Table 2
Characteristics of DLBCL cases

	All patients ($n = 67$)	Pre-HAART era (1987–1997) ($n = 37$)	HAART era (1997–2005) ($n = 30$)	P	HAART(−) ($n = 56$)	HAART(+) ($n = 11$)	P
Sex (% female)	6.0%	8.1%	3.3%	0.390 (F)	5.3%	9.1%	0.877 (F)
Age (year), median (mean, range)	43 (43.0, 12–74)	41 (41.9, 12–74)	44.5 (44.3, 30–64)	0.384 (MW)	42 (42.3, 12–74)	49 (46.3, 29–63)	0.348 (MW)
CD4 (cells/μl), median (mean, range)	20 (67.0, 0–496)	7 (47.6, 0–496)	67.5 (91.0, 0–414)	<0.01 (MW)	14 (58.5, 0–496)	58 (110.5, 0–414)	0.101 (MW)
EBER (%, positive)	79.4%	91.4%	64.0%	<0.01 (C)	78.9%	81.8%	0.595 (F)
KSHV, cases (%)	4.5%	5.4%	3.3%	0.579 (F)	5.4%	0%	0.579 (F)
Risk factor (% sexual)	88.1%	83.8%	93.3%	0.208 (F)	87.5%	90.9%	0.609 (F)
Prognosis (% death)	80.6%	89.1%	70.0%	0.048(C)	82.1%	63.6%	0.163 (F)
CNS involvement* (%)	55.1%	61.8%	40.0%	0.158 (C)	57.1%	42.9%	0.382 (F)
LN involvement* (%)	24.5%	9.1%	56.3%	<0.01 (F)	21.4%	42.9%	0.222 (C)

* CNS and LN involvements were investigated only by autopsy. See Table 1 for abbreviations.

of these KSHV-positive cases have been published previously [11,33,34].

To analyze whether histological subtype or occurrence of EBV infection are related to the degree of immunodeficiency, we reviewed CD4 cell counts (Fig. 2). The median of CD4 cell count of whole patients was 25.5 cells/μl (mean, 89.8 cells/μl; range, 0–519). CD4 cell counts of patients with DLBCL (median, 20 cells/μl; mean, 67 cells/μl) were significantly lower than those with BL (median, 177.5 cells/μl; mean, 250 cells/μl) (Fig. 2a, $P < 0.01$, Mann–Whitney test). When CD4 cell counts were plotted against EBV status, there was a significant difference between EBV-positive-lymphoma cases (median, 11 cells/μl; mean, 71 cells/μl) and EBV-negative-lymphoma cases (median, 101 cells/μl; mean, 166 cells/μl) ($P < 0.01$, Mann–Whitney test), revealing a lower CD4 cell count in EBV-positive lymphoma (Fig. 2b).

3.2. Comparison of AIDS-related lymphoma between the pre-HAART era and the HAART era, and between HAART users and non-users

To estimate the changes in the characteristics of AIDS-related lymphoma before and after the introduction of HAART, a total of 86 patients were divided into two groups: the pre-HAART era (1987–1997) including 47 patients and the HAART era (1998–2005) including 39 patients. The HAART era group contained only 15 patients who had received HAART prior to the diagnosis of lymphoma. Thus, we also compared the group of HAART users and non-users. Table 1 and Fig. 1 show the characteristics of patients in the pre-HAART era and the HAART era, and HAART users and non-users.

Lymphoma was found in 37 of 127 (29%) autopsies in the pre-HAART era, and 16 of 71 (23%) autopsies in the HAART era. The incidences detected by autopsy did not differ statistically between the pre-HAART era and the HAART era ($P = 0.31$, Chi-square test). With regard to histological type, the prevalence of DLBCL was stable in both the pre-HAART era (78%) and the HAART era (77%) (Fig. 1a). A similar trend was observed among HAART users (73%) and non-users (79%). In contrast, we found an increase in patients with BL from one (2%) in the pre-HAART era to five (13%) in the HAART era. Among five cases of BL in the HAART era, only one had received HAART before diagnosis, suggesting that HAART might not be related to the increase of BL.

Patients had a significantly higher CD4 cell count at the time of diagnosis in the HAART era (median, 75 cells/μl)

Table 3
Characteristics of BL cases

	All patients ($n = 6$)	Pre-HAART era (1987–1997) ($n = 1$)	HAART era (1997–2005) ($n = 5$)	HAART(−) ($n = 5$)	HAART(+) ($n = 1$)
Sex (% female)	0%	0%	0%	0%	0%
Age (year), median (mean, range)	37.5 (34.7, 25–41)	41 (41, 41–41)	36 (33.4, 25–40)	39 (34.4, 25–41)	36 (36, 36–36)
CD4 (cells/μl), median (mean, range)	177.5 (250.2, 128–519)	205 (205, 205–205)	150 (259.2, 128–519)	150 (196.4, 128–361)	519 (519, 519–519)
EBER (%, positive)	1/6 (16.7%)	0/1 (0%)	1/5 (20%)	1/5 (20%)	0/1 (0%)
KSHV, cases (%)	0%	0%	0%	0%	0%
Risk factor (% sexual)	6/6 (100%)	1/1 (100%)	5/5 (100%)	5/5 (100%)	1/1 (100%)
Prognosis (% death)	1/6 (16.7%)	1/1 (100%)	0/5 (0%)	1/5 (20%)	0/1 (0%)
CNS involvement* (%)	1/1 (100%)	1/1 (100%)	–	1/1 (100%)	–
LN involvement* (%)	1/1 (100%)	1/1 (100%)	–	1/1 (100%)	–

* CNS and LN involvements were investigated only by autopsy. See Table 1 for abbreviations.

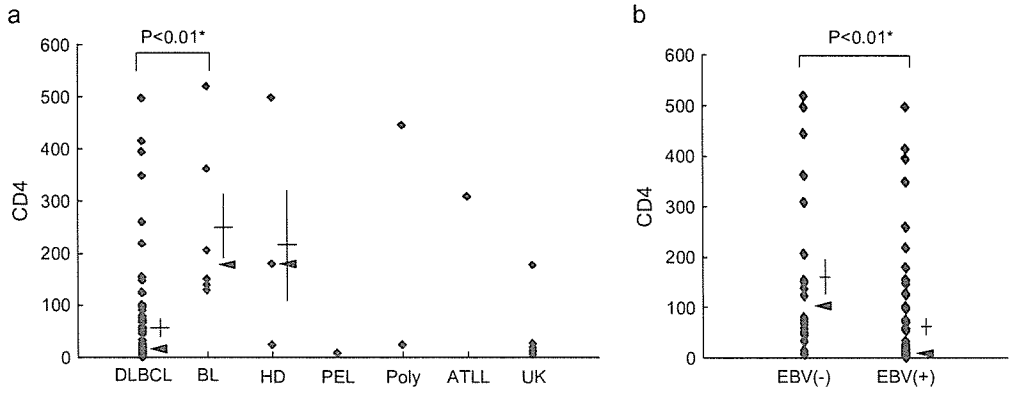

Fig. 2. Comparison of CD4 cell counts among histological subtypes and EBV-positive rate. CD4 cell counts (cells/ml) were compared among histological subtypes (a), and EBV-positive rate (b). Horizontal and vertical bars indicate means and standard errors, respectively. Arrowheads indicate medians. *P value was calculated using the Mann–Whitney test.

than in the pre-HAART era (8 cells/μl, $P < 0.01$, Mann–Whitney test). The CD4 cell count was also significantly higher in the HAART users (median, 154 cells/μl) than in the non-users (20 cells/μl, $P < 0.01$, Mann–Whitney test). In so far as the HAART era, the CD4 cell count was still higher in the HAART user (15 cases, median 154 cells/μl) than in the non-users (26 cases, median, 69.5 cells/μl), however, the difference is not significant ($P = 0.13$, Mann–Whitney test). We also compared CD4 cell counts in non-HAART users between the pre-HAART era and the HAART era. CD4 cell counts of non-HAART users in the HAART era (median, 69.5 cells/μl; mean, 89.6 cells/μl; range, 3–394 cells/μl) were significantly higher than those in the pre-HAART era (median, 8 cells/μl; mean, 57.2 cells/μl; range, 0–496 cells/μl, $P < 0.01$, Mann–Whitney test), implying that lymphoma occurred at higher CD4 cell count in the HAART era than in the pre-HAART era, regardless of HAART introduction. Although we observed a significant decrease in mortality from 87% in the pre-HAART era to 56% in the HAART era ($P < 0.01$), it did not differ significantly between the HAART users (60%) and non-users (62%) ($P = 0.887$). With respect to EBV status, EBV was detected less frequently in the HAART era (58%) than in the pre-HAART era (88%) ($P < 0.01$), but the prevalence of EBV-positive cases was indistinguishable between HAART users (73%) and non-users (74%) (Fig. 1b). This suggests that, at least in these cases, HAART did not affect the EBV-positive rate of AIDS-related lymphoma. Detection of KSHV was a rare event both in the pre-HAART era and in the HAART era. Decreased CNS involvement and increased LN involvement were observed between the pre-HAART era and the HAART era; however, the differences were not statistically significant. There was no significant change in sex, age and risk factors for HIV transmission during the two periods and between HAART users and non-users (Table 1, Fig. 1c and d).

The changing characteristics of the two major types, DLBCL and BL, are summarized in Tables 2 and 3. Interestingly, the EBV-positive rate in DLBCL significantly decreased from 91% in the pre-HAART era to 64% in the HAART era (Table 2). A significant increase in LN involvement in DLBCL was observed during that period. However, there were no significant differences in CD4 cell count, EBV- and KSHV-positive rates, risk factors, prognosis, and CNS or LN involvement in DLBCL between HAART users and non-users (Table 2). From the data on BL, the prognosis of BL in the HAART era was better than that in the pre-HAART era; however, it is impossible to determine any trend because of the small number of cases (Table 3).

4. Discussion

In the present study, we demonstrated changes in incidence and other clinicopathological aspects of Japanese AIDS-related lymphoma in the HAART era. Our data demonstrated a decrease in EBV-positive lymphoma and an increase in BL among AIDS-related lymphoma patients in Japan. A correlation between the change in the EBV-positive rate and use of HAART is not clear, because the EBV-positive rate did not change between HAART users and non-users. To our knowledge, this is the first report describing a change in the EBV-positive rate in AIDS-related lymphoma between the pre-HAART era and the HAART era. As already reported from European countries and the US [12], decreased CNS involvement and increased BL involvement in the HAART era were also observed among cases of AIDS-related lymphoma in Japan.

The incidence of AIDS-related lymphoma detected at autopsy was higher in Japan (27%) than in the US (12%) [35]. However, histological subtypes of AIDS-related lymphoma in Japan seem to be similar to those in western countries. BL, which represents 7–10% of all AIDS-related lymphoma in western countries [18], was observed in 7% of all AIDS-related lymphoma in Japan. PEL and Hodgkin lymphoma are also very rare in Japan, and there was no plasmablastic lymphoma in our cases. DLBCL is the most common subtype of AIDS-related lymphoma also in Japan. Seventy-eight percent of AIDS-related lymphoma in Japan were DLBCL, whereas about 60% of AIDS-related lymphoma were immunoblastic or primary brain lymphoma that might belong to DLBCL in an European study [18]. It is known that DLBCL is heterogeneous and that it contains some different variants

[36]. DLBCL contains both EBV-positive and EBV-negative cases. The EBV-positive rate of non-AIDS-related DLBCL is about 10% in Japan [29], suggesting heterogeneity in EBV status of DLBCL among non-HIV-1-infected individuals. In the pre-HAART era, about 90% of AIDS-related lymphoma cases were EBV-positive in Japan (Table 1). Many of those cases were classified as DLBCL, immunoblastic variant expressing LMP-1, which was typical of EBV-associated opportunistic lymphoma. In that sense, cases of Japanese AIDS-related lymphoma in the pre-HAART era could be classified into a single category of EBV-associated opportunistic lymphoma. Although the percentage of DLBCL did not change between the pre-HAART era and the HAART era, the EBV-positive rate among DLBCL cases decreased from 91% in the pre-HAART era to 64% in the HAART era (Table 2). It might be said, therefore, that EBV-negative DLBCL increased in the HAART era. This suggests that composition of DLBCL subtypes may have changed during that period.

There is no report so far describing any change in EBV-positive rates in AIDS-related lymphoma between HAART users and non-users. In our cases, only four of 15 patients with EBV-negative lymphoma in the HAART era received HAART, suggesting that the decrease in EBV-positive lymphoma did not correlate with use of HAART. Moreover, only one of six cases of BL received HAART, implying that the increase in BL also did not correlate with use of HAART. Together, although the number of HAART users in the present study was relatively small ($n = 15$), the recent changes in AIDS-related lymphoma might not correlate with use of HAART. Then, what did cause the change in AIDS-related lymphoma in Japan? The EBV-positive rate has been decreasing in nodular sclerosis subtype Hodgkin lymphoma in Tokyo between 1955 and 1999 [37]. Thus, an increase in EBV-negative lymphoma cases may be a recent trend in Japan. Among Japanese children, the sero-positivity to EBV is decreasing prominently in those decades, from 95% in 1980 to around 70% in 2000 [38]. Moreover, seroprevalence of herpes simplex viruses 1 and 2 and cytomegalovirus has declined in recent decades [39,40]. Therefore, we assume that decrease of EBV seroprevalence in the general Japanese population would be associated with the change in AIDS-related lymphoma. Alternatively, lymphoma may have been affected by some environmental changes in Japan. High CD4 cell counts also be partly responsible for changes in AIDS-related lymphoma in Japan. Recent cases of AIDS-related lymphoma occurred at high CD4 cell counts, regardless of HAART (Tables 1 and 2). Since the immune system of HIV-1-infected individuals remains relatively intact with high CD4 cell counts in the HAART era, it may be able to exclude EBV-infected B cells just like in case of immunocompetent individuals. However, some lymphoma cells without EBV infection might evade the immune system, resulting in formation of lymphoma. Thus, it is possible that the increase in EBV-negative lymphoma may be explained by the difference in tumor immunity to lymphoma and infectious immunity to EBV-infected cells.

In conclusion, we reported here the recent change in AIDS-related lymphoma in terms of EBV status, CD4 cell count, and CNS and LN involvements. Although DLBCL is still the predominant subtype of AIDS-related lymphoma in Japan, recent cases of DLBCL in AIDS patients may consist of several subtypes with different pathogenesis from monospecific EBV-associated opportunistic lymphoma with DLBCL morphology. Finally, even though the prognosis of AIDS-related lymphoma is better in the HAART era than in the pre-HAART era, 57% of the patients with AIDS-related lymphoma died of this disease even in the HAART era, indicating that AIDS-related lymphoma is still a very important complication of patients with AIDS in the HAART era.

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The authors declare that they have no competing financial interests.

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Urinary β_2-Microglobulin as a Possible Sensitive Marker for Renal Injury Caused by Tenofovir Disoproxil Fumarate

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ABSTRACT

Tenofovir disoproxil fumarate (TDF) is renally excreted by a combination of glomerular filtration and active tubular secretion, and its renal safety profiles have been reported based on a limited increase of serum creatinine (sCr) levels. However, renal tubular function has not previously been well monitored. We measured sCr and urinary β_2-microglobulin (U-β_2MG) levels cross-sectionally in 70 patients treated with TDF [TDF(+)] and 90 patients on other antiretroviral therapy who had never been exposed to TDF [TDF(−)]. The mean U-β_2MG was significantly higher in TDF(+) patients than that in TDF(−) patients ($p < 0.0001$), though no statistical difference was detected in their creatinine clearance estimated by using the Cockcroft–Gault equation. Multivariate analysis showed that coadministration of boosted lopinavir (LPV) and patients' body weight were associated with U-β_2MG levels in TDF(+) patients. U-β_2MG levels were significantly higher in those who also received boosted LPV [TDF(+)LPV(+)] ($p = 0.0007$), and abnormally high levels were noted in 67.7% of them. Furthermore, in the TDF(+)LPV(+) group, U-β_2MG levels showed significant negative correlation with patients' body weight ($p = 0.0029$) and abnormal U-β_2MG was observed in all six patients with body weight less than 55 kg. In four patients, a rapid fall in U-β_2MG occurred after cessation of TDF. Relative to sCr, U-β_2MG could be a more sensitive marker of renal tubular injury caused by TDF. Boosted LPV co-administration and low body weight may be risk factors for TDF-induced renal tubular dysfunction, probably because these factors are associated with an increase in TDF concentration.

INTRODUCTION

TENOFOVIR DISOPROXIL FUMARATE (TDF) is an orally bioavailable ester prodrug of tenofovir, an acyclic nucleotide analogue with activity against HIV-1, HIV-2, and hepatitis B virus. TDF is administered once daily in HIV treatment and a combination formula with emtricitabine is currently available. TDF does not have high mitochondrial toxicity compared with stavudine[1,2] and it does not induce a systemic hypersensitivity reaction like abacavir.[3] TDF is currently being prescribed for a growing number of HIV-infected patients. One concern regarding use of TDF is its renal toxicity. Several studies showed a limited incidence of renal dysfunction based on the monitoring of serum creatinine (sCr).[4–7] Some cases of TDF-related renal impairment have occurred in patients with underlying systemic or renal diseases.[8–11] However, the majority of the cases of TDF-related renal dysfunction have occurred in patients without any identified risk factor.[12–15] Therefore, careful monitoring of renal function is necessary for the follow-up of TDF-treated patients.

TDF is excreted unchanged in the urine via a combination of glomerular filtration and active tubular secretion.[16] Proximal renal tubular dysfunction and Fanconi's syndrome have been reported to be associated with TDF usage.[10–15,17–19] β_2-Microglobulin is commonly measured in urine by enzyme-linked immunosorbent assay (ELISA) or latex agglutination assay. It is freely filtered at the glomerulus and is avidly taken up and catabolized by the proximal renal tubules. Therefore, high levels of urinary β_2-microglobulin (U-β_2MG) are associated with various pathological conditions involving the proximal renal tubule.[20] In the present study, we compared U-β_2MG in TDF-treated patients and those on other antiretroviral treatment who had never been exposed to TDF, and assessed the suitability of U-β_2MG as a sensitive marker of TDF-induced proximal renal

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tubular injury compared with creatinine clearance (CrCl) estimated by using the Cockcroft–Gault equation.

MATERIALS AND METHODS

Patients

Between February 2004 and June 2005, U-β_2MG was measured cross-sectionally in 70 TDF-treated patients and 90 patients on other antiretroviral treatments who had never been exposed to TDF in the outpatient clinic of the AIDS Clinical Center, International Medical Center of Japan. The U-β_2MG levels were determined by latex agglutination assay. The normal range of U-β_2MG was <500 μg/liter, determined by the analysis of more than 100 healthy volunteers. A signed consent form was obtained from each participant of this study.

Statistical analysis

The sCr concentrations, body weight, CD4 count, HIV-1 viral load measured on the same day with U-β_2MG, and duration of antiretroviral treatment were also compared. U-β_2MG was analyzed logarithmically because U-β_2MG levels change logarithmically in the cases of renal tubular dysfunction and logarithmic analysis compensated the skewed distribution of U-β_2MG levels in the patients who had never been exposed to TDF [distribution skewness: U-β_2MG, 2.971; log(U-β_2MG), 0.412]. CrCl was calculated using the Cockcroft–Gault equation, which estimates CrCl on the basis of sCr level, body weight, and sex of the patient.[21] All data were expressed as mean ± SD. Differences between groups were examined for statistical significance using the Student's *t*-test. Correlations between values were examined using the Pearson's correlation coefficient and the Fisher's *z* transformation. Multivariate least-squares linear regression was used to assess the associations of multiple factors with high U-β_2MG level. A p value less than 0.05 denoted the presence of statistical significance. Statistical analysis was performed using StatView software (SAS Institute).

RESULTS

Patients

Between February 2004 and June 2005, U-β_2MG was measured in 70 TDF-treated patients [TDF(+) group] and 90 patients on other antiretroviral therapy who had never been exposed to TDF [TDF(−) group]. No enrolled patient was taking renal toxic drugs such as ganciclovir or adefovir. In both groups, the HIV-1 RNA loads were less than 400 copies/ml in more than 90% of the patients, suggesting that most patients maintained excellent adherence (Table 1). Most of the analyzed patients were Asian, and around 90% were males. There were no significant differences in age, body weight, CD4 cell count, sCr level, and duration of antiretroviral treatment between TDF(−) and TDF(+) groups.

High U-β_2MG in TDF-treated patients

U-β_2MG was measured at least 1 month after the introduction of TDF treatment in TDF(+) patients and the introduction of antiretroviral therapy in TDF(−) patients. TDF(+) patients had significantly higher logarithmic values of U-β_2MG than TDF(−) patients [2.79 ± 0.85 vs. 2.09 ± 0.43 (log (μg/liter)), $p < 0.0001$], though there was no significant difference in sCr (0.76 ± 0.18 vs. 0.76 ± 0.15 mg/dl) and in estimated CrCl (114.2 ± 34.3 vs. 120.0 ± 29.8 ml/min) between the two groups (Fig. 1A–C). Thirty of 70 (42.9%) TDF(+) patients had abnormally high U-β_2MG levels (>500 μg/liter), although abnormal sCr (>1.1 mg/dl) and abnormal CrCl (<90 ml/min) was observed in only 3 and 11 of them, respectively. There was no significant relation between the duration of TDF treatment and U-β_2MG values in the TDF(+) group. Six patients had abnormally high U-β_2MG values within 3 months after the initiation of TDF treatment. In the TDF(−) group, logarithmic U-β_2MG values showed a normal distribution and abnormally high U-β_2MG values were observed in only 7 of 90 (7.8%). Eleven of the TDF(−) patients had abnormal CrCl and only one TDF(−) patient with compromised CrCl showed abnormal U-β_2MG values. There was a significant negative correlation between CrCl

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF ENROLLED PATIENTS

Characteristics	*TDF (−) (n = 90)*	*TDF (+) (n = 70)*
Sex, no. (%) male	83 (92.2)	61 (87.1)
Ethnicity, no. (%)		
Asian	84 (93.3)	69 (98.6)
White	1 (1.1)	1 (1.4)
African	3 (3.3)	0 (0)
Half Hispanic half Asian	2 (2.2)	0 (0)
Age, mean ± SD (years)	40.4 ± 9.9	42.1 ± 12.3
Body weight, mean ± SD (kg)	64.3 ± 10.2	61.6 ± 9.1
CD4 cell count, mean ± SD (cells/mm^3)	461.9 ± 199.8	437.4 ± 224.0
HIV-1 RNA load, no. (%) <400 copies/ml	82 (91.1)	64 (91.4)
Serum creatinine level, mean ± SD (mg/dl)	0.76 ± 0.15	0.76 ± 0.18
Duration of ART,[a] mean ± SD (months)	40.2 ± 30.7	56.6 ± 30.8
Duration of TDF, mean ± SD (months)	Not applicable	13.0 ± 9.2

[a]ART, antiretroviral therapy.

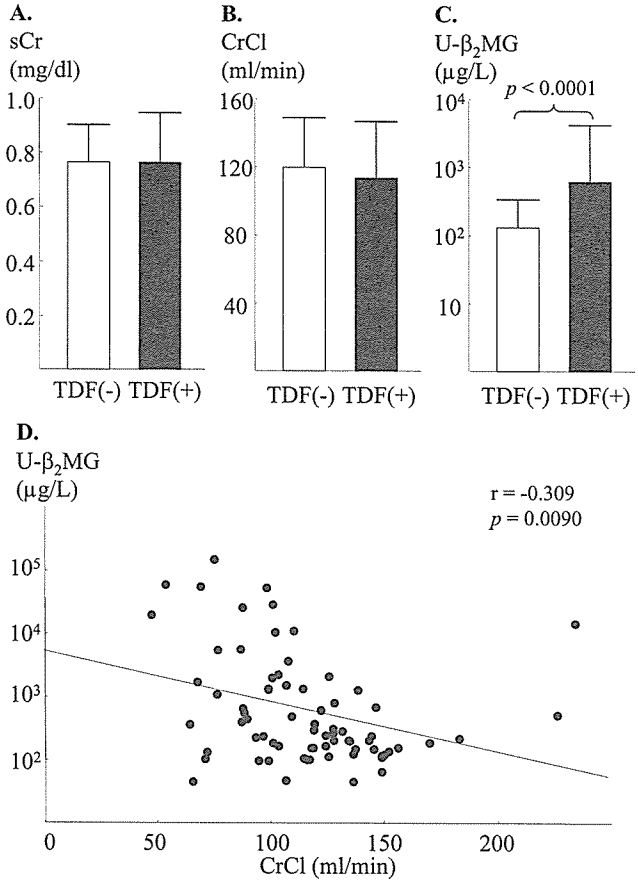

FIG. 1. Renal function of 90 TDF(−) and 70 TDF(+) patients. sCr (**A**), CrCl estimated by using Cockcroft–Gault formula (**B**), and U-β_2MG values (**C**) were compared between 90 TDF(−) (open bar) and 70 TDF(+) (closed bar) patients. Means and SDs are indicated with bars and horizontal lines, respectively. Logarithmic U-β_2MG values of 90 TDF(+) patients are plotted against CrCl estimated by using the Cockcroft–Gault formula (**D**). The regression line is also shown.

and U-β_2MG values in the TDF(+) group ($r = -0.309$, $p = 0.0090$) (Fig. 1D) though it was not significant in the TDF(−) group, which suggests renal insufficiency observed in TDF(+) patients was specifically associated with renal tubular damage.

Co-administration of boosted LPV as a risk factor

According to the results of a pharmacokinetic study,[22] TDF exposure was increased by 32% when administered with lopinavir (LPV)/ritonavir (RTV) therapy, compared with TDF monotherapy, which can be considered to accelerate TDF-induced renal injury. In order to analyze the effect of coadministration of RTV-boosted LPV on TDF-induced renal tubular damage, we classified TDF(+) patients into three subgroups by the usage of LPV and RTV, and compared their U-β_2MG values; those who did not receive LPV or RTV at the time of measurement of U-β_2MG [TDF(+)LPV(−)RTV(−) group, $n = 29$], those on RTV-boosted protease inhibitor-containing treatment other than LPV [TDF(+)LPV(−)RTV(+) group, $n = 10$], and those on the coadministration of RTV-boosted LPV [TDF(+)LPV(+)RTV(+), $n = 31$] (Fig. 2). There was no significant difference in U-β_2MG values between the TDF(+)LPV(−)RTV(−) group and the TDF(+)LPV(−)RTV(+) group [2.50 ± 0.78 vs. 2.49 ± 0.37 (log (μg/liter)), $p = 0.966$], suggesting RTV coadministration had little effect on TDF-induced renal injury. However, the U-β_2MG values of TDF(+)LPV(+)RTV(+) patients [3.17 ± 0.89 (log (μg/liter))] were significantly higher than those in the TDF(+)LPV(−)RTV(−) group ($p = 0.0032$) and those in the TDF(+)LPV(−)RTV(+) group ($p = 0.0257$), and abnormally high levels were noted in 67.7% of TDF(+)LPV(+)RTV(+) patients, indicating that boosted LPV cousage accelerated renal damage by TDF. There were no significant differences in CrCl among the three groups, suggesting that estimated CrCl is less sensitive in detecting TDF-induced renal injury than U-β_2MG.

Low body weight as a risk factor

When multivariate least-squares linear regression was used to assess the multiple factors including age, body weight, CD4 cell count, and TDF usage, TDF usage was a most significant factor associated with a high U-β_2MG value (partial regression coefficient = 0.477, $F = 46.7$). In order to identify other risk factors associated with TDF-induced renal injury, the multiple factors including age, body weight, CD4 cell count, duration of TDF treatment, and boosted LPV usage were assessed in TDF(+) patients. Boosted LPV usage had the greatest positive impact on the U-β_2MG value (partial regression coefficient = 0.394, $F = 12.5$) and patients' body weight had the second greatest impact though it was negative (partial regression coefficient = −0.305, $F = 6.97$) (Table 2). In order to confirm the effect of patients' body weight, U-β_2MG values were plotted against patients' body weight. In the TDF(+)LPV(+)RTV(+)

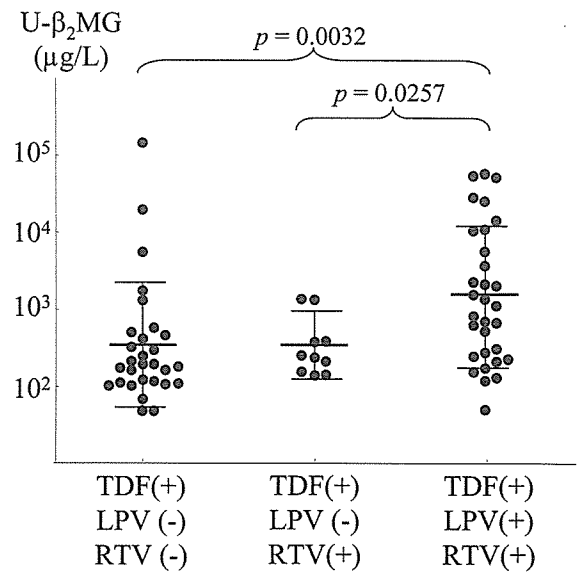

FIG. 2. U-β_2MG levels of TDF-treated patients and LPV and RTV usage. U-β_2MG values were compared among three subgroups of TDF(+) patients divided by usage of LPV and RTV; 29 TDF(+)LPV(−)RTV(−) patients, 10 TDF(+)LPV(−)RTV(+) patients, and 31 TDF(+)LPV(+)RTV(+) patients. Logarithmic means and SDs are indicated with bold and thin horizontal lines, respectively.

TABLE 2. FACTORS ASSOCIATED WITH U-β_2MG LEVEL IN TDF-TREATED PATIENTS

Factor	*Partial regression coefficient*	F *value*
Age (years)	0.131	1.19
Body weight (kg)	−0.305	6.97
CD4 cell count (cells/mm^3)	−0.237	4.03
Duration of TDF (months)	0.208	3.06
LPV usage	0.394	12.5

group, U-β_2MG levels showed significant negative correlation with patients' body weight ($r = -0.511$, $p = 0.0029$) and abnormal U-β_2MG was observed in all six patients with body weight less than 55 kg (Fig. 3). In the TDF(+)LPV(−) group including TDF(+)LPV(−)RTV(−) patients and TDF(+)LPV(−)RTV(+) patients, patients with lower body weight also tended to have higher U-β_2MG values, albeit statistically insignificant ($r = -0.151$, $p = 0.361$) (data not shown). When estimated CrCl was plotted against patients' body weight, CrCl showed significant positive correlation with patients' body weight in the TDF(+)LPV(−) group ($r = 0.467$, $p = 0.0024$), and patients with lower body weight also tended to have lower CrCl in the TDF(+)LPV(+) group, albeit statistically insignificant ($r = 0.181$, $p = 0.334$) (data not shown).

Rapid fall in U-β_2MG after cessation of TDF

Renal injury induced by TDF has been reported to be reversible after cessation of TDF.[10–15,17,18] To confirm this, we examined changes in U-β_2MG over time after switching from TDF to other antiretroviral agents. During this study period, TDF was switched to other agents in four cases that had abnormally high U-β_2MG levels before switching the treatment regimen. The U-β_2MG levels were substantially decreased by 0.86–2.15 log at 5–8 months after switching treatment, and in one case the level was normalized (<500 μg/liter). Thus, as reported by other investigators,[10–15,17,18] TDF-induced renal injury seems reversible, as evident by changes in U-β_2MG values.

DISCUSSION

Renal safety of TDF has been reported based on minimal change in sCr within the normal range during TDF treatment.[4–7] However, we found that 30 of 70 TDF-treated patients had abnormally high U-β_2MG while only 3 of them had abnormal sCr and only 11 had abnormal CrCl, suggesting that the incidence of renal tubular injury in TDF-treated patients is larger than previously estimated, and that U-β_2MG can be used as a more sensitive marker of TDF-induced renal injury than sCr.

U-β_2MG levels have been reported to be reproducible in urine samples collected from the same subjects on multiple occasions,[23] and its measurement is considered a useful tool for noninvasive monitoring of the renal allograft after kidney transplantation.[24] Measurement of urinary protein may be also helpful for monitoring TDF-induced renal injury, though its low specificity might be problematic because not only renal tubular injury but also glomerular damage such as diabetic nephropathy could increase protein excretion in the urine.[20] Furthermore, it was reported that proteinuria is often observed in HIV-infected patients on initial evaluation.[25,26] Therefore, monitoring markers specific to renal tubular injury, such as U-β_2MG, may be useful for HIV-infected patients treated with TDF.

Our results also showed that boosted LPV-containing treatment and low body weight were associated with high U-β_2MG in TDF-treated patients. Surprisingly, all TDF- and boosted LPV-treated patients with body weight <55 kg had abnormally high U-β_2MG. It is possible that boosted LPV usage and low body weight accelerate TDF-induced renal injury by increasing TDF plasma concentrations. In fact, many of the reported cases of TDF-induced renal injury have been in those treated with boosted LPV-containing regimens,[10,12–15,17] and Peyriere *et al.*[10] reported seven cases of TDF-induced renal tubular dysfunction and 6 of them had a low body weight (<60 kg). Patients with low body weight have unusually low sCr levels because creatinine is mainly derived from muscle and lean patients have disproportionately less muscle mass.[27] Therefore, body weight should be taken into consideration when evaluating renal function with sCr values, but this may not be sufficient for full assessment. A simple renal tubular injury is often not associated with an increase in sCr,[20] and mild TDF tubular toxic effects may be missed by the simple and convenient approach of the formula-based estimation of ClCr based on sCr and patient demographics. Thus, sensitive markers, such as U-β_2MG values, may be of use in monitoring HIV-infected patients during TDF treatment, especially when boosted LPV is coadministered or the patient has low body weight.

Abnormally high U-β_2MG levels indicate renal tubular injury, though its clinical significance in TDF-treated patients is still unknown. Recently, Gallant *et al.*[5] reported that TDF treat-

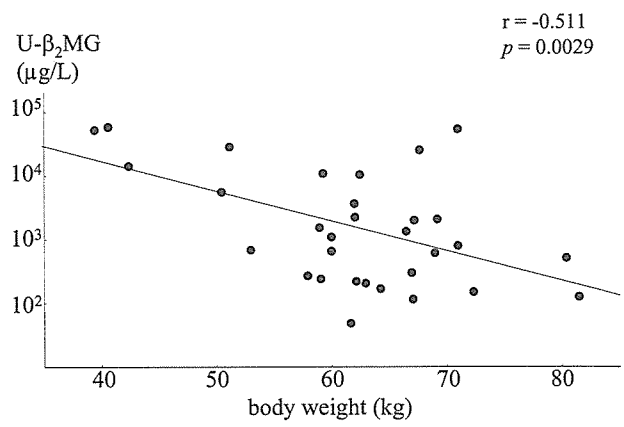

FIG. 3. U-β_2MG values and estimated CrCl in TDF-treated patients. U-β_2MG values of 31 TDF(+)LPV(+) patients are plotted against patients' body weight. The regression line is also shown.

ment causes a mild decrease of bone density but this was not associated with increased frequency of bone fractures. However, persistence of renal tubular injury in TDF-treated patients may result in significant mineral loss and osteoporosis. It was reported that bone deformities and/or fractures were found in 28% of the pediatric patients with hereditary renal tubular disorders during 10 years of observation.[28] Only a longer trial looking at outcomes of TDF-treated patients with high U-β_2MG levels would confirm its clinical significance. It is also possible that since most of our patients were Asians, TDF excretion might be different from that reported in other races. Our study is limited by its cross-sectional design and lack of TDF concentrations in the plasma. Prospective and longitudinal analysis of U-β_2MG value and TDF plasma concentration in a larger cohort is warranted.

ACKNOWLEDGMENTS

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CD4+ Count–Guided Interruption of Antiretroviral Treatment

The Strategies for Management of Antiretroviral Therapy (SMART) Study Group*

ABSTRACT

BACKGROUND

Despite declines in morbidity and mortality with the use of combination antiretroviral therapy, its effectiveness is limited by adverse events, problems with adherence, and resistance of the human immunodeficiency virus (HIV).

METHODS

We randomly assigned persons infected with HIV who had a CD4+ cell count of more than 350 per cubic millimeter to the continuous use of antiretroviral therapy (the viral suppression group) or the episodic use of antiretroviral therapy (the drug conservation group). Episodic use involved the deferral of therapy until the CD4+ count decreased to less than 250 per cubic millimeter and then the use of therapy until the CD4+ count increased to more than 350 per cubic millimeter. The primary end point was the development of an opportunistic disease or death from any cause. An important secondary end point was major cardiovascular, renal, or hepatic disease.

RESULTS

A total of 5472 participants (2720 assigned to drug conservation and 2752 to viral suppression) were followed for an average of 16 months before the protocol was modified for the drug conservation group. At baseline, the median and nadir CD4+ counts were 597 per cubic millimeter and 250 per cubic millimeter, respectively, and 71.7% of participants had plasma HIV RNA levels of 400 copies or less per milliliter. Opportunistic disease or death from any cause occurred in 120 participants (3.3 events per 100 person-years) in the drug conservation group and 47 participants (1.3 per 100 person-years) in the viral suppression group (hazard ratio for the drug conservation group vs. the viral suppression group, 2.6; 95% confidence interval [CI], 1.9 to 3.7; P<0.001). Hazard ratios for death from any cause and for major cardiovascular, renal, and hepatic disease were 1.8 (95% CI, 1.2 to 2.9; P=0.007) and 1.7 (95% CI, 1.1 to 2.5; P=0.009), respectively. Adjustment for the latest CD4+ count and HIV RNA level (as time-updated covariates) reduced the hazard ratio for the primary end point from 2.6 to 1.5 (95% CI, 1.0 to 2.1).

CONCLUSIONS

Episodic antiretroviral therapy guided by the CD4+ count, as used in our study, significantly increased the risk of opportunistic disease or death from any cause, as compared with continuous antiretroviral therapy, largely as a consequence of lowering the CD4+ cell count and increasing the viral load. Episodic antiretroviral therapy does not reduce the risk of adverse events that have been associated with antiretroviral therapy. (ClinicalTrials.gov number, NCT00027352.)

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*The members of the SMART study group are listed in the Appendix.

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With the advent of potent combination antiretroviral therapy came the hope that such therapy might lead to the eradication of infection with the human immunodeficiency virus (HIV).[1] It was soon recognized, however, that this goal was unlikely to be achieved owing to the existence of latent reservoirs; people infected with HIV would need to receive antiretroviral therapy for many years, if not for life.[2,3] Potent antiretroviral therapy is associated with substantial benefits with regard to morbidity and mortality.[4-6] However, the therapy is also associated with both short-term and long-term adverse events.[7,8] Major metabolic and cardiovascular complications have been a particular concern.[9,10] In addition, HIV can become resistant to available antiretroviral therapy, particularly if adherence is poor, which can lead to cross-resistance within a class of drugs and, ultimately, to multidrug resistance.[11] Prolonged use of antiretroviral therapy is also expensive.[12]

The inherent risks and problems associated with lifelong antiretroviral therapy have led to the study of treatment-sparing strategies that might provide the benefits of antiretroviral therapy while minimizing the risk of adverse events and other risks associated with long-term use. We conducted the Strategies for Management of Antiretroviral Therapy (SMART) trial in order to compare a treatment strategy of episodic use of antiretroviral therapy according to the CD4+ count with the current practice of continuous antiretroviral therapy.

METHODS

The SMART study was initiated by the Terry Beirn Community Programs for Clinical Research on the Acquired Immunodeficiency Syndrome (AIDS) and implemented in collaboration with regional coordinating centers in Copenhagen (the Copenhagen HIV Programme), London (the Clinical Trials Unit of the Medical Research Council), and Sydney (the National Centre in HIV Epidemiology and Clinical Research). Some members of the SMART writing group developed the study protocol with the sponsor, the Division of AIDS of the National Institute of Allergy and Infectious Diseases (NIAID). The writing group takes full responsibility for the completeness and veracity of the data, data analyses, and this article. Drugs used in the study were purchased by patients either directly or through insurance, Social Security, or public access programs.

PARTICIPANTS

Persons infected with HIV who were older than 13 years and were not pregnant or breast-feeding were eligible for the study if their CD4+ count exceeded 350 cells per cubic millimeter and they were willing to initiate, modify, or stop antiretroviral therapy according to study guidelines. Participants were eligible whether or not they had received or were currently receiving antiretroviral therapy. The study was approved by the institutional review board at each site, and written informed consent was obtained from all participants.

STUDY DESIGN

The SMART study is a randomized trial comparing two antiretroviral treatment strategies. Investigators and participants were aware of the treatment assignments. The viral suppression strategy, which was the control strategy, was defined to be consistent with the 2003 guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents[13]: available antiretroviral regimens were to be used in an uninterrupted manner with the goal of maximal and continuous suppression of HIV replication. The experimental drug conservation strategy entailed the episodic use of antiretroviral therapy according to CD4+ count thresholds: the use of antiretroviral therapy was deferred until the CD4+ count decreased to less than 250 cells per cubic millimeter, at which time antiretroviral therapy was to be initiated (or reinitiated) and continued until the CD4+ count increased to more than 350 cells per cubic millimeter. The protocol also permitted antiretroviral therapy to be initiated (or reinitiated) if symptoms of disease from HIV infection (e.g., oral thrush) developed or the percentage of CD4+ lymphocytes (CD4+ percentage) was less than 15%. On confirmation that the CD4+ count was more than 350 cells per cubic millimeter, antiretroviral therapy was to be stopped and then resumed when the CD4+ count was less than 250 cells per cubic millimeter. During periods of antiretroviral therapy, the goal was to achieve maximal viral suppression. The CD4+ count thresholds for stopping and starting antiretroviral therapy were chosen on the basis of reported associations between CD4+ counts and the risks of opportunistic diseases and death.[13-16]

The primary end point was new or recurrent opportunistic disease or death from any cause. Qualifying clinical events included those in the revised case definition for AIDS of the Centers for

Disease Control and Prevention,[17] as well as additional conditions related to immunodeficiency (see the Supplementary Appendix, available with the full text of this article at www.nejm.org). Important secondary end points included death from any cause; serious opportunistic disease[18]; major cardiovascular, renal, or hepatic disease (see the Supplementary Appendix); and grade 4 adverse events (not including opportunistic disease) or death from any cause. Grade 4 adverse events were defined as potentially life-threatening symptomatic events requiring medical intervention, according to the toxicity table of the Division of AIDS of the NIAID. Data on lower-grade toxic effects were not collected.

Using preestablished criteria, an end-point review committee that was unaware of the treatment assignments reviewed the events classified as opportunistic disease, death from any cause, or major cardiovascular, hepatic, or renal disease. The end-point review committee classified the underlying cause of death using the Coding of Death in HIV (CoDe) project system.[19] Opportunistic disease and major cardiovascular, renal, or hepatic diseases classified as confirmed or probable by the end-point review committee were considered end points, as were all deaths, irrespective of the cause.

We calculated that 6000 patients would need to be enrolled for the study to have a statistical power of 80% to detect a 17% relative reduction in the rate of opportunistic disease or death from any cause in the drug conservation group as compared with the viral suppression group, with a two-sided alpha level of 0.05. Follow-up was to continue until 910 primary end points had occurred (estimated to be at least 6 years for each participant), assuming an event rate in the viral suppression group of 1.3% in each of the first 2 years and 2.6% per year thereafter.[20] Randomization was stratified according to clinical site with the use of permuted blocks of random sizes.

DATA COLLECTION AND FOLLOW-UP

Before randomization, participants' antiretroviral therapy history and medical history were obtained, as were the nadir CD4+ count; the highest recorded plasma HIV RNA level; the CD4+ count, CD4+ percentage, and HIV RNA level at baseline; and the three most recent CD4+ counts, CD4+ percentages, and HIV RNA levels before baseline. Follow-up visits were scheduled at 1 month and 2 months, every 2 months thereafter for the first year, and every 4 months in the second and subsequent years. At each visit, a history was taken and an examination conducted, and the CD4+ count and HIV RNA level were measured. More frequent assessments could be carried out if clinical care was required. At baseline and at each annual visit, a 12-lead electrocardiogram was obtained; the data were electronically transmitted to a reading center for detection of any changes indicative of a silent myocardial infarction.[21-23]

INTERIM MONITORING OF SAFETY AND EFFICACY

An independent data and safety monitoring board reviewed interim analyses from the SMART study at least annually. According to protocol guidelines, the board was to consider early termination of the study or modification of the protocol if findings concerning the primary end point (opportunistic disease or death from any cause) and the secondary end point of major cardiovascular, renal, or hepatic disease were consistent — both favoring the same treatment group — and there was clear and substantial evidence of benefit or harm. An O'Brien–Fleming boundary and the Lan–DeMets spending function were used to control the type I error with regard to the primary end point.[24,25]

On January 10, 2006, at its sixth meeting, the board recommended stopping enrollment in the SMART trial because of a safety risk in the drug conservation group and because it appeared to be very unlikely that superiority of the drug conservation treatment would be shown. On January 11, 2006, investigators and participants were notified of these findings, enrollment was stopped, and participants in the drug conservation group were advised to restart antiretroviral therapy. All participants continued in follow-up. This article describes findings through the closure of enrollment.

STATISTICAL ANALYSIS

The drug conservation and viral suppression groups were compared according to the intention-to-treat principle. Time-to-event methods (Kaplan–Meier survival curves and Cox proportional-hazards models) were used to compare the drug conservation group and the viral suppression group with respect to event rates for opportunistic disease or death from any cause; death from any cause; serious and nonserious opportunistic disease; major cardiovascular, renal, or hepatic disease; and grade 4 events.[26] Follow-up data were censored

Table 1. Baseline Characteristics of Study Participants.*

Characteristic	Drug Conservation Group (N = 2720)	Viral Suppression Group (N = 2752)	All (N = 5472)
Age (yr)			
Median	43	44	43
Interquartile range			38–50
Female sex (%)	26.3	28.0	27.2
Race (%)†			
Black	28.5	29.8	29.1
White	56.4	54.8	55.6
Other	15.1	15.4	15.3
Mode of infection with HIV (%)‡			
Sexual contact			
With person of same sex	51.4	48.5	49.9
With person of opposite sex	44.4	45.6	45.0
Injection-drug use	9.8	9.5	9.7
Other or unknown	7.5	8.7	8.1
CD4+ count (cells/mm^3)			
Median	597	597	597
Interquartile range			466–790
CD4+ nadir (cells/mm^3)			
Median	250	250	250
Interquartile range			155–359
HIV RNA ≤400 copies/ml (%)	71.8	71.5	71.7
Prior recorded highest HIV RNA level (log copies/ml)			
Median	4.8	4.8	4.8
Interquartile range			4.2–5.3
Cardiovascular risk factor			
Current smoker (%)	41.3	39.6	40.5
Diabetes (%)	7.0	7.1	7.0
Prior cardiovascular disease (%)	6.7	6.1	6.4
Blood pressure–lowering drugs (%)	19.2	18.1	18.6
Lipid-lowering drugs (%)	15.7	15.6	15.6
Total cholesterol (mg/dl)			
Median	191	189	190
Interquartile range			163–220
High-density lipoprotein cholesterol (mg/dl)			
Median	40	41	40
Interquartile range			33–50

either when participants were lost to follow-up before January 11, 2006, or on that date.

The hazard ratios for the comparison of the drug conservation group with the viral suppression group were estimated from Cox models with a single binary treatment group indicator. We tested the proportional-hazards assumption by including an interaction term between the treat-

Table 1. (Continued.)

Characteristic	Drug Conservation Group (N=2720)	Viral Suppression Group (N=2752)	All (N=5472)
History of ART (%)			
Never received ART	4.4	4.8	4.6
Previous PI use	69.5	67.7	68.6
Previous NNRTI use	64.7	63.9	64.3
Use of ART at baseline	84.3	83.6	83.9
Time since first ART (yr)			
Median	6	6	6
Interquartile range			3–8
Type of ART at baseline (% of patients receiving ART)			
PI	45.1	45.2	45.2
NNRTI	50.0	47.9	49.0
PI and NNRTI	6.3	5.3	5.8
Most common drug combinations			
Zidovudine, lamivudine, efavirenz	10.3	9.9	10.0
Zidovudine, lamivudine, nevirapine	7.4	7.7	7.5
Zidovudine, lamivudine, abacavir	6.5	7.1	6.8
Zidovudine, lamivudine, nelfinavir	4.6	3.9	4.3
Prior AIDS-related illness (%)	24.7	23.1	23.9
Hepatitis B (%)	2.4	2.2	2.3
Hepatitis C (%)	15.3	14.4	14.8

* None of the characteristics differed significantly between treatment groups. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. Percentages may not total 100 because of rounding. ART denotes antiretroviral therapy, PI protease inhibitor, and NNRTI nonnucleoside reverse-transcriptase inhibitor.
† Race was self-reported.
‡ Mode of infection was self-reported. Percentages do not total 100 because some participants reported more than one mode.

ment indicator and the log-transformed follow-up time.[26]

The primary end point was summarized for selected subgroups that were predefined according to baseline characteristics. The heterogeneity of hazard ratio estimates for subgroups was assessed by including an interaction term between treatment and subgroup in expanded Cox models.

Cox proportional-hazards models were used to assess the effects of CD4+ counts and HIV RNA levels as time-dependent covariates during follow-up on the hazard ratios for opportunistic disease or death from any cause. Statistical analyses were performed with the use of SAS software, version 8.2. All reported P values are two-sided and have not been adjusted for multiple examinations of the data.

RESULTS

BASELINE CHARACTERISTICS

Between January 8, 2002, and January 11, 2006, 5472 participants were randomly assigned to treatment groups (2720 to the drug conservation group and 2752 to the viral suppression group). Participants were enrolled at 318 sites in 33 countries. Table 1 summarizes key baseline characteristics. The treatment groups were well balanced at entry.

FOLLOW-UP

Total follow-up time was approximately 3700 person-years in each group, with a mean follow-up time of 16 months. Approximately 26% of participants were followed for more than 2 years. Participants attended 96.5% of follow-up visits in

the drug conservation group and 94.8% of follow-up visits in the viral suppression group. On January 11, 2006, the status with regard to the primary end point was unknown for 32 participants (1.2%) in the drug conservation group and 41 participants (1.5%) in the viral suppression group (Fig. I in the Supplementary Appendix).

USE OF ANTIRETROVIRAL THERAPY AND CHANGES IN CD4+ AND HIV RNA LEVELS

After randomization, the median duration of the first period of interruption of antiretroviral therapy for participants in the drug conservation group was 16.8 months (interquartile range, 5.7 to 42.3) (Fig. IIA in the Supplementary Appendix shows the percentage of participants who received antiretroviral therapy through follow-up). A total of 343 participants stopped antiretroviral therapy a second time, and 62 participants stopped three or more times. The average CD4+ count decreased by 87 cells per cubic millimeter per month during the first 2 months after randomization among participants in the drug conservation group (Fig. IIB in the Supplementary Appendix); thereafter, it continued to decline but at a lower rate. On average, throughout follow-up, the CD4+ count was 206 cells per cubic millimeter lower in the drug conservation group than in the viral suppression group. HIV RNA levels also changed rapidly in the drug conservation group after randomization: within 2 months, the percentage of participants with HIV RNA levels of 400 copies per milliliter or less decreased from 71.8% to 6.0% (Fig. IIC in the Supplementary Appendix). After reinitiation of antiretroviral therapy in the drug conservation group, the median time to an HIV RNA level of 400 copies per milliliter or less was 3.1 months (Fig. IIIA in the Supplementary Appendix); the CD4+ count increased by an average of 166 cells per cubic millimeter within 8 months (Fig. IIIB in the Supplementary Appendix).

On average, participants in the drug conservation group and the viral suppression group received antiretroviral therapy during 33.4% and 93.7% of the follow-up time, respectively. Participants in both groups had CD4+ counts of 350 cells per cubic millimeter or more during the majority of the follow-up time (67.9% of the time in the drug conservation group and 92.7% of the time in the viral suppression group) (Fig. 1). Participants in the drug conservation group had CD4+ counts of less than 250 cells per cubic millimeter during 8.6% of the follow-up time, as compared with 1.8% of the time in the viral suppression group. The percentage of follow-up time during which participants had HIV RNA levels

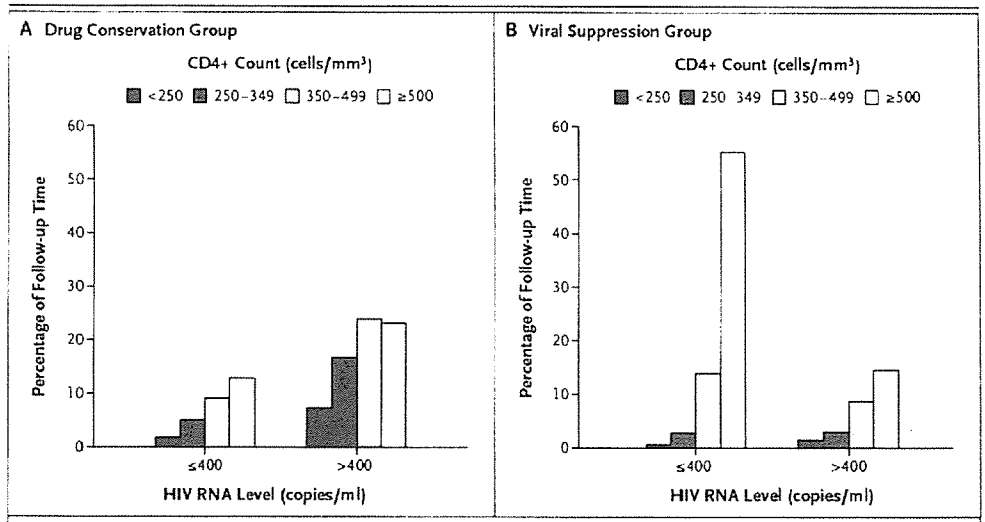


Figure 1. Percentage of Follow-up Time during Which Participants Had a Specified CD4+ Count and HIV RNA Level.
For 28.8% of the 3666 person-years of follow-up in the drug conservation group (Panel A), the HIV RNA level was 400 copies per milliliter or less. Patients received antiretroviral therapy during 33% of the follow-up time. For 72.3% of the 3701 person-years of follow-up in the viral suppression group (Panel B), the HIV RNA level was 400 copies per milliliter or less. Patients received antiretroviral therapy during 94% of the follow-up time.

of 400 copies per milliliter or less was substantially greater in the viral suppression group than in the drug conservation group (72.3% and 28.8%, respectively).

PRIMARY END POINT AND ITS COMPONENTS

There were 120 participants in the drug conservation group and 47 in the viral suppression group who had an opportunistic disease or died from any cause (Table 2). The cumulative probabilities of opportunistic disease or death from any cause after 12, 24, and 36 months were 0.030, 0.067, and 0.091 in the drug conservation group and 0.010, 0.021, and 0.042 in the viral suppression group, respectively (Fig. 2A).

The estimated hazard ratio for opportunistic disease or death from any cause was 2.6 (95% confidence interval [CI], 1.9 to 3.7; P<0.001) (Table 2 and Fig. 2A), and it did not vary significantly over the follow-up period (P=0.78 for proportional hazards). Among patients in the drug conservation group who reached the primary end point, death from any cause was the most common individual event (39.2%), followed by esophageal candidiasis (20.0%) and pneumonia from *Pneumocystis jiroveci* infection (6.7%). Among patients in the viral suppression group who reached the primary end point, the most common event was death from any cause (57.4%), followed by esophageal candidiasis (14.9%) (Table 1 in the Supplementary Appendix).

The estimated hazard ratio for serious opportunistic disease, a component of the primary end point, was 6.6 (95% CI, 1.5 to 29.1; P=0.01); the hazard ratio for nonserious events associated with opportunistic disease was 3.6 (95% CI, 2.1 to 6.1; P<0.001); and the hazard ratio for death from any cause was 1.8 (95% CI, 1.2 to 2.9; P=0.007) (Table 2 and Fig. 2B).

Only 8% of deaths were due to opportunistic disease. The most common underlying causes of death were cancers other than those considered to be opportunistic disease (in 11 participants in the drug conservation group and 5 participants in the viral suppression group); cardiovascular disease (7 participants and 4 participants, respectively); substance abuse (3 participants and 5 participants, respectively); accident, violence, or suicide (3 participants and 4 participants, respectively); and infection other than that considered to be

Table 2. Primary and Major Secondary End Points.*

End Point	Drug Conservation Group (N=2720)		Viral Suppression Group (N=2752)		Hazard Ratio for Drug Conservation Group vs. Viral Suppression Group (95% CI)	P Value
	No. of Participants with Event	Event Rate (per 100 Person-Yr)	No. of Participants with Event	Event Rate (per 100 Person-Yr)		
Primary end point	120	3.3	47	1.3	2.6 (1.9–3.7)	<0.001
Death from any cause	55	1.5	30	0.8	1.8 (1.2–2.9)	0.007
Opportunistic disease						
Serious	13	0.4	2	0.1	6.6 (1.5–29.1)	0.01
Nonserious	63	1.7	18	0.5	3.6 (2.1–6.1)	<0.001
Major cardiovascular, renal, or hepatic disease	65	1.8	39	1.1	1.7 (1.1–2.5)	0.009
Fatal or nonfatal cardiovascular disease	48	1.3	31	0.8	1.6 (1.0–2.5)	0.05
Fatal or nonfatal renal disease	9	0.2	2	0.1	4.5 (1.0–20.9)	0.05
Fatal or nonfatal liver disease	10	0.3	7	0.2	1.4 (0.6–3.8)	0.46
Grade 4 event	173	5.0	148	4.2	1.2 (1.0–1.5)	0.13
Grade 4 event or death from any cause	205	5.9	164	4.7	1.3 (1.0–1.6)	0.03

* Numbers of individual events of each type do not sum to the total number because some participants had more than one event. End-point definitions are listed in the Supplementary Appendix. Grade 4 events were determined on the basis of toxicity grades developed by the Division of AIDS of the NIAID. CI denotes confidence interval.

an opportunistic disease (3 participants and 1 participant, respectively). For 18 participants who died (15 in the drug conservation group and 3 in the viral suppression group), the underlying cause of death could not be determined (Table II in the Supplementary Appendix).

We examined the robustness of the primary end-point findings by considering three additional outcomes: opportunistic diseases (restricted to those that were new [nonrecurrent]) or death from any cause; fatal and nonfatal cases of opportunistic disease (excluding deaths from causes other than opportunistic disease); and all reported cases of opportunistic diseases and deaths irrespective of the classification after review by the end-point review committee. The estimated hazard ratios for these three outcomes in the drug conservation group versus the viral suppression group were 2.6 (95% CI, 1.8 to 3.7; P<0.001), 3.6 (95% CI, 2.2 to 5.9; P<0.001), and 2.5 (95% CI, 1.9 to 3.3; P<0.001), respectively.

MAJOR CARDIOVASCULAR, RENAL, AND HEPATIC DISEASE

Among the participants in the drug conservation group, 65 had at least one episode of major cardiovascular, renal, or hepatic disease, as did 39 participants in the viral suppression group (hazard ratio, 1.7; 95% CI, 1.1 to 2.5; P=0.009) (Table 2 and Fig. 2C). Estimated hazard ratios for each type of disease were all greater than 1.0, favoring the viral suppression group.

GRADE 4 EVENTS

Grade 4 adverse symptomatic events occurred in 173 participants in the drug conservation group and 148 participants in the viral suppression group (hazard ratio, 1.2; 95% CI, 1.0 to 1.5; P=0.13) (Table 2 and Fig. 2D, and Table III in the Supplementary Appendix). The hazard ratio for the composite outcome of a grade 4 event or death from any cause was 1.3 (95% CI, 1.0 to 1.6; P=0.03).

PRIMARY END POINT ACCORDING TO SUBGROUP

Estimated hazard ratios varied significantly according to race, baseline HIV RNA level, and baseline CD4+ cell count (Fig. 3). Among participants who were receiving antiretroviral therapy at baseline, for those with an HIV RNA level of 400 copies per milliliter or less at baseline, the hazard ratio for opportunistic disease or death from any cause was 4.0, whereas those with levels of more than 400 copies per milliliter had a hazard ratio of 1.2 (P<0.001). This significant difference was due to different rates of opportunistic disease or death from any cause in the viral suppression group between the subgroups with HIV RNA levels of 400 copies per milliliter or less and those with HIV RNA levels of more than 400 copies per milliliter (0.8 and 2.6 events per 100 person-years, respectively), in contrast to similar rates for those subgroups in the drug conservation group (3.2 and 3.1, respectively).

Figure 2 (facing page). Cumulative Probability of the Primary End Point (Panel A); Death from Any Cause (Panel B); Major Cardiovascular, Renal, or Hepatic Disease (Panel C); and Grade 4 Adverse Events (Panel D).

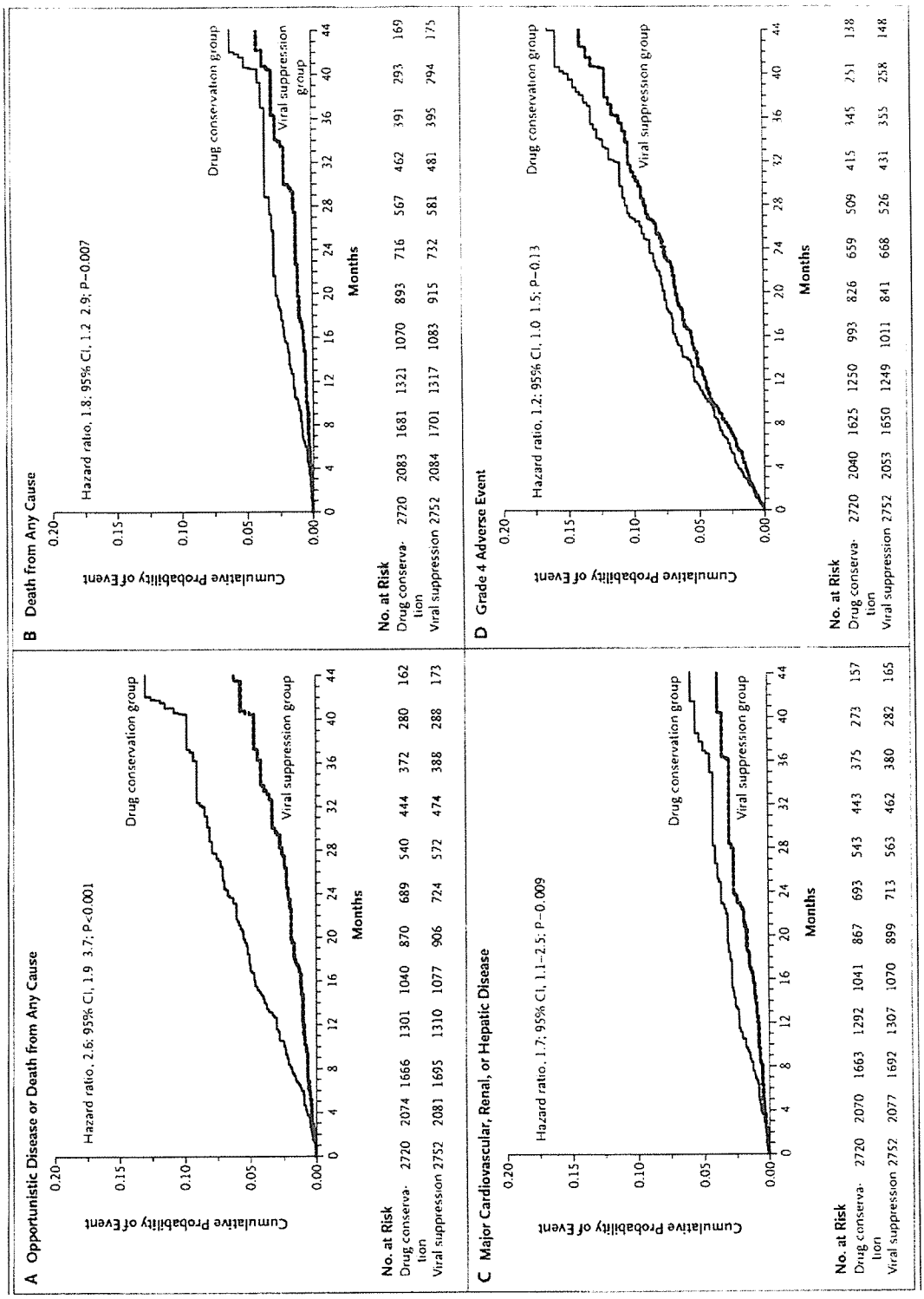
Grade 4 adverse events were determined on the basis of toxicity grades developed by the Division of AIDS of the NIAID. End-point definitions are listed in the Supplementary Appendix.

ADJUSTMENT FOR LATEST CD4+ COUNT AND HIV RNA LEVEL

Proportional-hazards models were used to assess the effects of time-dependent covariates corresponding to the latest CD4+ counts and latest HIV RNA levels, considered separately and together, on the hazard ratio (Fig. 4). The hazard ratio for opportunistic disease or death from any cause was reduced from 2.6 to 1.7 (95% CI, 1.2 to 2.5) after adjustment for the latest CD4+ count; it was further reduced to 1.5 (95% CI, 1.0 to 2.1) after adjustment for both the latest HIV RNA level and the latest CD4+ count. Adjusted for both the latest HIV RNA level and the latest CD4+ count, the hazard ratios for opportunistic disease and for death from causes other than opportunistic disease were 1.7 (95% CI, 1.0 to 2.9) and 1.2 (95% CI, 0.7 to 2.2), respectively; unadjusted, these hazard ratios were 3.6 (95% CI, 2.2 to 5.9) for opportunistic disease and 1.8 (95% CI, 1.1 to 2.9) for death from causes other than opportunistic disease, respectively.

DISCUSSION

Our data demonstrate that continuous use of antiretroviral therapy is superior to its episodic use as guided by the CD4+ count, with antiretroviral therapy deferred until the CD4+ count is less than 250 cells per cubic millimeter. The superiority of the viral suppression strategy, designed to achieve


A Opportunistic Disease or Death from Any Cause
Hazard ratio, 2.6; 95% CI, 1.9–3.7; P<0.001
Drug conservation group
Viral suppression group
Cumulative Probability of Event
Months
No. at Risk
Drug conservation 2720 2074 1666 1301 1040 870 689 540 444 372 280 162
Viral suppression 2752 2081 1695 1310 1077 906 724 572 474 388 288 173
B Death from Any Cause
Hazard ratio, 1.8; 95% CI, 1.2–2.9; P=0.007
Drug conservation group
Viral suppression group
Cumulative Probability of Event
Months
No. at Risk
Drug conservation 2720 2083 1681 1321 1070 893 716 567 462 391 293 169
Viral suppression 2752 2084 1701 1317 1083 915 732 581 481 395 294 175
C Major Cardiovascular, Renal, or Hepatic Disease
Hazard ratio, 1.7; 95% CI, 1.1–2.5; P=0.009
Drug conservation group
Viral suppression group
Cumulative Probability of Event
Months
No. at Risk
Drug conservation 2720 2070 1663 1292 1041 867 693 543 443 375 273 157
Viral suppression 2752 2077 1692 1307 1070 899 713 563 462 380 282 165
D Grade 4 Adverse Event
Hazard ratio, 1.2; 95% CI, 1.0–1.5; P=0.13
Drug conservation group
Viral suppression group
Cumulative Probability of Event
Months
No. at Risk
Drug conservation 2720 2040 1625 1250 993 826 659 509 415 345 251 138
Viral suppression 2752 2053 1650 1249 1011 841 668 526 431 355 258 148

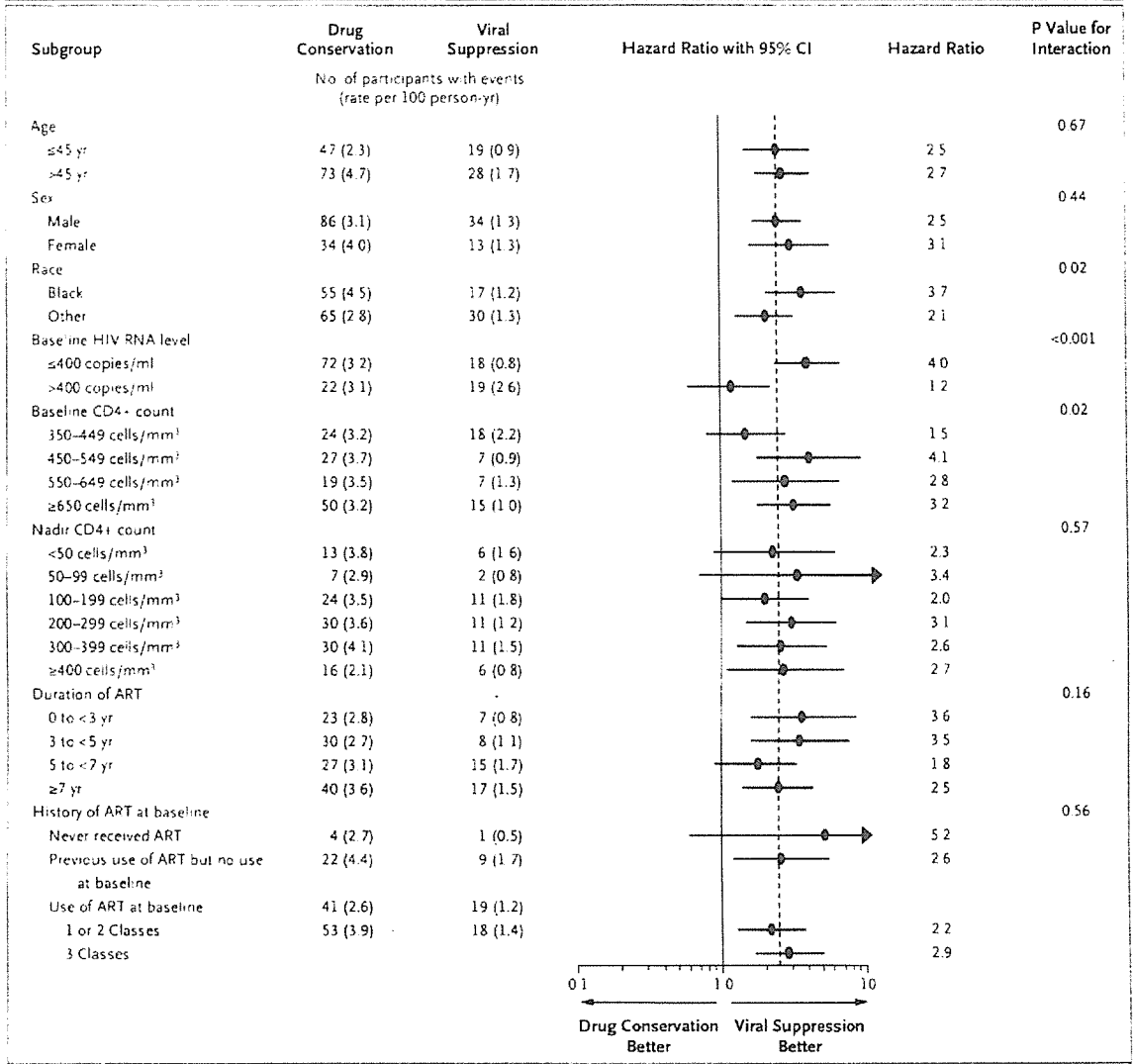


Figure 3. Hazard Ratios for Opportunistic Disease or Death from Any Cause.

The dashed line represents the overall hazard ratio (drug conservation group vs. viral suppression group) of 2.6. Horizontal lines indicate 95% CIs. Subgroup data for HIV RNA levels were restricted to participants who were receiving antiretroviral therapy at baseline. Race was self-reported. ART denotes antiretroviral therapy.

maximal and continuous suppression of HIV replication with the use of antiretroviral therapy, was evident with regard to the primary end point (opportunistic disease or death from any cause), as well as death from any cause, serious opportunistic disease, and an important secondary end point, major cardiovascular, renal, or hepatic disease.

Interruption of antiretroviral therapy has been advocated as a treatment strategy to enhance the quality of life, limit adverse events, and allow for the emergence of the predominant wild-type virus in patients infected with multidrug-resistant HIV.[27,28] Two randomized studies have used higher CD4+ count thresholds than those used in our study for the initiation of antiretroviral therapy, but they involved only 69 patients[29] and 74 pa-

tients[30,31] and were therefore too small to allow for the reliable assessment of effects of treatment interruption on clinical outcomes.

More recently, the findings of two other larger, randomized trials were reported. In the Staccato study, in which the primary end point was virologic suppression and the amount of exposure to antiretroviral drugs, 284 patients were randomly assigned to receive antiretroviral therapy guided by the CD4+ count (with therapy deferred until the CD4+ count was less than 350 cells per cubic millimeter and then used until the CD4+ count was more than 350 cells per cubic millimeter) and 146 patients were randomly assigned to receive continuous antiretroviral therapy.[32] After approximately 2 years of follow-up, diarrhea and neuropathy were more common among those receiving continuous therapy, and oral and vaginal candidiasis were more common among those receiving episodic therapy. In the Trivacan study, 216 patients were randomly assigned to episodic therapy with the same CD4+ count thresholds as those in our study, and 110 patients were randomly assigned to continuous antiretroviral therapy.[33] An increased risk of bacterial infections and other complications were noted in the episodic treatment group.

When we began our study, data indicated that the risk of AIDS was low among patients who had never received antiretroviral therapy and among those who had received it but who also had CD4+ counts of more than 200 cells per cubic millimeter.[14-16] Consequently, we chose to use a CD4+ count threshold of 250 cells per cubic millimeter for initiation (or reinitiation) of antiretroviral therapy in the drug conservation group, and CD4+ counts and symptoms were monitored closely. Data also indicated that complications and deaths among patients with higher CD4+ levels were largely due to grade 4 adverse events and deaths from causes other than opportunistic disease that were either associated with antiretroviral therapy or attributable to non-HIV causes.[8] Thus, we expected the risk of death from causes other than opportunistic disease to decrease with the interruption of antiretroviral therapy, rather than to increase.

In our study, deaths represented a large percentage of the primary events (44.3%) and, as in other reports, deaths from causes other than opportunistic disease were common.[8,34-36] The excess of deaths from causes other than opportunistic disease in the drug conservation group was surprising. Furthermore, contrary to available data and to the assumptions underlying our study design, participants in the drug conservation group had a higher rate of major cardiovascular, renal, or hepatic disease than did those in the viral suppression group. On the basis of prior findings, and as a consequence of less exposure to antiretroviral therapy in the drug conservation group,[10,37] we expected the rate of cardiovascular disease to be 15% lower in the drug conservation group than in the viral suppression group.

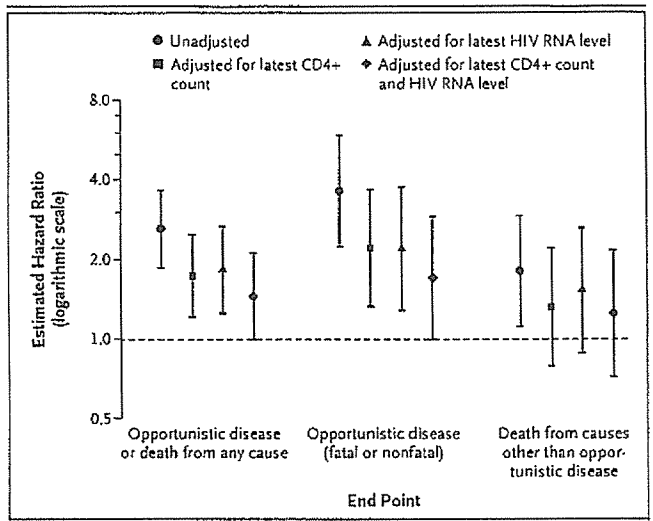


Figure 4. Estimated Unadjusted and Adjusted Hazard Ratios (Drug Conservation Group vs. Viral Suppression Group) for Opportunistic Disease or Death from Any Cause, Opportunistic Disease (Fatal or Nonfatal), and Death from Causes Other Than Opportunistic Disease.

End-point definitions are listed in the Supplementary Appendix. I bars denote the 95% CIs.

There were fewer occurrences of major hepatic or renal disease than of cardiovascular disease in our study, but hepatic or renal disease was still more frequent in the drug conservation group than in the viral suppression group. Some antiretroviral drugs have been associated with adverse hepatic and renal events,[7,38,39] but recent findings indicate that these events are also related to the level of immunodeficiency[40,41] and that antiretroviral therapy improves these outcomes either directly by inhibiting viral replication or indirectly by improving immune function.[39] Our data support these findings. Adjustment for the latest CD4+ count reduced the hazard ratio in the drug conservation group versus the viral suppression