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Effects of Early Antiretroviral Treatment on HIV-1 RNA in Blood and Lymphoid Tissue: A Randomized Trial of Double Versus Triple Therapy

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Summary: To assess the effects of early initiation of antiretroviral therapy on cell-free and cell-associated viral load in blood and lymphoid tissue, we performed a randomized, open-label, multicenter trial comparing a double (zidovudine + lamivudine) and triple (zidovudine + lamivudine + ritonavir) drug combination in treatment-naive, asymptomatic patients with CD4 counts >400 cells/μl. HIV-1 RNA was measured in plasma, peripheral blood mononuclear cells, and sequential tonsil or lymph node biopsies (27 patients); the study follow-up was 2 years. Among 42 randomized patients, the proportion with plasma HIV-1 RNA <50 copies/ml was 16% and 74% at week 24 ($p < .001$) in those randomized to double and triple therapy, respectively, necessitating frequent treatment intensification in the double arm. After a rapid decline within 4 weeks in both arms, cell-associated HIV-1 RNA decreased further only in those patients with sustained suppression of plasma viral load, but remained almost always detectable at low levels, indicating persisting transcription of viral RNA. CD4 counts increased by 200 to 250 cells/μl at week 96 in both arms without significant differences (intent-to-treat analyses). Thus, even if treatment is initiated early in asymptomatic patients with preserved CD4 counts, three drugs are necessary to achieve sustained decreases of HIV load in blood and lymphoid tissue. **Key Words:** Early antiretroviral therapy—Combination therapy—Lymphoid tissue—Transcription—Viral load—Randomized study—Clinical trials.

In patients with advanced HIV infection, highly active antiretroviral treatment (HAART), usually consisting of the triple combination of two nucleoside analogues and one protease inhibitor, has been shown to produce better HIV-1 RNA suppression and to decrease clinical complications in comparison to a combination of two nucleoside analogues alone (1,2). The magnitude of HIV-1 RNA suppression, however, does not depend only on the potency of the treatment but also on the patient population studied. Treatment-naive and asymptomatic subjects usually respond better than pretreated patients (3–5). Additionally, high HIV-1 RNA, low CD4 lymphocyte count, or the syncytium-inducing virus phenotype at baseline, and failure to reach an as low HIV-1 RNA nadir as possible on treatment have been associated with treatment failure (5–11), and these factors are more likely to be present in patients with advanced HIV infection.

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Thus, patients with favorable prognostic markers treated during early HIV infection might show a better virologic response and possibly allow for a less intense therapy.

In 1996, when this study started, antiretroviral treatment recommendations were based on clinical stage and laboratory markers. Treatment was not recommended in asymptomatic patients with CD4 counts >500 cells/μl, and triple combination therapy was reserved for patients with more advanced disease (12). The present study was therefore designed to evaluate the virologic and immunologic effects, as well as the clinical feasibility and tolerability, of early initiation of antiretroviral therapy in asymptomatic patients with preserved CD4 lymphocyte counts. The main goal was to compare the effects of double nucleoside analogue therapy with a protease inhibitor–containing triple regimen in this patient population, assessed by determination of cell-free and cell-associated viral load in blood and lymphoid tissue. Results from the first 24 months of follow-up are presented here.

METHODS

Study Design and Patient Population

The study included asymptomatic, treatment-naive HIV-1–infected individuals with CD4 lymphocyte counts >400 cells/μl, documented within 1 month before the first baseline visit of the study. HIV infection was diagnosed by detection using enzyme-linked immunosorbent assay (ELISA) of anti-p24 antibodies. Exclusion criteria were hemoglobin <9.5 g/dl, neutrophil count $<1.5 \times 10^3$/μl, platelet count $<75 \times 10^6$/μl, clinically significant hepatic, renal, or metabolic disease, and pregnancy. Female participants of childbearing potential were required to use adequate birth control. Each participating center received institutional ethics committee approval to conduct the study and all patients gave written informed consent. Patients had two baseline examinations 4 weeks apart before the treatment started and were randomized to either double (zidovudine and lamivudine) or triple therapy arm (zidovudine, lamivudine, and ritonavir). Participants were stratified by center and by consent to undergo tonsil biopsies. Randomization was performed centrally using a permuted block algorithm. Open-label antiretroviral therapy was administered at a dosage of 2 × 300 mg/day for zidovudine, 2 × 150 mg/day for lamivudine, and 2 × 600 mg/day for ritonavir.

Patients received follow-up with clinic visits at weeks −4, 0, 2, 4, 8, and every 4 weeks thereafter; beyond 48 weeks, the visits took place every 6 weeks. At each visit, routine laboratory tests were performed according to the protocol and 24-ml anticoagulated blood (CPT Vacutainer, Becton Dickinson, Franklin Lakes, NJ, U.S.A.) was stored as plasma and peripheral blood mononuclear cell (PBMC) fractions. Serial bilateral tonsil biopsies were obtained at weeks 0, 4, 24, 48, and 96 in 26 patients who consented to the procedure. Tonsil biopsies were selected to access the lymphoid tissue because of the suitability for repeated biopsies and that tonsil biopsies consist of more than 90% lymphoid tissue (13,14). In 1 additional patient, an ultrasound-guided needle biopsy of an inguinal lymph node was performed at weeks 0 and 24 and the viral load was included in the lymphoid tissue data.

The study design incorporated a recommendation for a treatment switch in case of inadequate treatment response or therapeutic failure, with the earliest switch being allowed after 16 weeks of trial therapy, based on the following criteria: HIV-1 RNA >1000 copies/ml plasma, confirmed in a second blood draw 4 weeks apart; or decrease in absolute CD4 lymphocyte counts by >20% in comparison to the average of the baseline (week −4) and the pretherapy (week 0) values, confirmed in a second blood draw 4 weeks apart; or development of an AIDS-defining illness. In the event of treatment failure, the suggested new treatment was at least one new nucleoside analogue plus at least one protease inhibitor for patients switching from the double therapy arm, and stavudine + ritonavir (600 mg twice daily) + saquinavir (400 mg twice daily) + nevirapine for patients failing in the triple therapy arm. In January 1998, the study was amended to redefine virologic failure and allow for a treatment switch if HIV-1 RNA was repeatedly detectable by the ultrasensitive test (detection limit = 50 copies/ml). If patients experienced treatment-limiting intolerance while HIV-1 RNA was suppressed, changing only one drug within the regimen was allowed.

Using these criteria, the study continues. Here, we present the complete data for the first 24 months of treatment.

Plasma HIV-1 RNA

The standard Amplicor HIV-1 Monitor Test, version 1.5 (Roche Diagnostic Systems, Branchburg, NJ, U.S.A.), with a lower limit of quantitation of 400 copies/ml, was used according to the manufacturer's instructions. Specimens with undetectable plasma HIV-1 RNA using the standard test and specimens taken after 8 weeks of trial medication were tested with modified versions of the Monitor test that enhanced the quantitation limit to 50 HIV-1 RNA copies/ml, as described previously (15,16).

Lymphoid Tissue and Peripheral Blood Mononuclear Cell HIV-1 Reverse Transcription Polymerase Chain Reaction

Tonsil or lymph node biopsies were frozen by submersion in liquid nitrogen within 2 minutes of excision. Each entire frozen biopsy (~2 × 4 mm) was completely cut into 6-μm serial frozen sections. The section series from each biopsy was grouped into levels of 18 serial sections, 10 to 12 frozen sections from each level were placed in a polypropylene screw-top microfuge tube (Sarstedt, Nurnbrecht, Germany) and stored at −70°C until needed for RNA extraction. For PBMC, "dry" pellets (−70°C) containing approximately 1 to 5×10^6 cells were used for RNA extraction. RNA extraction including DNase digestion from frozen tissue sections or PBMC using the RNeasy Kit (Qiagen Ltd., Hilde, Germany) was carried out as described previously (16). RNA yields (in nanograms per microliter) were fluorometrically determined using RiboGreen fluorescent dye (Molecular Probes, Eugene, OR, U.S.A.). Purified total cellular RNA was used for polymerase chain reaction (PCR) by a modification of the Amplicor HIV-1 Monitor Test, as described previously (16). Results are reported as HIV-1 RNA copies/μg total RNA. The median (maximum/minimum) lower limit of detection using this assay was 2 (5/1) copies/μg total RNA for PBMC and 2 (49/1) copies/μg RNA for lymphoid tissue specimens.

T-Lymphocyte Subpopulations

The absolute number and percentage of peripheral blood $CD4^+$ and $CD8^+$ T lymphocytes (henceforth referred to as CD4 and CD8 cells) were prospectively assessed using a whole-blood method of sample

preparation and two-color flow cytometry (Coulter, Hialeah, FL, U.S.A.). Absolute values were calculated using a particle concentration method based on precalibrated fluorescent microspheres (Flow-Check Fluorospheres, Coulter). For each analysis, 10,000 events were counted.

Statistical Analysis

A sample-size calculation performed before the start of the study indicated that 44 participants were required to detect a difference in virologic efficacy of 80% (proportion of patients with HIV-1 RNA < detection limit) on triple therapy versus 40% on double therapy with $\alpha = 0.05$ and $\beta = 0.2$. In all, 45 participants were randomized. Two withdrew before the treatment started, and 1 was randomized for a violation of the inclusion criteria (CD4 lymphocyte count <400 cells/μl) and was thus excluded before the start of therapy. The intent-to-treat population reported here consists of 42 patients who received at least one dose of study medication.

Assessment of proportion of patients with HIV-1 RNA below detection limit was based on the intent-to-treat principle, with missing data counting as virologic failure except when immediately preceded and immediately followed by values <50 copies/ml. Because of the relatively high number of patients who underwent treatment modification, virologic data were further evaluated using observed data on treatment. For calculations of $\log_{10}$ decreases in viral load, measurements below the quantitation limit were assigned the value of the corresponding quantitation limit. Potential treatment group differences were evaluated using a two-sample *t*-test for continuous measurements and Fisher's exact test for discrete measurements. The difference in CD4 and CD8 responses between the treatment groups was analyzed using repeated measures analysis (with imputation of missing data) and by the comparison of the area under the curve minus baseline.

RESULTS

Patient Characteristics

The study group consisted of 42 asymptomatic, treatment-naive individuals (31 males, 11 females) with a mean age of 33 years (Table 1). At baseline, the mean plasma HIV-1 RNA level was 4.00 ± 0.74 $\log_{10}$ copies/ml and the CD4 lymphocyte count was 516 ± 173/μl. Nineteen patients were randomized to the double-therapy group receiving zidovudine and lamivudine, and 23 patients received triple therapy with zidovudine, lamivudine, and ritonavir.

Patients started the treatment between September 1996 and July 1997. Moreover, 3 patients in the triple therapy arm withdrew from treatment after 5, 19, and 36 weeks and 1 in the double therapy arm withdrew after 72 weeks; these 4 patients were lost to follow-up.

HIV-1 RNA in Plasma

The proportions of patients with plasma HIV-1 RNA <50 copies/ml were 16% and 74% at week 24 ($p < .001$), 37% and 78% at week 48 ($p = .011$) and 42% and 74% at week 96 ($p = .059$) in patients randomized to double and triple therapy, respectively (intent-to-treat analysis, missing values counting as virologic failure; Fig. 1*A*).

When the HIV-1 RNA course on assigned treatment was evaluated (Table 2), only 1 patient randomized to double therapy achieved a sustained RNA suppression <50 copies/ml whereas another patient in this group had HIV-1 RNA <50 copies/ml at baseline and remained so. In all, 9 patients had consistently detectable HIV-1 RNA at levels between 50 and 10,000 copies/ml but elected to remain on double therapy and refused a suggested treatment switch. Eight patients switched from double to a new triple therapy (protease inhibitor and at least one new nucleoside analogue) between weeks 24 and 72 be-

TABLE 1. *Patient characteristics*

Characteristic	Double therapy	Triple therapy	All
No. of patients	19	23	42
Age (y)	35 ± 8	32 ± 10	33 ± 9
No. male (%)	14 (74)	17 (74)	31 (74)
HIV risk factor			
No. homosexual (%)	11 (58)	10 (43)	21 (50)
No. heterosexual (%)	6 (32)	8 (35)	14 (33)
No. injection drug use (%)	2 (11)	5 (22)	7 (17)
CD4 lymphocytes (cells/μl)	499 ± 176	531 ± 174	516 ± 173
CD4 lymphocytes (%)	30.1 ± 8.0	28.9 ± 8.7	29.5 ± 8.3
CD8 lymphocytes (cells/μl)	960 ± 825	1024 ± 595	995 ± 700
CD8 lymphocytes (%)	49.0 ± 12.3	50.5 ± 13.4	49.8 ± 12.8
HIV-1 RNA in plasma ($\log_{10}$ copies/ml)	4.15 ± 0.81	3.87 ± 0.67	4.00 ± 0.74
HIV-1 RNA in PBMC ($\log_{10}$ copies/μg RNA)	2.76 ± 0.80	2.75 ± 0.46	2.75 ± 0.63
No. serial lymphoid tissue biopsies	12	15	27
HIV-1 RNA in lymphoid tissue ($\log_{10}$ copies/μg RNA)	4.49 ± 0.31	4.55 ± 0.49	4.52 ± 0.42

Data represent mean ± standard deviation. The numbers for HIV-1 RNA and for the lymphocyte subsets are the average of the two screening values at week −4 and week 0. None of the baseline parameters was statistically different between the double and triple therapy groups. Lymphoid tissue was obtained by tonsil biopsies in 26 patients and by lymph node biopsies in 1 patient.

PBMC, peripheral blood mononuclear cells.

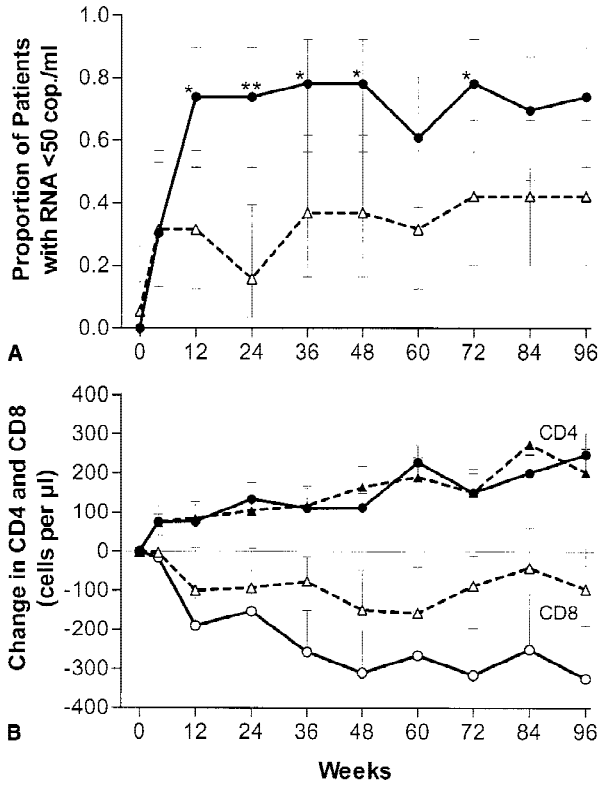

FIG. 1. Response to treatment in patients randomized to double (*dashed line*) versus triple (*solid line*) therapy, intent-to-treat analyses. (**A**) Proportion of patients with serum HIV-1 RNA <50 copies/ml. Missing data were considered as failure of RNA suppression except when immediately preceded and followed by values <50 copies/ml. Bars represent 95% confidence intervals. **p* < .05. ***p* < .001, Fischer's exact test for the difference between the arms. (**B**) Mean changes in CD4 and CD8 cell count from baseline. Bars represent one standard error of the mean (SEM).

cause their HIV-1 RNA never became fully suppressed. This new regimen achieved sustained HIV-1 RNA suppression <50 copies/ml in 7 (88%) and contributed to the increased proportion of patients with suppressed RNA seen in the intent-to-treat analysis. In contrast, 17 (74%) of 23 patients who started triple therapy achieved sustained HIV-1 RNA suppression <50 copies/ml. Among the 6 patients who did not achieve a sustained response, 3 completely withdrew from treatment because of intolerance or poor adherence and 1 patient had consistently poor adherence but remained in the study.

Four patients reduced their assigned triple regimen to only zidovudine + lamivudine due to intolerance of ritonavir at weeks 5, 7, 13, and 80, respectively. At the time of switch, HIV-1 RNA was <50 copies/ml in all 4 patients. Two remained suppressed during the remainder of their participation in this study (24 and 96 weeks, respectively). The HIV-1 RNA of the other 2 patients intermittently rose to 1390 and 54 copies/ml at weeks 63 and 71, respectively, but decreased again to 101 and <50 copies/ml at week 96.

CD4 and CD8 T-Lymphocyte Counts

Parallel to the suppression of HIV replication, CD4 lymphocytes increased in both groups to a similar extent (Fig. 1*B*). At week 96, the mean rise in CD4 lymphocytes was 201 and 247 cells/µl, corresponding to a rise from 30.1% to 36.1% and 28.9% to 39.8% of total lymphocytes, in patients randomized to double and triple therapy, respectively (intent-to-treat analysis). The levels of CD8 lymphocytes declined by 98 and 325 cells/µl at week 96, corresponding to a decrease from 49.0% to 40.7% and from 50.5% to 36.7%, in the double and triple therapy arms, respectively. The CD4 and CD8 responses did not differ significantly between the arms, neither by repeated measures analysis nor by the comparison of the area under the curve minus baseline.

HIV-1 RNA in Peripheral Blood Mononuclear Cells and Lymphoid Tissue

In conjunction with the reduction of HIV-1 RNA in plasma during the initial 4 weeks of treatment, cell-associated HIV-1 RNA decreased sharply by approximately 1.3 $\log_{10}$ in PBMC and 1.7 $\log_{10}$ in the lymphoid tissue in patients on triple therapy (Fig. 2). These decreases were $\approx$ 0.5 $\log_{10}$ less pronounced in patients on double therapy. Beyond week 4 of treatment, HIV-1 RNA in lymphoid tissue continued to decline in patients randomized to triple therapy only, leading to significant differences between the arms at week 24.

We further attempted to evaluate the effect of successful HIV suppression on the amount of cell-associated HIV-1 RNA over 2 years of treatment. For this purpose, *successful* HIV suppression was defined as sustained suppression of HIV-1 RNA in plasma below 50 copies/ml between week 48 and the end of the study observation at week 96, independent of the actual medication ("responders"). In all, 25 patients, 23 of whom received HAART at least during a part of the study, were classified as responders, whereas the remaining 17 nonresponders, mainly on double therapy, had an incomplete viral suppression in plasma with mean HIV-1 RNA values $\approx$ 2.5 $\log_{10}$ copies/ml (Fig. 3*A*). Within these two groups, we then assessed the time course of cell-associated HIV-1 RNA both in PBMCs and lymphoid tissue. Both groups showed similarly decreased HIV-1 RNA by over 1 $\log_{10}$ within the first 4 weeks of treatment, as previously mentioned (Fig. 3*B* and *C*). However, viral load continued to decrease in the responder

TABLE 2. *Virologic efficacy on treatment, assessed by plasma HIV-1 RNA levels and stratified by treatment modifications during the study*

Course of treatment	*N*	HIV-1 RNA decline <50 copies/ml	Sustained HIV-1 RNA suppression <50 copies/ml between weeks 48 and 96
Double			
Remained on double therapy	10	5 (50%)[a]	2 (20%)[a]
Switched to triple therapy	8	7 (88%)[b]	6 (75%)
Stopped therapy before study end	1	0 (0%)	NA
Triple			
Remained on triple therapy	16	15 (94%)	14 (88%)
Reduced to double therapy (AZT + 3TC)	4	4 (100%)[c]	3 (75%)
Stopped therapy/lost to follow up	3	1 (33%)	0 (0%)

[a] One patient had plasma HIV-1 RNA <50 copies/ml at baseline and remained so during the study.
[b] Intensification resulting from lack of full HIV-1 RNA suppression on double therapy; HIV-1 RNA decreased <50 copies/ml in all patients only after the treatment switch. One patient with alcoholism and poor adherence failed to reach HIV-1 RNA <50 copies/ml.
[c] HIV-1 RNA suppression occurred during triple therapy, before the switch to double therapy.

group, leading to a reduction of HIV-1 RNA in lymphoid tissue from 4.59 to 1.34 $\log_{10}$ HIV-1 RNA copies/μg RNA at week 48, and of HIV-1 RNA in PBMC from 2.76 to 1.20 $\log_{10}$ HIV-1 RNA copies/μg RNA at week 96. However, cell-associated HIV-1 RNA remained almost always detectable at low levels even in patients whose plasma HIV-1 RNA was fully suppressed. Despite relatively low HIV-1 RNA levels in the nonresponder group (average, 2.5 $\log_{10}$ HIV-1 RNA, corresponding to 300–400 copies/ml), cell-associated viral load showed only an initial decline within the first 4 weeks and remained rather stable thereafter. Thus, suboptimal therapy in this group produced and maintained viral suppression in the lymphoid tissue, less than fully successful therapy, but still clearly below the levels in untreated patients.

Clinical Course and Adverse Events

None of the patients suffered any HIV-associated opportunistic events during the study. Tolerability of the double therapy with zidovudine + lamivudine was good, no patient experienced dose-limiting toxicity. In contrast, triple therapy including ritonavir was not tolerated by 7 of 23 patients. Of them, 4 discontinued ritonavir (discussed previously) and 3 others replaced ritonavir with another protease inhibitor (nelfinavir or indinavir). Another 3 patients stopped the entire triple combination and were thus lost to follow-up. Overall, grade 2 or 3 toxicity occurred in 3 patients on double therapy (e.g., abdominal disturbances, dry skin) and in 12 patients on triple therapy, consisting mainly of nausea, vomiting, diarrhea, and elevation of pancreatic amylase levels in 1 patient. No grade 4 toxicity and no cases of lipodystrophy were reported during the 2 years of study duration.

DISCUSSION

This prospective, randomized trial demonstrates that double nucleoside analogue therapy with zidovudine and lamivudine in treatment-naive, asymptomatic patients with CD4 lymphocyte counts >400 cells/μl almost always fails to fully and continuously suppress plasma HIV-1 RNA, even when treatment is initiated early in the course of HIV infection. In an intent-to-treat analysis (counting missing values as failures), 74% of patients randomized to triple therapy had suppressed HIV-1 RNA <50 copies/ml at week 96. A significantly lower percentage (41%) of patients randomized to double therapy achieved this goal and suppression in this group was mostly the result of switching to HAART because of inadequate response to double nucleoside therapy. The viral response in the two arms in our study beyond week 24 therefore represents the comparison of an immediate versus a sequential or deferred start of HAART. Similar results have previously been shown in patients whose HIV infection was more advanced at the time of treatment initiation, both from prospective clinical trials and cohort data (1,2,17,18). In contrast to previous studies, our patients were asymptomatic and had well-preserved immune systems before treatment initiation. Thus, this study provides evidence that HAART is virologically necessary even in patients with favorable prognostic markers.

Measurement of cell-associated messenger HIV-1 RNA is considered primarily to represent recent viral transcription and allows assessment of the biologic activity of HIV in infected cells even when HIV-1 RNA is suppressed in plasma (16,19–21). Both double and triple therapy induced a sharp decline in PBMC and lymphoid tissue RNA within the first 4 weeks; however, the de-

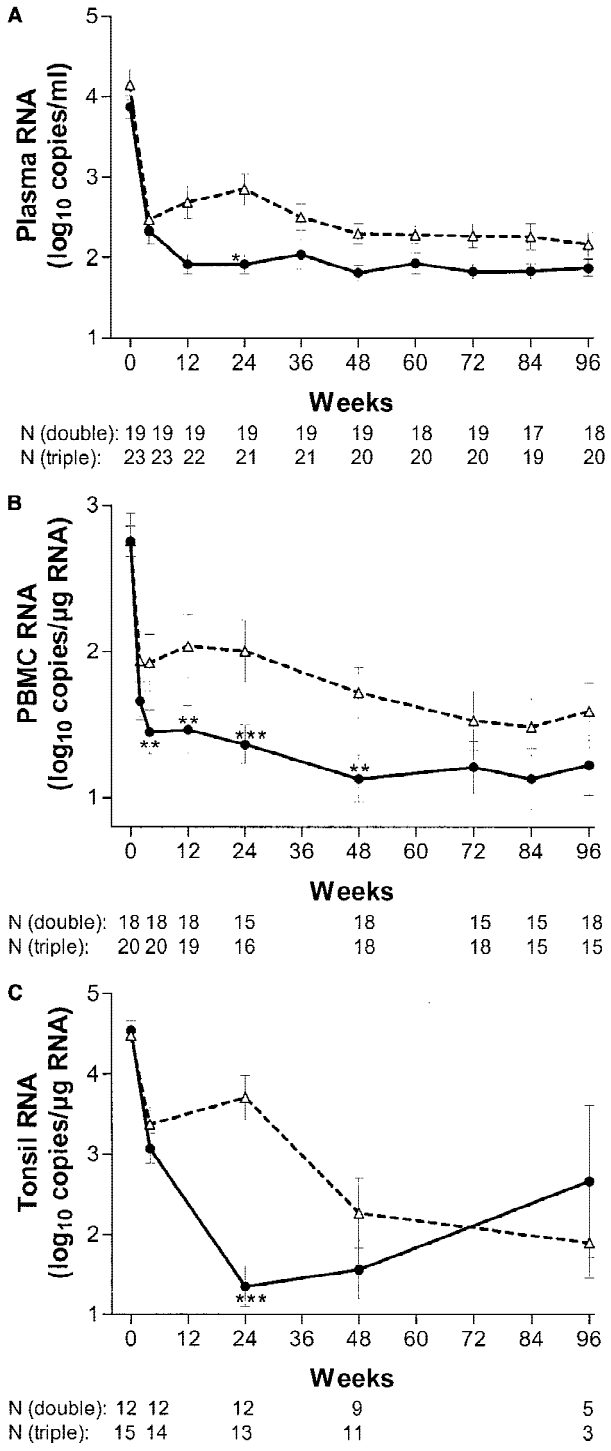

FIG. 2. Time course of HIV-1 RNA in patients randomized to double (*dashed line*) versus triple (*solid line*) therapy, intent-to-treat analysis. Mean $\log_{10}$ values and standarde error of the mean (SEM) (*bars*). Three compartments were analyzed: HIV-1 RNA (**A**) in plasma, (**B**) in peripheral blood mononuclear cells, and (**C**) in the lymphoid tissue ($N = 27$). HIV-1 RNA in PBMC was also measured at week 2. *$p \leq .05$. **$p < .005$. ***$p < .001$ for the comparison of changes from baseline between the groups.

cline was steeper and by approximately 0.5 $\log_{10}$ more pronounced on triple therapy. This fast decline is consistent with data from studies with frequent sampling of material (22,23), whereas less frequent sampling seems to miss the initial sharp drop in viral load (19,20). Our data show that the desired goal of further depletion of viral load in the lymphoid tissue reservoir is only accomplished in patients who achieve sustained suppression of HIV-1 RNA in plasma. In most of our patients, this only occurred on a protease inhibitor–containing combination. Conversely, patients who continued therapy that was not fully suppressive and whose plasma HIV-1 RNA values were between 150 and 1050 copies/ml (interquartile range, 25%–75%) were able to maintain a moderate degree of suppression of cell-associated HIV-1 RNA. Such partial viral load depletion may explain why combination regimens that are not fully suppressive provide clinical benefit (3,24).

Even if treatment decreased the HIV-1 RNA in plasma <50 copies/ml, cell-associated HIV-1 RNA remained detectable in each such patient, both in PBMCs and in the lymphoid tissue, with only a few datapoints yielding occasionally undetectable values. Persistence of cell-associated HIV-1 RNA has been recently demonstrated in successfully treated patients, most of whom, however, were at a more advanced stage of HIV infection at the time when treatment had started (19–21). Together with data demonstrating that slow evolution of proviral HIV-1 DNA takes place despite seemingly effective HAART (21,25), these studies indicate that low level viral transcription and possibly replication persist in some of these patients, and our data confirm that this remains true even in patients with high initial CD4 counts and HAART that persisted for 2 years. Consequently, and supported by the calculation of an extremely long half-life of the latently infected CD4 cells of 44 months (26), the chance of virus eradication by conventional HAART remains miniscule, independent of whether treatment initiation occurs at an early stage of HIV infection or later.

Reassessing the principle of "hit HIV early and hard" (27), our study indicates that even if antiretroviral treatment is initiated early, but after the establishment of a chronic HIV infection, only "hitting hard" will substantially reduce cell-free and cell-associated viral load. Suppression of plasma HIV-1 RNA below 20 to 50 copies/ml seems necessary to prevent ongoing mutations and development of drug resistance, which eventually lead to treatment failure (21,28,29). Although virologic response was eventually good in those of our patients who started double therapy, failed, and were rescued with subsequent HAART, this procedure has probably led to development of resistance to the initial double regimen, thus dimin-

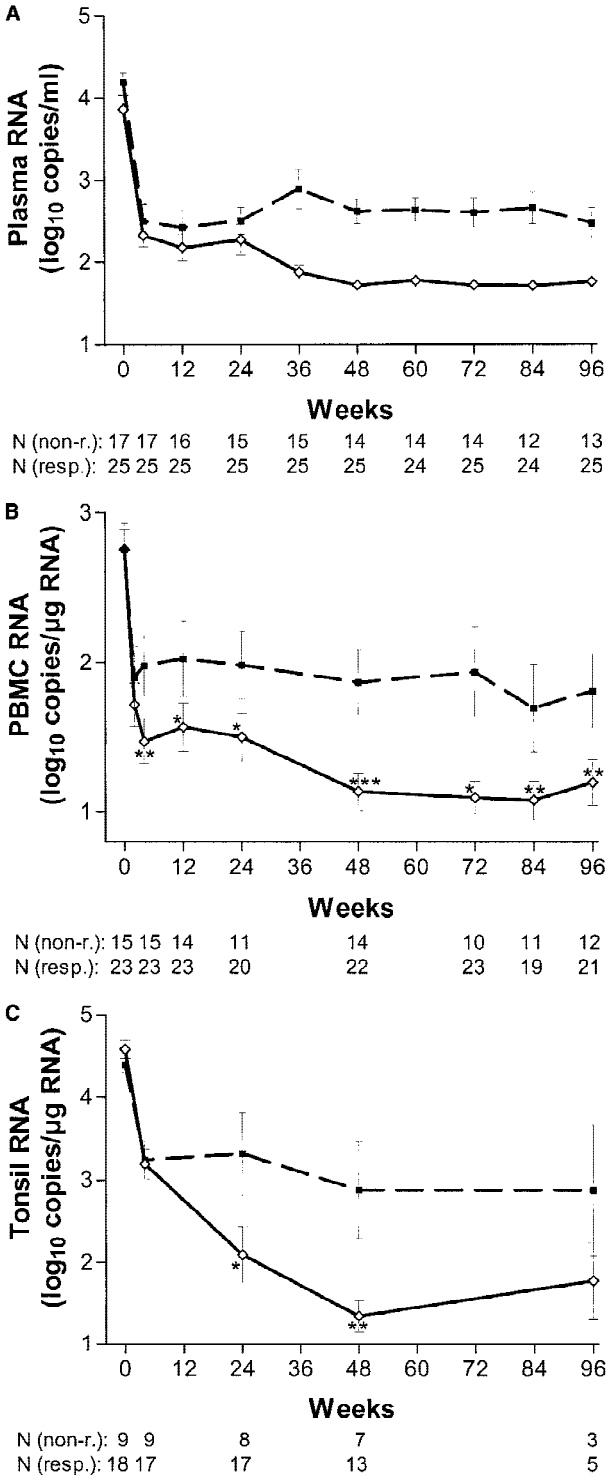

FIG. 3. (**A**) Time course of HIV-1 RNA in plasma, stratified by patients with (N = 25, "responders," *solid line*) and without (N = 17; "nonresponders," *dashed line*) sustained viral suppression in plasma, defined as HIV-1 RNA <50 copies/ml between weeks 48 and 96. The "responder" group showed a significantly stronger decrease of cell-associated HIV-1 RNA, both in (**B**) peripheral blood mononuclear cells and in (**C**) lymphoid tissue. Mean $\log_{10}$ values and standard error of the mean (*bars*). *$p \leq .05$. **$p < .005$. ***$p < .001$ for the comparison of changes from baseline between the groups.

ishing further treatment options. The same applies to those patients who remained on double therapy during the 2-year duration of the study. Although avoiding the side effects and inconvenience of protease inhibitors, their virologic and immunologic values were more favorable than before treatment initiation, but inferior to those of patients on HAART. The newest antiretroviral treatment guidelines (30,31), aimed at achieving the best possible response and stating that an initial combination of only two nucleoside analogues is no longer recommended thus also apply to patients who initiate treatment very early. This is in contrast to the real-life situation in which more than 40% of patients who initiated therapy in the United Kingdom between January and June 1998 did not receive triple therapy (32).

Our study did not address the controversy of early versus late initiation of therapy (33). Overall virologic response was no better in our patients in comparison with those reported in the literature in whom treatment started later. Treatment-induced sustained reduction in plasma HIV-1 RNA correlates strongly with the eventual clinical benefit (7,18,34) and on a long-term basis, an additional lowering of the risk of progression to AIDS and death can be extrapolated for these patients. However, no clinical data exist at present to support this assumption and comparative trials with clinical endpoints are not likely to ever be feasible in this low-risk population.

In our study, treatment over 2 years induced a continuously rising beneficial effect on CD4 and CD8 T lymphocytes. In an immunologic substudy in 15 participants from this study receiving triple therapy, we previously demonstrated that high levels of CD8 cell activation exist in these patients and that early initiation of treatment is capable of reconstituting the number of naive CD4 cells and of significantly decreasing the high activation level of CD8 cells (35). Because high levels of CD8 activation markers (HLA-DR$^+$ or CD38$^+$) are predictive of HIV progression or of the development of AIDS (36), treatment-induced normalization of these abnormalities may counteract further immune-mediated disease due to uncontrolled HIV replication. The stronger decline in CD8 cells in the triple therapy arm, shown predominantly to represent a decrease in the activation state of cytotoxic T lymphocytes (35), indirectly suggests a reduced risk of subsequent clinical progression. These beneficial effects, however, must be weighed against the burden of a lifelong daily intake of pills and at least a temporary impairment of qualify of life in patients on triple therapy, as documented by the evaluation of the quality-of-life questionnaires within this study and reported previously (37). Although lipodystrophy was not reported in any of our patients up to week 96, adverse

events associated with long-term use of protease inhibitors such as hyperlipidemia, lipodystrophy, and insulin resistance may impose an additional, delayed burden on patients who initiate treatment early (38). Thus, appropriate risk assessment and thorough information of the patient is necessary before treatment is initiated (31).

The observation that four patients who started triple therapy discontinued ritonavir due to adverse events, and partially maintained suppression of HIV-1 RNA with only zidovudine and lamivudine, supports the hypothesis raised within the INCAS study (39) that simpler maintenance treatment after effective induction therapy may be feasible in selected patients. However, in view of the failures of the induction-maintenance treatment principle reported in three controlled trials (40–42), this observation must be judged with caution and may be related to particularly favorable prognostic baseline parameters in these patients.

In conclusion, the data obtained in our study demonstrate that even when antiretroviral therapy is initiated early in this low-risk population, it must consist of HAART to achieve durable viral suppression in plasma and substantial decrease of cell-associated HIV-1 RNA in PBMC and lymphoid tissue. However, ongoing low-level viral transcription despite 2 years of treatment indicates that continued rounds of viral replication may occur that would render eradication of HIV by conventional HAART unlikely. Further studies are needed to determine whether the continuous beneficial effects on both CD4 and CD8 lymphocyte subsets and on viral load warrant such an early treatment intervention in patients with low risk of clinical progression.

APPENDIX

The members of the Swiss HIV Cohort Study are M. Battegay (Chairman of the Scientific Board), E. Bernasconi, Ph. Bürgisser, M. Egger, P. Erb, W. Fierz, M. Flepp (Chairman of the Group "Clinics"), P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, CH-1011—Lausanne), H.J. Furrer, P. Grob, B. Hirschel, B. Ledergerber, L. Matter (Chairman of the Group "Laboratories"), A. Meynard, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, W. Pichler, J-C. Piffaretti, M. Rickenbach (Head of Coordination and Data Center), C. Rudin, P. Sudre, J. Schupbach, A. Telenti, P. Vernazza, and R. Weber.

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REFERENCES

1. Gulick RM, Mellors JW, Havlir D, et al. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med* 1997;337:734–9.
2. Hammer SM, Squires KE, Hughes MD, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. *N Engl J Med* 1997;337:725–33.
3. Delta Coordinating Committee. Delta: a randomised double-blind controlled trial comparing combinations of zidovudine plus didanosine or zalcitabine with zidovudine alone in HIV-infected individuals. *Lancet* 1996;348:283–91.
4. Hammer SM, Katzenstein DA, Hughes MD, et al. A trial comparing nucleoside monotherapy with combination therapy in HIV-infected adults with CD4 cell counts from 200 to 500 per cubic millimeter. *N Engl J Med* 1996;335:1081–90.
5. Opravil M, Hill AM, DeMasi R, Dawson D. Prediction of HIV-1 RNA suppression and its durability during treatment with zidovudine/lamivudine. *Antiviral Ther* 1998;3:169–76.
6. O'Brien WA, Hartigan PM, Daar ES, Simberkoff MS, Hamilton JD, for the VA Cooperative Study Group on AIDS. Changes in plasma HIV RNA levels and $CD4^+$ lymphocyte counts predict both response to antiretroviral therapy and therapeutic failure. *Ann Intern Med* 1997;126:939–45.
7. Marschner IC, Collier AC, Coombs RW, et al. Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. *J Infect Dis* 1998;177:40–7.
8. Katzenstein DA, Hammer SM, Hughes MD, et al. The relation of virologic and immunologic markers to clinical outcomes after nucleoside therapy in HIV-infected adults with 200 to 500 CD4 cells per cubic millimeter. *N Engl J Med* 1996;335:1091–8.
9. Spijkerman I, de Wolf F, Langendam M, Schuitemaker H, Coutinho R. Emergence of syncytium-inducing human immunodeficiency virus type 1 variants coincides with a transient increase in viral RNA level and is an independent predictor for progression to AIDS. *J Infect Dis* 1998;178:397–403.
10. Kempf DJ, Rode RA, Xu Y, et al. The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir. *AIDS* 1998;12:F9–14.
11. Raboud JM, Montaner JS, Conway B, et al. Suppression of plasma viral load below 20 copies/ml is required to achieve a long-term response to therapy. *AIDS* 1998;12:1619–24.
12. Carpenter CC, Fischl MA, Hammer SM, et al. Antiretroviral therapy for HIV infection in 1996. Recommendations of an international panel. *JAMA* 1996;276:146–54.
13. Faust RA, Henry K, Dailey P, et al. Outpatient biopsies of the palatine tonsil: access to lymphoid tissue for assessment of human immunodeficiency virus RNA titers. *Otolaryngol Head Neck Surg* 1996;114:593–8.
14. Haase AT, Henry K, Zupancic M, et al. Quantitative image analysis of HIV-1 infection in lymphoid tissue. *Science* 1996;274: 985–9.
15. Schockmel GA, Yerly S, Perrin L. Detection of low HIV-1 RNA levels in plasma. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;14:179–83.
16. Fischer M, Huber W, Kallivroussis A, et al. Highly sensitive methods for quantitation of human immunodeficiency virus type 1 RNA from plasma, cells, and tissues. *J Clin Microbiol* 1999;37:1260–4.
17. Hogg RS, Rhone SA, Yip B, et al. Antiviral effect of double and triple drug combinations amongst HIV-infected adults: lessons from the implementation of viral load-driven antiretroviral therapy. *AIDS* 1998;12:279–84.

18. Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy in HIV-1 patients: a prospective cohort study. *Lancet* 1999;353:863–8.
19. Romano L, Venturi G, Catucci M, De Milito A, Valensin PE, Zazzi M. Evaluation of cell-free and cell-associated peripheral blood human immunodeficiency virus type 1 RNA response to antiretroviral therapy. *J Infect Dis* 1999;179:361–6.
20. Furtado MR, Callaway DS, Phair JP, et al. Persistence of HIV-1 transcription in peripheral-blood mononuclear cells in patients receiving potent antiretroviral therapy. *N Engl J Med* 1999;340: 1614–22.
21. Zhang LQ, Ramratnam B, Tenner-Racz K, et al. Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med* 1999;340:1605–13.
22. Cavert W, Notermans DW, Staskus K, et al. Kinetics of response in lymphoid tissues to antiretroviral therapy of HIV-1 infection. *Science* 1997;276:960–4.
23. Tenner-Racz K, Stellbrink HJ, van Lunzen J, et al. The unenlarged lymph nodes of HIV-1-infected, asymptomatic patients with high CD4 T cell counts are sites for virus replication and CD4 T cell proliferation. The impact of highly active antiretroviral therapy. *J Exp Med* 1998;187:949–59.
24. CAESAR Coordinating Committee. Randomised trial of addition of lamivudine or lamivudine plus loviride to zidovudine-containing regimens for patients with HIV-1 infection: the CAESAR trial. *Lancet* 1997;349:1413–21.
25. Martinez MA, Cabana M, Ibanez A, Clotet B, Arno A, Ruiz L. Human immunodeficiency virus type 1 genetic evolution in patients with prolonged suppression of plasma viremia. *Virology* 1999;256:180–7.
26. Finzi D, Blankson J, Siliciano JD, et al. Latent infection of $CD4^+$ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med* 1999; 5:512–7.
27. Ho DD. Time to hit HIV, early and hard. *N Engl J Med* 1995;333: 450–1.
28. Gunthard HF, Wong JK, Ignacio CC, et al. Human immunodeficiency virus replication and genotypic resistance in blood and lymph nodes after a year of potent antiretroviral therapy. *J Virol* 1998;72:2422–8.
29. Wong JK, Gunthard HF, Havlir DV, et al. Reduction of HIV-1 in blood and lymph nodes following potent antiretroviral therapy and the virologic correlates of treatment failure. *Proc Natl Acad Sci USA* 1997;94:12574–9.
30. Carpenter CC, Fischl MA, Hammer SM, et al. Antiretroviral therapy for HIV infection in 1998: updated recommendations of the International AIDS Society-USA Panel. *JAMA* 1998;280:78–86.
31. Gazzard BG, Moyle G, on behalf of the BHIVA Guidelines Writing Committee. 1998 revision to the British HIV Association guidelines for antiretroviral treatment of HIV seropositive individuals. *Lancet* 1998;352:314–6.
32. The UK Register of HIV Seroconverters Steering Committee. How soon after HIV seroconversion is antiretroviral therapy initiated? *AIDS* 1999;13:1241–7.
33. Levy JA. Caution: should we be treating HIV infection early? *Lancet* 1998;352:982–3.
34. Fiscus SA, Hughes MD, Lathey JL, et al. Changes in virologic markers as predictors of CD4 cell decline and progression of disease in human immunodeficiency virus type 1-infected adults treated with nucleosides. *J Infect Dis* 1998;177:625–33.
35. Bisset LR, Cone RW, Huber W, et al. Highly active antiretroviral therapy during early HIV infection reverses T-cell activation and maturation abnormalities. *AIDS* 1998;12:2115–23.
36. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. Elevated CD38 antigen expression on $CD8^+$ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than $CD4^+$ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;16:83–92.
37. Zinkernagel C, Ledergerber B, Battegay M, et al. Quality of life in asymptomatic patients with early HIV infection initiating antiretroviral therapy [letter]. *AIDS* 1999;13:1587–9.
38. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet* 1999;353:2093–9.
39. Hall DB, Montaner JG, Reiss P, et al. Induction-maintenance antiretroviral therapy: proof of concept. *AIDS* 1998;12:F41–4.
40. Havlir DV, Marschner IC, Hirsch MS, et al. Maintenance antiretroviral therapies in HIV infected patients with undetectable plasma HIV RNA after triple-drug therapy. *N Engl J Med* 1998;339: 1261–8.
41. Pialoux G, Raffi F, Brun VF, et al. A randomized trial of three maintenance regimens given after three months of induction therapy with zidovudine, lamivudine, and indinavir in previously untreated HIV-1-infected patients. *N Engl J Med* 1998;339:1269–76.
42. Reijers MHE, Weverling GJ, Jurriaans S, et al. Maintenance therapy after quadruple induction therapy in HIV-1 infected individuals: Amsterdam Duration of Antiretroviral Medication (ADAM) study. *Lancet* 1998;352:185–90.