingdom; ‡Department of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom; §Department of Immunology, University Medical Centre, Utrecht, The Netherlands; and ‖Center for Infection and Immunity, Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Centre of the University of Amsterdam, Amsterdam, The Netherlands.

Presented in part at the 13th Conference on Retroviruses and Opportunistic Infections, Denver, February 5–8, 2006 [poster 530].

Reprints: Luuk Gras, MSc, HIV Monitoring Foundation, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands (e-mail: l.a.gras@amc.uva.nl).

The choice of when to begin highly active antiretroviral therapy (HAART) is based on a trade-off between the complications of long-term antiretroviral drug use[1–4] and the benefits of timely reversal of the deterioration of the immune system. Current guidelines[5–7] recommend HAART initiation before asymptomatic patients drop to 200 CD4 cells/mm^3. Delaying the start of HAART until after this threshold (ie, in a relatively late stage of infection) is associated with faster disease progression and death as compared with starting when counts are still greater than 200 cells/mm^3.[8–10] Further studies have concluded that the prognosis is improved when patients start HAART when CD4 counts are still greater than 350 cells/mm^3.[11]

Residual HIV replication,[12,13] impaired thymic function,[14,15] advanced age,[16] enhanced T-cell activation,[17,18] apoptosis,[19,20] and, possibly, viral coinfection[21,22] have been associated with more limited immune restoration in patients on HAART. After an initial rapid increase, because of redistribution of cells trapped in the lymphoreticular system to the peripheral blood, CD4 cell counts may plateau after the first year of HAART.[23–28] These studies described follow-up of <3 years and included a mixture of pretreated and naive patients or a small number of patients. Studies with longer follow-up in naive patients likewise reported a plateau effect, however.[29,30] In contrast, no evidence of a plateau effect was found in patients who had suppressed plasma HIV-RNA levels to less than 1000 copies/mL.[31]

Here, we explore the capacity of patients on long-term HAART to improve CD4 cell counts. We assess how these improvements, 7 years after starting HAART, compare with CD4 cell levels in the non–HIV-infected population. In addition, we describe the determinants of reaching a plateau in CD4 cell restoration between 5 and 7 years of uninterrupted HAART.

MATERIALS AND METHODS

Study Population

Patients were selected from the AIDS Therapy Evaluation Project, Netherlands (ATHENA) national observational HIV cohort.[32] HAART was defined as a combination of 3 or more drugs from at least 2 drug classes or a combination of

3 or more nucleoside reverse transcriptase inhibitors, including abacavir or tenofovir. All patients were 16 years of age or older and had a recorded pre-HAART CD4 cell count. We performed 3 analyses.

Time to CD4 Cell Counts ≥800 Cells/mm^3

In the first analysis, we used a longitudinally followed cohort of 5299 antiretroviral therapy–naive patients who initiated HAART between July 1, 1996 and December 31, 2004 to analyze the probability of reaching CD4 cell counts ≥800 cells/mm^3 in relation to pre-HAART values and other baseline characteristics. Most studies[25,28,30] have used a threshold of 500 CD4 cells/mm^3, the lower limit of the normal range in uninfected individuals. The mean observed CD4 counts in HIV-negative adults are 1050, 840, and 800 cells/mm^3 for women, heterosexual men, and men who have sex with men (MSM), respectively.[33] Because our cohort is largely MSM, we chose ≥800 CD4 cells/mm^3 as an endpoint. Potential predictors tested for association with time taken to reach the endpoint included pre-HAART CD4 count (<50, 50–200, 200–350, 350–500, and ≥500 cells/mm^3), pre-HAART CD8 count (<600, 600–1300, and ≥1300 cells/mm^3), pre-HAART HIV RNA plasma levels (<3, 3–4, and ≥4 $\log_{10}$ copies/mL), Centers for Disease Control and Prevention category C (CDC-C) events before starting HAART, age at starting HAART, gender, and region of birth (Western or Central Europe, North America, or Australia combined [WCE/NA/A] and sub-Saharan Africa, Caribbean, Latin America, Southeast Asia, and all other regions combined were tested for association with time taken to reach the endpoint). The CD4 cell count measured closest to starting HAART from 6 months before to 7 days after starting was selected as the pre-HAART CD4 cell count. Patients were allowed to change or interrupt regimens and were retained in the analysis regardless of the level of HIV RNA.

Long-Term CD4 Cell Response in Patients on Uninterrupted HAART for 7 Years

Second, we analyzed the immune system's maximum capacity to restore CD4 cell numbers in a longitudinally followed subcohort of 554 patients who started HAART between July 1, 1996, and June 30, 1998, and took HAART continuously for at least 7 years. These patients were also part of the first analysis; they were included regardless of whether they had HIV RNA measurements greater than detectable limits because we were interested in estimating the effect of periods of viremia on the rate of change in CD4 cell count.

We selected CD4 cell counts measured closest to weeks 24, 48, and 72 after initiating HAART (within a time frame of 3 months) and, subsequently, at 24-week intervals up to 360 weeks. Median increases in CD4 cell count at these time points were calculated and graphically summarized.

All CD4 cell counts measured between starting HAART and 7 years thereafter were longitudinally modeled. The same potential predictors as in the first analysis were tested for their association with slopes of CD4 cell counts over time. Given the smaller sample size for this analysis, the predictors were subdivided differently: region of origin (WCE/NA/A and all other regions combined), pre-HAART HIV RNA plasma concentration (<4.5 $\log_{10}$ copies/mL and ≥4.5 $\log_{10}$ copies/mL), and CD8 count (<1300 cells/mm^3 and ≥1300 cells/mm^3). To study the effect of viremia, we created a time-updated variable with values between 0% and 100% and denoting the percentage of time a patient's plasma HIV RNA concentration was ≥500 copies/mL after the initial 6 months of HAART. The value was 0% when a patient never had HIV RNA levels <500 copies/mL after the initial 6 months and 100% when a patient always had levels <500 copies/mL after the initial 6 months. We also tried to distinguish the effect of low-level viremia and high-level viremia on the slopes of CD4 cell count using different cutoffs (500–1000, 1000–10,000, and >10,000 copies/mL). Given our inclusion criteria, however, the number of HIV RNA measurements greater than 500 copies/mL was limited, and we lacked statistical power to detect significant differences in slopes of CD4 cell count during periods of low-level and high-level viremia.

Decreases in CD4 Cell Count Between 5 and 7 Years in Virologically Suppressed Patients

The third analysis determined predictors for reaching a plateau in CD4 cell restoration between years 5 and 7. To counter random fluctuations in CD4 cell measurements, we used the individual slopes between 5 and 7 years derived from a longitudinal model similar to that used in the second analysis (ie, with 5 intercepts for the 5 pre-HAART CD4 cell strata and 4 slopes for the 4 time intervals but without any other covariates) to determine whether a patient had reached a plateau in CD4 cell restoration. This plateau can be interpreted as an on-average decreasing CD4 cell count between years 5 and 7. Included in this model was a subset of 366 patients on uninterrupted HAART who were among those included in the second analysis. Additional inclusion criterion for this subset was that all HIV RNA measurements between 6 months and 5 years after starting HAART were <500 copies/mL. Variables included in the analysis were the same as in the second analysis, with the exception of HIV viremia, which was now defined as at least 1 HIV RNA measurement ≥1000 copies/mL between 5 and 7 years after starting HAART (yes/no). This excludes HIV RNA measurements between 500 and 1000 copies/mL from the definition because these are unlikely to cause a plateau in CD4 cell restoration.

Statistical Analysis

The Cox proportional hazards model and Kaplan-Meier estimates were used for the first analysis of time to ≥800 CD4 cells/mm^3. Time was censored at the end of follow-up or time of death, whichever occurred first. The statistical model used for the longitudinal analyses was a mixed-effects model with a random intercept and 4 random slopes for each patient. A first-order autoregressive covariance structure was used to correlate intraindividual serial measurements. We divided the 7-year time period into 4 intervals: 0 to 6 months after starting HAART, 6 months to 3 years, 3 to 5 years, and 5 to 7 years; slopes were allowed to differ between them. The intervals were chosen by visual inspection of the graphs of median CD4 cell response. CD4 cell counts were square root transformed to comply with model assumptions. Slopes of CD4 cell count

increase during each interval were estimated for the 5 pre-HAART CD4 cell count strata (<50, 50–200, 200–350, 350–500, and ≥500 cells/mm^3). The other variables were allowed to have 1 effect on the slopes between the start of HAART and 7 years thereafter (ie, the whole period) or to have an effect in each of the 4 previously stated time intervals. Model fit was determined by the Akaike Information Criterion statistic.[34] Logistic regression was used for the third analysis of decreasing CD4 cell counts between 5 and 7 years after first starting HAART. All calculations were performed using SAS 9.1.3 (SAS Institute, Cary, NC).

RESULTS

Time to CD4 Cell Counts ≥800 Cells/mm^3

The characteristics of the 5299 patients at the start of HAART are shown in Table 1. Most (76%) were male, 50% were MSM, and 63% originated from WCE/NA/A. The median HIV RNA concentration in plasma was 5.0 $\log_{10}$ copies/mL. A pre-HAART CD4 count of <200 cells/mm^3 was found in 2703 patients (51%).

The time required to restore CD4 counts to ≥800 cells/mm^3 was associated with a higher pre-HAART CD4 cell count. After 7 years of HAART, Kaplan-Meier estimates of the percentage of patients reaching ≥800 CD4 cells/mm^3 were 20%, 26%, 46%, 73%, and 87% for those with a pre-HAART CD4 count of <50, 50 to 200, 200 to 350, 350 to 500, and ≥500 cells/mm^3, respectively. Adjusted hazard ratios compared with those of patients with a pre-HAART CD4 count of 200 to 350 cells/mm^3 were 0.26 and 0.46, respectively, for those with <50 and 50 to 200 cells/mm^3 and were 2.84 and 6.79, respectively, for those with 350 to 500 and ≥500 cells/mm^3 (Table 2). Female gender and higher pre-HAART HIV RNA levels were associated with a shorter time to CD4 cell counts ≥800 cells/mm^3. Older age, Southeast Asian or sub-Saharan African origin, and HIV infection through intravenous drug use were associated with a longer time to this endpoint. There were no significant differences according

TABLE 1. Characteristics at the Start of HAART of 5299 Antiretroviral Therapy–Naive Patients and of the 554 Patients on Uninterrupted HAART for 7 Years or More

	Antiretroviral Therapy–Naive Patients Starting HAART		Subset of Patients on Uninterrupted HAART for 7 Years	
	N	%	N	%
Total	5299	100.0	554	100.0
Gender				
Male	4013	75.7	498	89.9
Region of origin				
WCE/NA/A	3349	63.2	464	83.8
Sub-Saharan Africa	1090	20.6	36	6.5
Caribbean	182	3.4	12	2.2
Latin America	381	7.2	24	4.4
Southeast Asia	171	3.2	9	1.6
Other	126	2.4	9	1.6
Transmission risk group				
Homosexual	2636	49.7	367	66.2
Injection drug user	212	4.0	12	2.2
Heterosexual	1878	35.4	123	22.2
Other	573	10.8	52	9.4
Pre-HAART CD4 count (cells/mm^3)				
<50	930	17.6	98	17.7
50–200	1773	33.4	155	28.0
200–350	1513	28.6	172	31.0
350–500	694	13.1	78	14.1
≥500	389	7.3	51	9.2
Pre-HAART clinical stage				
CDC-C	1494	28.2	147	26.5

	N	Median	IQR	N	Median	IQR
Age at starting HAART (y)	5299	37	32–44	554	37.7	33.0–44.3
Pre-HAART HIV RNA ($\log_{10}$ copies/mL)	4908	5.0	4.5–5.4	507	5.0	4.4–5.4
Pre-HAART CD8 count (cells/mm^3)	4672	820	530–1220	488	903	560–1345
Pre-HAART CD4 count (cells/mm^3)	5299	190	80–314	554	221	80–340
WCE/NA/A	3349	200	80–330	464	240	80–340
Non-WCE/NA/A	1950	175	70–290	90	180	80–290

TABLE 2. Predictors of Reaching ≥800 CD4 Cells/mm^3 After Starting HAART

	Univariate Hazard Ratio (95% CI)	*P*	Multivariate Hazard Ratio (95% CI)	*P*
Gender				
Male	1.00		1.00	
Female	1.10 (0.97 to 1.25)	0.14	1.26 (1.05 to 1.52)	0.01
Transmission risk group				
Homosexual	1.00		1.00	
Heterosexual	0.82 (0.72 to 0.92)	0.001	0.93 (0.78 to 1.10)	0.39
Injection drug user	0.71 (0.52 to 0.96)	0.02	0.56 (0.42 to 0.77)	0.0003
Other	0.72 (0.5 to 0.88)	0.002	0.97 (0.79 to 1.21)	0.81
Region of origin				
WCE/NA/A	1.00		1.00	
Sub-Saharan Africa	0.58 (0.49 to 0.69)	<0.0001	0.63 (0.51 to 0.77)	<0.0001
Caribbean	0.79 (0.57 to 1.08)	0.14	0.88 (0.63 to 1.21)	0.42
Latin America	0.85 (0.69 to 1.06)	0.16	0.91 (0.73 to 1.14)	0.41
Southeast Asia	0.67 (0.47 to 0.94)	0.02	0.68 (0.48 to 0.96)	0.03
Other	0.92 (0.65 to 1.33)	0.68	0.94 (0.65 to 1.35)	0.73
Pre-HAART CD4 count (cells/mm^3)				
<50	0.28 (0.22 to 0.36)	<0.0001	0.26 (0.20 to 0.34)	<0.0001
50–200	0.46 (0.39 to 0.55)	<0.0001	0.46 (0.38 to 0.54)	<0.0001
200–350	1.00		1.00	
350–500	2.76 (2.39 to 3.19)	<0.0001	2.84 (2.45 to 3.28)	<0.0001
>500	6.62 (5.67 to 7.73)	<0.0001	6.79 (5.79 to 7.95)	<0.0001
Pre- HAART clinical stage				
CDC-A, B	1.00		1.00	
CDC-C	0.69 (0.64 to 0.74)	<0.0001	1.07 (0.99 to 1.16)	0.09
Age at starting HAART				
Per 10-year increase	0.88 (0.84 to 0.94)	0.0001	0.92 (0.87 to 0.98)	0.01
Pre-HAART HIV RNA ($\log_{10}$ copies/mL)				
<4.0	1.00		1.00	
4.0–5.0	1.12 (0.94 to 1.33)	0.23	1.51 (1.26 to 1.81)	<0.0001
≥5.0	0.91 (0.76 to 2.20)	0.21	1.81 (1.49 to 2.19)	<0.0001
Pre-HAART CD8 count (cells/mm^3)				
<600	0.52 (0.44 to 0.60)	<0.0001		
600–1300	1.00			
≥1300	1.28 (1.12 to 1.46)	0.0002		

to different pre-HAART CD8 cell count strata in adjusted models ($P = 0.58$).

Long-Term CD4 Cell Response in Patients on Uninterrupted HAART

Also in Table 1 are the demographic and clinical characteristics of the subset of 554 patients on uninterrupted HAART for 7 years. These patients started HAART between July 1, 1996, and June 30, 1998, and were among those included in the first analysis. Because the inclusion criteria for the second analysis implied starting HAART in earlier calendar years, there was a higher proportion of men, MSM, and patients originating from WCE/NA/A.

The median CD4 count increased from 221 (interquartile range [IQR]: 80–340) cells/mm^3 at the start to 607 (IQR: 440–800) cells/mm^3 after 7 years of HAART. The median CD4 counts at 7 years were 410, 548, 660, 780, and 870 cells/mm^3 for those with pre-HAART CD4 counts of <50, 50 to 200, 200 to 350, 350 to 500, and ≥500 cells/mm^3, respectively (Fig. 1A). Overall, increases were a median of 136 cells/mm^3 during the first 24 weeks and leveled off over time to 40 cells/mm^3 in weeks 96 through 144 and to 0 cells/mm^3 in weeks 312 through 360. Median increases in CD4 cell counts after 7 years of HAART were between 367 and 410 cells/mm^3 for the 4 pre-HAART CD4 count strata <500 cells/mm^3, whereas increases were 287 cells/mm^3 for patients in the ≥500 cells/mm^3 stratum (see Fig. 1B; Wilcoxon test, $P = 0.007$).

Of 554 patients, 344 (62.1%) had HIV RNA plasma concentrations <500 copies/mL at all measurements taken between 6 months and 7 years after starting HAART. The remaining 210 patients had at least 1 HIV RNA result ≥500 copies/mL. In 80 of them, plasma concentrations were between 500 and 1000 copies/mL, which, in the majority (54 patients), occurred between 6 months and 3 years after the start of HAART. Periods of HIV viremia occurring after initial virologic success were found in 27.6% of the patients from WCE/NA/A and 42.2% of the patients ($P = 0.006$) with a non-WCE/NA/A origin.

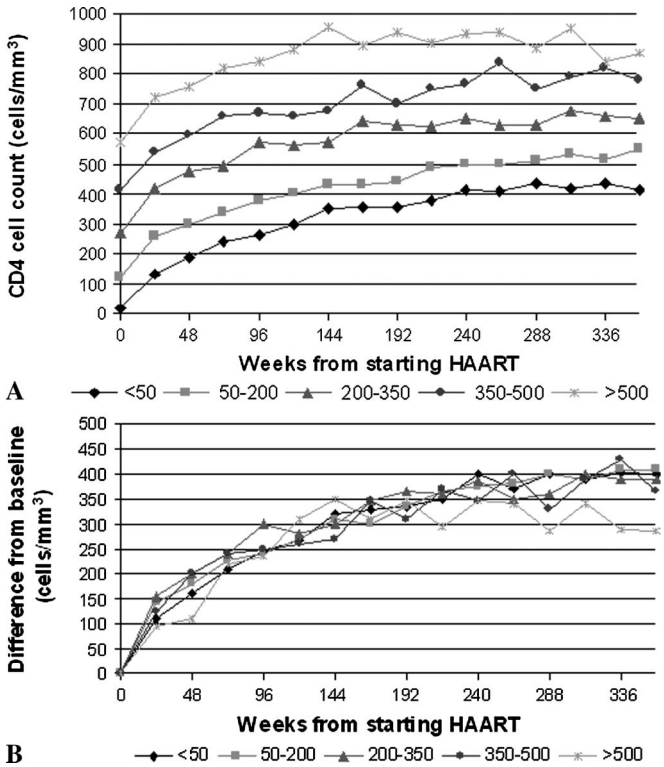

FIGURE 1. Median CD4 cell count (A) and median difference between current CD4 and pre-HAART CD4 cell counts (B) according to pre-HAART CD4 cell strata of <50 (♦), 50 to 200 (■), 200 to 350 (▲), 350 to 500 (●), and ≥500 cells/mm^3 (*) among a subset of 554 patients on uninterrupted HAART for 7 years.

The multivariate longitudinal analyses included 13,528 CD4 cell count measurements for the 554 patients. The median number of measurements per patient was 25 (minimum to maximum: 6–58). The model estimates can be interpreted as the slope or annual rate of change in CD4 cell count (on a square root scale). During the first 6 months, the slope of CD4 cell count was higher in patients with a pre-HAART HIV RNA measurement ≥4.5 $\log_{10}$ copies/mL than in those with <4.5 $\log_{10}$ copies/mL ($P = 0.009$). Furthermore, the slopes of CD4 cell count during the first 6 months were higher in women than in men ($P = 0.003$), in patients originating from regions other than WCE/NA/A ($P = 0.04$), and in patients with a pre-HAART CD8 count <1300 cells/mm^3 as compared with ≥1300 cells/mm^3 ($P = 0.0007$). The effect of body weight at the start of HAART on the slope of CD4 cell count was not significant in univariate or multivariate models (data not shown). Between 6 months and 7 years after starting HAART, the slopes did not differ significantly between men and women, between patients from various origins, or between patients with various levels of pre-HAART CD8 cells or pre-HAART HIV RNA. The slopes of CD4 cell count between 6 months and 7 years were significantly higher in patients <50 years of age as compared with those ≥50 years of age at the start of HAART ($P < 0.0001$). There were no significant differences in CD4 cell count increases between 0 and 6 months according to age. Finally, during periods of viremia (HIV RNA >500 copies/mL after the initial 6 months of HAART), the slope of CD4 cell count was estimated to be lower than when HIV RNA levels were less than 500 copies/mL ($P < 0.0001$).

To facilitate interpretation, estimates from the longitudinal mixed-effect model were back-transformed from the square root scale to the usual absolute CD4 cell count scale. The estimated median CD4 count after 6 months of HAART was 448 (95% confidence interval [CI]: 419 to 478) cells/mm^3 for a male reference patient of Western origin aged <50 years who started HAART with <1300 CD8 cells/mm^3, 310 CD4 cells/mm^3, and HIV RNA <4.5 $\log_{10}$ copies/mL. This is compared with 504 (459–551) CD4 cells/mm^3 for a female patient, 483 (457–509) cells/mm^3 for a patient with a pre-HAART HIV RNA level ≥4.5 $\log_{10}$ copies/mL, 403 (370–437) cells/mm^3 for a patient with ≥1300 CD8 cells at the start of HAART, and 418 (381–456) cells/mm^3 for a patient not born in WCE/NA/A. The estimated median CD4 count after 7 years of uninterrupted HAART for the reference patient was 704 (656–754) cells/mm^3 compared with 585 (522–652) cells/mm^3 for a patient aged ≥50 years at the start of HAART and 648 (598–701) cells/mm^3 for a patient with viremia >500 copies/mL at all HIV RNA measurements between 5 and 7 years. The estimated median CD4 cell values and values at other time points are graphically depicted in Figure 2.

Decreases in CD4 Cell Count in Virologically Suppressed Patients

Finally, we selected 366 patients who took 7 years of uninterrupted HAART and in whom all measured HIV RNA levels between 6 months and 5 years after starting HAART were <500 copies/mL. Their distribution over the 5 pre-HAART CD4 cell strata and the other baseline variables was similar to that in the previous longitudinal analysis. The estimated median CD4 count at 5 years after the start of HAART for patients aged <50 years was 631 (IQR: 459–812) cells/mm^3 and 489 (IQR: 412–725) cells/mm^3 for those aged ≥50 years (Wilcoxon test, $P = 0.03$). In total, 150 patients had negative CD4 cell slopes between 5 and 7 years of uninterrupted HAART use. Variables independently associated with this outcome, as identified in a multivariate logistic regression analysis, are shown in Table 3 and were age ≥50 years at the start of HAART (odds ratio [OR] for a negative slope between 5 and 7 years = 3.01 [95% CI: 1.60 to 5.67] compared with age <50 years); at least 1 HIV RNA measurement ≥1000 copies/mL compared with all HIV RNA measurements <1000 copies/mL between 5 and 7 years after starting HAART (OR = 6.10 [95% CI: 2.12 to 17.51]); and, finally, a higher CD4 cell count at 5 years. Compared with patients with ≥800 cells/mm^3 at 5 years, the OR for patients with <400 cells/mm^3 was 2.23 (IQR: 1.10–4.51). The OR for patients with 400 to 600 CD4 cells/mm^3 at 5 years was 1.07 (IQR: 0.54–2.11; $P = 0.85$), and the OR for patients with 600 to 800 cells/mm^3 at 5 years was 1.08 (IQR: 0.54–2.15; $P = 0.82$); neither was significantly different from those for patients with <400 cells/mm^3 at 5 years. When age was

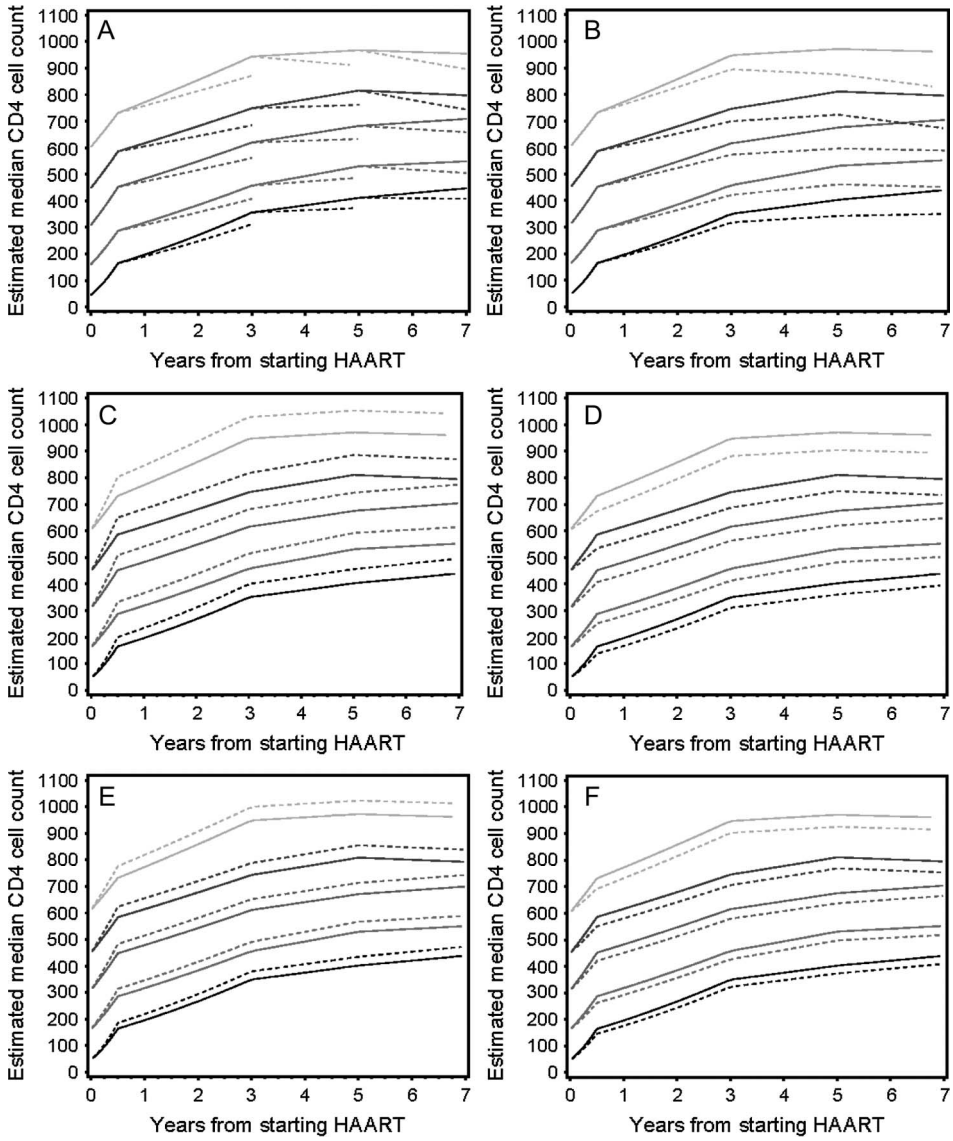

FIGURE 2. A–F, Estimated long-term median CD4 cell response according to the 5 pre-HAART CD4 cell count strata of <50, 50 to 200, 200 to 350, 350 to 500, and ≥500 cells/mm^3. The solid lines in each graph denote the median CD4 cell response for the reference patient: a male patient originating from WCE/NA/A who is <50 years of age at the start of HAART with a pre-HAART HIV RNA level <4.5 $\log_{10}$ copies/mL and a pre-HAART CD8 count <1300 cells/mm^3 and in whom all HIV RNA measurements between 6 months and 7 years after starting HAART were <500 copies/mL. The dashed lines in A display the median CD4 cell response for patients with all HIV RNA measurements ≥500 copes/mL between 6 months and 3 years, between 3 and 5 years, and between 5 and 7 years after starting HAART. The dashed lines in the other parts of this figure display the median CD4 cell response for patients ≥50 years of age at the start of HAART (B), female patients (C), patients with a pre-HAART CD8 cell count ≥1300 cells/mm^3 (D), patients with a pre-HAART HIV RNA level ≥4.5 $\log_{10}$ copies/mL (E), and patients not from WCE/NA/A origin (F).

included in the model as a continuous variable, the OR was 1.027 (95% CI: 1.003 to 1.052, $P = 0.02$) for each year by which the starting age was increased. In multivariate analysis, region of origin ($P = 0.81$), pre-HAART HIV RNA level ($P = 0.55$), or gender ($P = 0.55$) was not associated with negative CD4 cell slopes between 5 and 7 years, nor was the pre-HAART CD4 cell count ($P = 0.66$) after controlling for the CD4 cell count at 5 years.

DISCUSSION

We performed 3 types of analyses. First, we evaluated determinants of CD4 cell count recovery up to 800 cells/mm^3 in a cohort of HAART-naive patients. Second, we evaluated changes in CD4 cell count in patients on uninterrupted HAART for 7 years to determine the maximum capacity of the immune system to restore CD4 cell numbers. Finally, we determined predictors for decreases in CD4 cell count after 5 years of virologically successful uninterrupted HAART. The first 2 analyses showed that 7 years after starting HAART, patients starting with lower pre-HAART CD4 counts experienced less restoration of CD4 cell counts than patients starting with higher pre-HAART CD4 cell counts. The third analysis showed that the "plateau effect" found after long-term CD4 cell restoration is associated with achievement of CD4 levels in the normal range. Plateauing of CD4 cell counts at a less than normal range is associated with insufficient suppression of HIV replication and with older age at the start of HAART. The strength of this study is the long follow-up (7 years) in a large number of naive patients with a variety of pre-HAART CD4 cell counts. We did not look at differences in CD4 cell response between individual drugs or drug classes because that is beyond the scope of this report and is the topic of a future analysis.

The largest gains in the number of CD4 cells occurred in the first 6 months after starting HAART, presumably because

TABLE 3. Predictors of a Plateauing CD4 Cell Count Between 5 and 7 Years After Initiating HAART in 366 Patients With HIV RNA Plasma Concentrations <500 Copies/mL Between 6 Months and 5 Years of Uninterrupted HAART

	No.	No. With Plateau	Univariate OR (95% CI)	*P*	Multivariate OR (95% CI)	*P*
Gender						
Male	328	136	1.00			
Female	38	14	0.82 (0.41 to 1.65)	0.58		
Transmission risk group						
Homosexual	238	100	1.00			
Heterosexual	84	33	0.89 (0.54 to 1.48)	0.66		
Injection drug user	7	3	1.04 (0.23 to 4.73)	0.96		
Other	37	14	0.84 (0.41 to 1.71)	0.63		
Region of origin						
WCE/NA/A	311	127	1.00			
Other	55	23	0.93 (0.52 to 1.68)	0.81		
Pre-HAART CD4 cells/mm^3						
<50	70	29	1.18 (0.65 to 2.17)	0.59		
50–200	88	33	1.00 (0.57 to 1.78)	0.99		
200–350	115	43	1.00			
350–500	53	24	1.39 (0.72 to 2.68)	0.33		
>500	40	21	1.85 (0.89 to 3.83)	0.10		
CD4 cells/mm^{3}at 5 years						
<400	60	23	1.00		1.00	
400–600	108	40	0.95 (0.49 to 1.81)	0.87	1.07 (0.54 to 2.11)	0.85
600–800	105	38	0.91 (0.47 to 1.76)	0.78	1.08 (0.54 to 2.15)	0.82
≥800	93	49	1.72 (0.88 to 3.35)	0.11	2.23 (1.10 to 4.51)	0.025
Clinical stage at 5 years						
CDC-A, B	185	111	1.00			
CDC-C	181	39	0.89 (0.56 to 1.43)	0.63		
Age at start of HAART (y)						
<50	317	121	1.00		1.00	
≥50	49	29	2.35 (1.27 to 4.34)	0.006	3.01 (1.60 to 5.67)	0.0006
Viremia between 5 and 7 years*						
None	346	135	1.00		1.00	
At least once	22	15	4.69 (1.66 to 13.19)	0.003	6.10 (2.12 to 17.51)	0.0008

*At least 1 HIV RNA measurement >1000 copies/mL.

of redistribution of CD4 cells from lymphoid tissue.[35] Thereafter, the rate of increase in CD4 cell counts gradually slowed. Between 5 and 7 years of uninterrupted HAART, CD4 cells still continued to increase in patients with a pre-HAART CD4 count less than 350 cells/mm^3. Because of the slow rate of increase, however, restoration to CD4 cell levels ≥800 cells/mm^3 is a lengthy process and may not be feasible for patients who start HAART with <200 CD4 cells/mm^3.

The association between periods of HIV production despite HAART and reaching a CD4 cell plateau earlier and (thus) at a lower level confirms the importance of monitoring of HIV RNA and keeping plasma levels to less than 500 copies/mL. The single study[31] that did not find a plateau effect, in contrast to our study and others,[29,30] might reflect different levels of ongoing viremia or different age distributions among studies. The association of a lower CD4 cell count plateau with older age (≥50 years of age at the start of HAART) could reflect the lower normal CD4 cell range reported in older healthy individuals.[36–38] Larger CD4 cell gain in treated patients has previously been associated with younger age,[16,28] and less gain has been attributed to lower thymic function with older age.[14,15] Because patients with low CD4 cell counts remain at risk of developing new AIDS events after starting HAART, it may be appropriate to start antiretroviral therapy in older (ie, ≥50 years of age) patients earlier than in younger patients.

Factors associated with differences in CD4 cell response during the first 6 months of HAART include gender, region of origin, pre-HAART HIV RNA plasma levels, and the number of pre-HAART CD8 cells. These differences persisted over the study period. The long-term CD4 cell response is, however, largely determined by age and by the degree of HIV RNA suppression. The findings that patients from sub-Saharan Africa, and possibly from Southeast Asia, have a slower recovery to 800 CD4 cells/mm^3 may indicate geographic variation in normal CD4 ranges but also differences in adherence. The latter may be confirmed by our finding of a higher proportion of patients experiencing periods of HIV viremia while on uninterrupted HAART. Normal CD4 cell counts in HIV-seronegative Dutch individuals are reported to be higher than

in HIV-seronegative individuals from Tanzania, Ethiopia, Kenya, and China[39–44] but lower than in such individuals in Cameroon and Uganda.[45,46] Seropositive patients from Ethiopia experience a slower decline in CD4 cells than seen in Dutch patients, but time to AIDS is not significantly different between these Dutch and Ethiopian patients.[47] This suggests that immune restoration in patients originating from regions with low normal CD4 cell numbers might be slower than in patients with high normal CD4 cell numbers even if they are fully adherent.

The higher increase in CD4 cell count in women than in men follows most probably from the higher CD4 cell counts in uninfected women than in uninfected men[33,38] but might also reflect prescription of different antiretroviral drugs between men and women. As in other studies,[48–50] our patients with a lower pre-HAART CD8 cell count experienced higher rates of CD4 cell increase during the first 6 months. The reason for this relation is unclear, but it might be related to the level of CD4 and CD8 cell activation.[17] CD4 and CD8 cell activation was not measured in this cohort, however.

It is well established that patients with pre-HAART CD4 cell counts <200 cells/mm^3 are much more likely to progress to AIDS or death.[10] Combined observational cohort data suggest that the long-term prognosis might be better for patients starting HAART when having ≥ 350 CD4 cells/mm^3 as compared with 200 to 350 cells/mm^3,[51] although the absolute risk difference is small. In our subset of patients who used HAART uninterrupted for 7 years, the restoration of CD4 cell counts was sufficient to minimize the risk for development of AIDS, even for those starting HAART with CD4 cell counts less than 200 cells/mm^3. These patients were likely to be adherent, and those who died were excluded from the analysis. Therefore, the results of the longitudinal model and the analysis of the decrease in CD4 cell response after 5 years of HAART use cannot be generalized to all patients using HAART but do give an estimate of the immune system's maximum capacity for CD4 cell restoration during 7 years of therapy.

CONCLUSIONS

HAART restoration of CD4 cell counts in HIV-infected individuals to levels normally seen in uninfected individuals takes a long time and is not feasible within 7 years in most patients who initiate HAART with CD4 cell counts <350 cells/mm^3. Patients ≥ 50 years of age when starting HAART and patients with periods of viremia (HIV RNA level >500 copies/mL) experience smaller increases and are more likely to reach a CD4 cell plateau earlier and at a lower level. Given the better toxicity profiles of the currently used antiretroviral combinations, particularly in patients older than 50 years of age, it may be beneficial to start HAART earlier than current guidelines recommend.

REFERENCES

1. Fellay J, Boubaker K, Ledergerber B, et al. Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. *Lancet*. 2001;358:1322–1327.
2. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med*. 2003;349: 1993–2003.
3. d'Arminio Monforte A, Sabin CA, Phillips AN, et al. Cardio- and cerebrovascular events in HIV-infected persons. *AIDS*. 2004;18:1811–1817.
4. Kuritzkes DR. Preventing and managing antiretroviral drug resistance. *AIDS Patient Care STDS*. 2004;18:259–273.
5. Borleffs J, Tuut MK, Boer K, et al. *Richtlijn antiretrovirale behandeling* [in Dutch]. Utrecht, The Netherlands: Nederlandse Vereniging van AIDS Behandelaren (NVAB); 2005.
6. Yeni PG, Hammer SM, Carpenter CC, et al. Antiretroviral treatment for adult HIV infection in 2002: updated recommendations of the International AIDS Society-USA Panel. *JAMA*. 2002;288:222–235.
7. Gazzard B. British HIV Association (BHIVA) guidelines for the treatment of HIV-infected adults with antiretroviral therapy (2005). *HIV Med*. 2005; 6 (Suppl 2) :1–61.
8. Palella FJ Jr, Oria-Knoll M, Chmiel JS, et al. Survival benefit of initiating antiretroviral therapy in HIV-infected persons in different CD4+ cell strata. *Ann Intern Med*. 2003;138:620–626.
9. Hogg RS, Yip B, Chan KJ, et al. Rates of disease progression by baseline CD4 cell count and viral load after initiating triple-drug therapy. *JAMA*. 2001;286:2568–2577.
10. Egger M, May M, Chene G, et al. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet*. 2002;360:119–129.
11. Opravil M, Ledergerber B, Furrer H, et al. Clinical efficacy of early initiation of HAART in patients with asymptomatic HIV infection and CD4 cell count $>350 \times 10(6)$/l. *AIDS*. 2002;16:1371–1381.
12. Zhang L, Ramratnam B, Tenner-Racz K, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med*. 1999;340:1605–1613.
13. Wood E, Yip B, Hogg RS, et al. Full suppression of viral load is needed to achieve an optimal CD4 cell count response among patients on triple drug antiretroviral therapy. *AIDS*. 2000;14:1955–1960.
14. Teixeira L, Valdez H, McCune JM, et al. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *AIDS*. 2001;15:1749–1756.
15. Smith KY, Valdez H, Landay A, et al. Thymic size and lymphocyte restoration in patients with human immunodeficiency virus infection after 48 weeks of zidovudine, lamivudine, and ritonavir therapy. *J Infect Dis*. 2000;181:141–147.
16. Viard JP, Mocroft A, Chiesi A, et al. Influence of age on CD4 cell recovery in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy: evidence from the EuroSIDA study. *J Infect Dis*. 2001;183:1290–1294.
17. Hunt PW, Martin JN, Sinclair E, et al. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *J Infect Dis*. 2003;187:1534–1543.
18. Giorgi JV, Hultin LE, McKeating JA, et al. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *J Infect Dis*. 1999;179:859–870.
19. Benveniste O, Flahault A, Rollot F, et al. Mechanisms involved in the low-level regeneration of CD4+ cells in HIV-1-infected patients receiving highly active antiretroviral therapy who have prolonged undetectable plasma viral loads. *J Infect Dis*. 2005;191:1670–1679.
20. Gougeon ML. Apoptosis as an HIV strategy to escape immune attack. *Nat Rev Immunol*. 2003;3:392–404.
21. Greub G, Ledergerber B, Battegay M, et al. Clinical progression, survival, and immune recovery during antiretroviral therapy in patients with HIV-1 and hepatitis C virus coinfection: the Swiss HIV Cohort Study. *Lancet*. 2000;356:1800–1805.
22. Al-Harthi L, Voris J, Du W, et al. Evaluating the impact of hepatitis C virus (HCV) on highly active antiretroviral therapy-mediated immune responses in HCV/HIV-coinfected women: role of HCV on expression of primed/memory T cells. *J Infect Dis*. 2006;193:1202–1210.
23. Tarwater PM, Margolick JB, Jin J, et al. Increase and plateau of CD4 T-cell counts in the 3(½) years after initiation of potent antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2001;27:168–175.
24. Notermans DW, Pakker NG, Hamann D, et al. Immune reconstitution after 2 years of successful potent antiretroviral therapy in previously untreated human immunodeficiency virus type 1-infected adults. *J Infect Dis*. 1999; 180:1050–1056.

25. 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; 163:2187–2195.
26. Valdez H, Connick E, Smith KY, et al. Limited immune restoration after 3 years' suppression of HIV-1 replication in patients with moderately advanced disease. *AIDS*. 2002;16:1859–1866.
27. Smith CJ, Sabin CA, Lampe FC, et al. The potential for CD4 cell increases in HIV-positive individuals who control viraemia with highly active antiretroviral therapy. *AIDS*. 2003;17:963–969.
28. Kaufmann GR, Bloch M, Finlayson R, et al. The extent of HIV-1-related immunodeficiency and age predict the long-term CD4 T lymphocyte response to potent antiretroviral therapy. *AIDS*. 2002;16:359–367.
29. Garcia F, de LE, Plana M, et al. Long-term CD4+ T-cell response to highly active antiretroviral therapy according to baseline CD4+ T-cell count. *J Acquir Immune Defic Syndr*. 2004;36:702–713.
30. Kaufmann GR, Furrer H, Ledergerber B, et al. Characteristics, determinants, and clinical relevance of CD4 T cell recovery to <500 cells/μL in HIV type 1-infected individuals receiving potent antiretroviral therapy. *Clin Infect Dis*. 2005;41:361–372.
31. Hunt PW, Deeks SG, Rodriguez B, et al. Continued CD4 cell count increases in HIV-infected adults experiencing 4 years of viral suppression on antiretroviral therapy. *AIDS*. 2003;17:1907–1915.
32. Gras L, van Sighem A, Smit C, et al. Monitoring of human immunodeficiency virus (HIV) infection in the Netherlands. 2006. Amsterdam, Stichting HIV Monitoring. Available at: http://www.hiv-monitoring.nl. Accessed March 12, 2007.
33. Bofill M, Janossy G, Lee CA, et al. Laboratory control values for CD4 and CD8 T lymphocytes. Implications for HIV-1 diagnosis. *Clin Exp Immunol*. 1992;88:243–252.
34. Burnham K, Anderson D. *Model Selection and Inference: A Practical Information-Theoretic Approach*. New York: Springer Verlag; 1988.
35. Pakker NG, Notermans DW, de Boer RJ, et al. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. *Nat Med*. 1998; 4:208–214.
36. McNerlan SE, Alexander HD, Rea IM. Age-related reference intervals for lymphocyte subsets in whole blood of healthy individuals. *Scand J Clin Lab Invest*. 1999;59:89–92.
37. Bisset LR, Lung TL, Kaelin M, et al. Reference values for peripheral blood lymphocyte phenotypes applicable to the healthy adult population in Switzerland. *Eur J Haematol*. 2004;72:203–212.
38. Jentsch-Ullrich K, Koenigsmann M, Mohren M, et al. Lymphocyte subsets' reference ranges in an age- and gender-balanced population of 100 healthy adults—a monocentric German study. *Clin Immunol*. 2005; 116:192–197.
39. Kassa E, Rinke de Wit TF, Hailu E, et al. Evaluation of the World Health Organization staging system for HIV infection and disease in Ethiopia: association between clinical stages and laboratory markers. *AIDS*. 1999; 13:381–389.
40. Kalinkovich A, Weisman Z, Burstein R, et al. Standard values of T-lymphocyte subsets in Africa. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1998;17:183–185.
41. Tsegaye A, Messele T, Tilahun T, et al. Immunohematological reference ranges for adult Ethiopians. *Clin Diagn Lab Immunol*. 1999; 6:410–414.
42. Messele T, Abdulkadir M, Fontanet AL, et al. Reduced naive and increased activated CD4 and CD8 cells in healthy adult Ethiopians compared with their Dutch counterparts. *Clin Exp Immunol*. 1999;115: 443–450.
43. Urassa W, Bakari M, Sandstrom E, et al. Rate of decline of absolute number and percentage of CD4 T lymphocytes among HIV-1-infected adults in Dar es Salaam, Tanzania. *AIDS*. 2004;18:433–438.
44. Kam KM, Leung WL, Kwok MY, et al. Lymphocyte subpopulation reference ranges for monitoring human immunodeficiency virus-infected Chinese adults. *Clin Diagn Lab Immunol*. 1996;3:326–330.
45. Zekeng L, Sadjo A, Meli J, et al. T-lymphocyte subset values among healthy Cameroonians. *J Acquir Immune Defic Syndr Hum Retrovirol*. 1997;14:82–83.
46. Tugume SB, Piwowar EM, Lutalo T, et al. Hematological reference ranges among healthy Ugandans. *Clin Diagn Lab Immunol*. 1995;2:233–235.
47. Mekonnen Y, Geskus RB, Hendriks JC, et al. Low CD4 T cell counts before HIV-1 seroconversion do not affect disease progression in Ethiopian factory workers. *J Infect Dis*. 2005;192:739–748.
48. Smith CJ, Sabin CA, Youle MS, et al. Factors influencing increases in CD4 cell counts of HIV-positive persons receiving long-term highly active antiretroviral therapy. *J Infect Dis*. 2004;190:1860–1868.
49. Kaufmann GR, Zaunders JJ, Cunningham P, et al. Rapid restoration of CD4 T cell subsets in subjects receiving antiretroviral therapy during primary HIV-1 infection. *AIDS*. 2000;14:2643–2651.
50. Kaufmann GR, Khanna N, Weber R, et al. Long-term virological response to multiple sequential regimens of highly active antiretroviral therapy for HIV infection. *Antivir Ther*. 2004;9:263–274.
51. Sterne J, May M, Costagliola D, et al, and the ART Cohort Collaboration. Estimating the optimum CD4 threshold for starting HAART in ART-naïve HIV-infected individuals [abstract 525]. Presented at: 13th Conference on Retroviruses and Opportunistic Infections; 2006; Denver.

APPENDIX

The ATHENA database is supported by a grant from the Dutch Health Minister and was set up and is maintained by the HIV Monitoring Foundation. The physicians and data analysts include the following (*site coordinating physicians): F. de Wolf (Director), D. O. Bezemer, L. A. J. Gras, A. M. Kesselring, A. I. van Sighem, C. Smit, and S. Zhang (data analysis group), and S. Zaheri (data collection), HIV Monitoring Foundation, Amsterdam; W. Bronsveld* and M. E. Hillebrand-Haverkort, Medical Center Alkmaar, Alkmaar; J. M. Prins*, J. Branger, J. K. M. Eeftinck Schattenkerk, J. Gisolf, M. H. Godfried, J. M. A. Lange, K. D. Lettinga, J. T. M. van der Meer, F. J. B. Nellen, T. van der Poll, P. Reiss, Th.A. Ruys, R. Steingrover, G. van Twillert, J.N. Vermeulen, S. M. E. Vrouenraets, M. van Vugt, and F. W. M. N. Wit, Academic Medical Center of the University of Amsterdam, Amsterdam; T. W. Kuijpers, D. Pajkrt, and H. J. Scherpbier, Emma Children's Hospital, Amsterdam; A. van Eeden, Medical Center Jan van Goyen, Amsterdam; K. Brinkman*, G. E. L. van den Berk, W. L. Blok, P. H. J. Frissen, J. C. Roos, W. E. M. Schouten, and H. M. Weigel, Onze Lieve Vrouwe Gasthuis, Amsterdam; J. W. Mulder*, E. C. M. van Gorp, and J. Wagenaar, Slotervaart Hospital, Amsterdam; J. Veenstra*, St. Lucas Andreas Hospital, Amsterdam; S. A. Danner*, M. A. van Agtmael, F. A. P. Claessen, R. M. Perenboom, A. Rijkeboer, and M. G. A. van Vonderen, Free University Medical Center, Amsterdam; C. Richter* and J. van der Berg, Hospital Rijnstate, Arnheim; R. Vriesendorp* and F. J. F. Jeurissen, Medical Center Haaglanden, Westeinde, Den Haag; R. H. Kauffmann* and K. Pogány, Haga Hospital, Leyenburg, Den Haag; B. Bravenboer*, Catharina Hospital, Eindhoven; C. H. H. ten Napel*, G. J. Kootstra, Medisch Spectrum Twente, Enschede; H. G. Sprenger*, S. van Assen, and J. T. M. van Leeuwen, University Medical Center Groningen, Groningen; R. Doedens and E. H. Scholvinck, University Medical Center Beatrix kliniek, Groningen; R. W. ten Kate* and R. Soetekouw, Kennemer Gasthuis, Haarlem; D. van Houte* and M. B. Polée, Medical Center Leeuwarden, Leeuwarden; F. P. Kroon*, P. J. van den Broek, J. T. van Dissel, and E. F. Schippers, Leiden University Medical Center, Leiden; G. Schreij*, S. van der Geest, S. Lowe, and A. Verbon, Academic Hospital Maastricht, Maastricht; P. P. Koopmans*, R. van Crevel, R. de Groot, M. Keuter, F. Post, A. J. A. M. van der Ven, and A. Warris, Radboud University Nijmegen Medical

Center, Nijmegen; M. E. van der Ende*, I. C. Gyssens, M. van der Feltz, J. L. Nouwen, B. J. A. Rijnders, and T. E. M. S. de Vries, Erasmus Medical Center, Rotterdam; G. Driessen, M. van der Flier, and N. G. Hartwig, Erasmus Medical Center Sophia, Rotterdam; J. R. Juttman*, M. E. E. van Kasteren, and C. van de Heul, St. Elisabeth Hospital, Tilburg; I. M. Hoepelman*, M. M. E. Schneider, M. J. M. Bonten, J. C. C. Borleffs, P. M. Ellerbroek, C. A. J. J. Jaspers, T. Mudrikove, C. A. M. Schurink, and E. H. Gisolf, University Medical Center Utrecht, Utrecht; S. P. M. Geelen, T. F. W. Wolfs, and T. Faber, Wilhelmina Children's Hospital, Utrecht; A. A. Tanis*, Hospital Walcheren, Vlissingen; P. H. P. Groeneveld*, Isala Clinics, Zwolle; J. G. den Hollander*, Medical Center Rijnmond-Zuid, Clara, Rotterdam; and A. J. Duits and K. Winkel, St. Elisabeth Hospitaal/Stichting Rode Kruis Bloedbank, Willemstad, Curaçao.