

Comparison of twice-daily stavudine plus once- or twice-daily didanosine and nevirapine in early stages of HIV infection: the Scan Study

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Objectives: To evaluate the safety and effectiveness of once-daily didanosine and nevirapine plus twice-daily stavudine versus twice-daily administration of all three drugs.

Methods: This open-label, randomized, multicentre study enrolled 94 antiretroviral-naive patients with chronic HIV infection, CD4+ cell counts $> 500 \times 10^6$ cells/l, and viral loads > 5000 copies/ml. Patients were treated with either 40 mg stavudine (twice daily) plus 400 mg didanosine (once daily) and 400 mg nevirapine (once daily) or 40 mg stavudine (twice daily) plus 200 mg didanosine (twice daily) and 200 mg nevirapine (twice daily).

Results: After 12 months, 68% of patients who received twice-daily didanosine and nevirapine had viral loads < 200 copies/ml in the intention-to-treat and 79% in the on-treatment analysis, respectively. The corresponding values for patients treated with didanosine and nevirapine, taken once-daily, were 73 and 85%. The percentages of patients in each group with viral loads < 5 copies/ml at 12 months were 40% (once daily) and 45% (twice daily) for the intention-to-treat analysis. Five of 11 patients (45%) with plasma viral loads < 5 copies/ml at 12 months had detectable virus in tonsillar tissue. Genotypic resistance to nevirapine was noted in seven of the 14 patients with detectable viral load at month 12. Mean changes in CD4+ cell counts for patients treated with stavudine plus once- or twice-daily didanosine and nevirapine were 154 and 132×10^6 cells/l, respectively. Treatment was interrupted due to adverse events in seven patients (8%) (four who received once-daily didanosine and nevirapine and three treated with twice-daily doses).

Conclusions: The combination of twice-daily stavudine plus once-daily didanosine and nevirapine was as safe and well tolerated as twice-daily administration of all three agents. Both regimens were equally effective in reducing viral loads and in increasing CD4+ cell counts.

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Introduction

First-line treatment for patients with HIV infection generally includes two nucleoside reverse transcriptase inhibitors (NRTI) and a protease inhibitor (PI) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) [1]. The results of large-scale clinical trials have demonstrated that these combinations are highly effective in reducing viral load and restoring immunologic function [2,3]. Nevertheless, this approach to initial antiretroviral therapy may not be suitable for all patients. One potentially important limitation of these treatment regimens is the requirement for multiple medications that may require dosing as many as three times per day. Such complicated dosing regimens and the short- or long-term intolerance to these treatments may decrease patient compliance and jeopardize the efficacy of antiretroviral therapy [4–6].

The issues raised above support continued evaluation of new antiretroviral combinations for initial therapy of patients with HIV infection. The results of several recent studies have suggested that the combinations including two NRTI, stavudine, didanosine, and the NNRTI, efavirenz or nevirapine, may provide effective initial therapy for patients with HIV infection [3,7–9]. In addition to effectively suppressing HIV replication, such combinations have the potential to significantly enhance the convenience of antiretroviral therapy. Didanosine has a long intracellular half-life [10] and is effective when administered once daily to patients with HIV infection [11–14]. In addition, *in vitro* results have shown that didanosine and stavudine have additive effectiveness in suppressing HIV replication [15]. Both efavirenz and nevirapine have plasma half-lives that exceed 24 h [16–18], and once-daily dosing results in plasma concentrations many times higher than those required to inhibit HIV replication in cell culture [18]. These results all suggest that the combination of twice-daily stavudine and once-daily didanosine and efavirenz or nevirapine may provide effective initial combination therapy for patients with HIV infection. However, almost all the studies have been performed in relatively advanced patients and it is not known if these results are also applicable to early stages. Therefore, it is also important to study these alternative regimens with single daily dosing also in early stages.

The present pilot study was carried out to evaluate the safety and effectiveness of once-daily didanosine and nevirapine plus twice-daily stavudine versus twice-daily administration of three antiretroviral drugs for reducing viral loads and increasing CD4+ cell counts, slowing disease progression, and enhancing survival in antiretroviral-naïve patients with HIV infection. We studied these combinations in a cohort of antiretroviral naïve chronic HIV-1-infected patients with a baseline viral

load above 5000 copies/ml and a CD4+ T-cell count above 500×10^6 cells/l.

Methods

Design and patients

This prospective pilot trial used an open-label, randomized design and was carried out at 10 centres in Spain. The study was approved by the institutional review boards of the participating institutions, and all patients gave written informed consent. Patients eligible for enrolment were antiretroviral-naïve and at least 18 years of age, with chronic HIV infection. Patients were required to have CD4+ cell counts $> 500 \times 10^6$ cells/l and viral loads > 5000 copies/ml. Exclusion criteria included pregnant or breast-feeding women, individuals with active substance abuse, absolute neutrophil count $< 1 \times 10^9$ /l, platelet count $< 0.8 \times 10^{12}$ /l, haemoglobin < 90 g/l, transaminase levels more than four times the upper limit of the normal range, alkaline phosphatase levels more than five times the upper limit of the normal range; serum creatinine level greater than twice the upper limit of the normal range, or a Karnofsky score < 90 points.

Treatments

Patients were randomized to receive 40 mg stavudine (twice daily) plus 150–200 mg didanosine (twice daily, based on body weight) and 200 mg nevirapine (twice daily) or 40 mg stavudine (twice daily) plus 300–400 mg didanosine (daily, based on body weight) plus 400 mg nevirapine daily. Patients in both treatment arms received nevirapine at a dosage of 200 mg daily for the first 2 weeks of treatment.

Evaluations

Viral loads and CD4+ cell counts were evaluated at baseline and at 1, 2, 4, 6, 9, and 12 months after the start of therapy. Viral load testing with the ultrasensitive assay was carried out at 6 and 12 months. Assessments of HIV RNA levels in peripheral blood (Amplicor HIV Monitor Test, Roche Diagnostics Systems, Inc, Branchburg, New Jersey, USA, limit of detection = 200 copies/ml) were carried out at baseline and at the end of 1, 2, 4, 6, 9, and 12 months of treatment. Viral loads were also assessed with an ultrasensitive assay (limit of detection = 5 copies/ml) [19] at 6 and 12 months. In brief, HIV particles were concentrated from 1 ml of plasma by ultracentrifugation of samples at 23 300 rpm ($50\,000 \times g$) for 80 min at 4°C (Heraeus Biofuge 28RS; Heraeus Instrument, Hanau, Germany). The pelleted virus particles were lysed, precipitated, and washed as with the standard assay, but the residual ethanol was removed in a speed-vac centrifuge in order to avoid inhibition of polymerase chain reaction (PCR). The dry pellet was resuspended in 55 µl speci-

men diluent, and reverse transcriptase (RT)-amplification, hybridization, and detection were performed by the standard method. The amount of QS (quantitation standard) added to the working lysis buffer was adjusted in order to have the same concentration as in the standard Amplicor PCR reaction. Calculation of RNA concentration was made by comparing the total HIV-1 optical density (OD at 450 nm) of the sample to the total QS OD of the sample, using a correction factor in the formula (1 instead of 40). This was done because the volume of starting sample and the volume of specimen diluent used for resuspending the precipitated RNA was different from the standard method.

Lymphoid HIV-1 RNA

Tonsillar biopsies were performed at baseline and after 12 months of treatment on all the individuals enrolled at the coordinating center who had accessible tonsillar tissue and who gave their written informed consent. Each tonsillar sample was split in two parts, one-half was paraffin-embedded and examined by a pathologist to confirm the presence of lymphoid tissue. The other half was frozen immediately and stored in liquid nitrogen. HIV-1 RNA in tonsillar biopsies was determined using the NucliSens HIV-1 RNA QT Assay (Organon Teknika, Turnhout, Belgium). RNA was extracted from lymphoid tissue using the Boom extraction method according to the manufacturer's (Organon Teknika) recommended protocol. In brief, 80 slices of each specimen (mean total weight 18 mg) were added to 1 ml guanidine thiocyanate-containing lysis buffer. After homogenization, 10 µl of each sample was added to 1 ml of new lysis buffer with RNA internal standards (NucliSens; Organon Teknika) and 50 µl silica suspension to bind nucleic acid. After centrifugation, the silica pellet was washed five times (twice with guanidine thiocyanate-based wash buffer, twice with 70% ethanol, and once with acetone). Subsequently, nucleic acid was eluted using 50 µl of Tris-EDTA-based elution buffer. Five microlitres of this nucleic acid solution used in the amplification reaction (NucliSens; Organon Teknika) were added to 45 µl of specimen diluent, and the sample was amplified using nucleic acid sequence-based amplification, following the manufacturer's protocol (NucliSens; Organon Teknika). The amount of nucleic acid was expressed as copies/mg of tissue, based on the internal RNA standards.

Genotypic resistance

For the analysis of genotypic resistance, viral RNA was extracted from plasma by the method of Chomczynski and Sacchi [20]. The viral RNA was retrotranscribed into complementary DNA and subsequently amplified by a single-tube RT-PCR using the TruGene HIV-1 assay (Visible Genetics, Inc. Toronto, Canada). This step produced a 1.3 kb amplicon covering the protease gene and the first 318 codons of the RT gene of HIV-1. Amplification products were sequenced simulta-

neously in three gene portions: the whole protease and RT codons 39–142 (beginning) and 135–244 (middle). This process used CLIP sequencing that generates a sequencing ladder in both forward and reverse directions. Each sequencing reaction was loaded into a MicroGene Clipper sequencer (Version 3.0; Visible Genetics Ltd.). For each sample sequenced, the six resulting assays were based called. Base-calling was performed using GeneObjects software (Version 3.0; Visible Genetics Ltd.), and the bases were aligned and assembled using GeneLibrarian software (Version 3.0; Visible Genetics Ltd.). The resulting sequences for each sample were compared with a database containing known drug resistance mutations.

T-lymphocyte subsets

Evaluation of T-lymphocyte subsets and proliferation assays were carried out at baseline and after 12 months of treatment. They were evaluated in the 11 patients enrolled in the coordinating center. The subpopulations of CD4+ lymphocytes RA+, and RO+ and CD8+ lymphocytes CD38+, CD28+, RA+ and RO+ were determined by three-colour flow cytometry at baseline and 12 months. Proliferative responses to HIV-1 antigens also were determined. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over Ficoll-Paque and resuspended at 2×10^6 cells/ml in serum-free medium x-VIVO10 (BioWhittaker, Walkersville, Maryland, USA). Cells were cultured into 96-well plates (TPP Europe, Trasadingen, Switzerland) and stimulated in triplicate with HIV p24 antigen (Protein Sciences, Meriden, Connecticut, USA). After 6 days of incubation at 37°C in 5% CO₂, cells were pulsed with 1 µCi of [³H]thymidine. After a further 18 h of incubation, cells were harvested, and [³H]thymidine incorporation was read as counts per minute of β-radioactivity by a scintillation counter (Betaplate; LKB, Wallac, Sweden). Stimulation index (SI) was defined as counts per minute in the presence of a stimulus divided by counts per minute in its absence. An SI > 3 was considered to represent a proliferative response.

Endpoints and definitions

The primary endpoints for the study were the percentage of patients with viral loads < 200 copies/ml at the end of 12 months of treatment and safety. Secondary endpoints included percentages of patients with viral loads < 5 copies/ml at 12 months, time to viral rebound in patients with viral loads that decreased to < 200 copies/ml, CD4 cell response, and disease progression and survival. Treatment safety was evaluated by the recording of adverse events and abnormal clinical laboratory values. In the assessment of safety, the outcome measure was the occurrence of adverse events defined as severe or worse according to the grading scheme of the AIDS Clinical Trial Group [21]. Patients were considered to have developed lipodystro-

phy syndrome if they fulfilled the criteria of the case definition of Carr *et al.* [22].

Statistical analyses

The sample size was calculated in order to demonstrate the equivalence between the two treatment regimens. It was assumed that 80% of patients in the twice-daily treatment arm would achieve plasma viral loads < 200 copies/ml after 1 year of therapy. Both therapies would be considered as equivalent if the higher limit of unilateral 95% confidence interval in the proportion of patients with a viral load above 200 copies/ml after 1 year of treatment in the once-daily arm was not > 35%. This value represents the lower limit of unilateral 95% confidence interval of the difference between the patients who did not have a viral load below 200 copies/ml in the INCAS study [8] that compared a similar triple therapy, zidovudine plus didanosine and nevirapine with zidovudine plus didanosine. If equivalence was demonstrated with this criterion, it could be concluded that these regimens were more efficacious than double therapy. The alpha error was set at 0.05 and the beta error at 0.2.

Results were evaluated on both an intent-to-treat (ITT) and on-treatment basis. Analysis of all the variables pertaining to efficacy was performed on an ITT basis that included data on all randomized patients and all available follow-up data (lost patients or missing data were considered as failures). Patients were followed until the last randomized patient completed 1 year of follow-up. Between-group differences for categorical variables were assessed using Fisher's exact tests. Survival analysis and log-rank tests were used to assess time to viral rebound. Between-group comparisons for these variables were made using proportional hazards methods.

For each pair of groups, Fisher's exact test was used to compare proportions of patients with a decrease of RNA viraemia to < 200 copies/ml, and the adverse events.

Change and durability in RNA viraemia and CD4+ T-cell counts over a period of 12 months were analysed by an area-under-the-curve measurement that incorporated the baseline value. All the patients with determinations made at baseline and at least once subsequently were included in the analysis (including those obtained after discontinuing or changing the study-assigned treatment). For the purpose of analysis, RNA values reported as undetectable (< 200 copies/ml) were considered equivalent to 200 copies/ml. The HIV RNA values underwent a log₁₀ transformation before analysis. The area-under-the-curve was compared among the different groups with an analysis of variance model. Pairwise comparisons were corrected by Bonferroni test.

Quantitative data for CD4+RA+, CD4+RO+, CD8+RA+, CD8+RO+, CD8+CD38+, CD8+CD28+ cells were compared from baseline to 12 months with a *t*-test for paired data. The same populations and proliferative SIs were compared between groups with *t*-tests for independent samples.

Results

Patients enrolled

A total of 94 patients were enrolled. Twenty-four patients (83%) were males in the twice-daily arm and 26 (81%) in the once-daily arm. Five patients (17%) were drug users in the twice-daily arm and nine (28%) in the once-daily arm. The baseline virologic and immunologic data for these individuals are summarized in Table 1. The mean \pm SE of log baseline viral load for the patients randomized to twice-daily dosing with didanosine and nevirapine was 4.41 ± 0.09 , and that for the patients assigned to once-daily didanosine and nevirapine was 4.34 ± 0.07 . The respective mean \pm SE of CD4+ cell counts for patients in the two treatment arms were 681 ± 32 and $700 \pm 28 \times 10^6$ cells/l. There were no statistically significant differences between treatment groups with respect to these variables.

In both treatment groups, most of the patients completed the study. Three patients randomized to twice-daily dosing with didanosine and nevirapine and two of those randomized to once-daily treatment did not begin treatment with study drugs and were excluded from all analyses. Three patients assigned to twice-daily didanosine and nevirapine (7%) withdrew consent, two were lost to follow-up (4%), and one became pregnant (2%). Five of the patients assigned to once-daily didanosine and nevirapine (11%) withdrew consent and one was lost to follow-up (2%). Adverse events leading to discontinuation of treatment are discussed below (see Safety). There were no deaths during the study and none of the patients experienced AIDS-related clinical events.

Effects of treatment on plasma viral load

Reductions in viral load achieved over 80 weeks with the two treatment regimens (ITT analysis) are illustrated in Fig. 1. The mean reductions in viral load at 12 months for the patients who received once- versus twice-daily didanosine and nevirapine were 1.84 ± 0.13 log and 1.78 ± 0.13 log copies/ml, respectively. After 12 months of treatment, the respective percentages of patients in each group with viral loads < 200 copies/ml were 73 and 68% for the ITT analysis and 85 and 79% for the on-treatment analysis. The relationships between treatment time and percentages of patients with viral loads < 200 copies/ml for the ITT and on-treatment populations are shown in Fig. 2.

Table 1. Baseline virologic and immunologic data for patients enrolled^a.

	Twice-daily ddl and NVP	Once-daily ddl and NVP	Total cohort
Number recruited	47	47	94
Number evaluated	44	45	89
Gender (males)	24	26	50
Risk factors (drug addicts)	5	9	14
Baseline viral load (mean log ± SE)	4.41 ± 0.09	4.34 ± 0.07	4.38 ± 0.03
Patients with viral load >10 ⁵ copies/mL (%)	21	16	37
Baseline CD4+ cell count (mean ± SE)	681 ± 32	700 ± 28	690 ± 17

^aNone of the small between-group differences were statistically significant. Three patients randomized to twice-daily dosing with didanosine (ddl) and nevirapine (NVP) and two of those randomized to once-daily treatment did not begin study drugs and were excluded from all analyses.

The respective percentages of patients in each group with viral loads < 5 copies/ml at 12 months were 40 and 45% for the ITT analysis and 46 and 53% for the on-treatment analysis. There was no significant difference between the two treatment arms in probability of treatment failure over the course of the study (*P* = 0.39; hazard ratio, 1.62; 95% confidence interval, 0.54–4.8).

Tonsillar HIV RNA

Tonsillar biopsies were performed in a subset of 11 patients (five from the once-daily arm and six from the twice-daily arm) with plasma viral loads <5 copies/ml at 12 months. Five (two from the once-daily arm and three from the twice-daily arm) out of 11 patients (45%) had detectable virus in lymph nodes (median viral load, 7750 copies/mg tissue; range, 1020–33 077 copies/mg). There were no differential characteristics between these patients with detectable HIV-1

RNA in tonsillar tissue compared with the rest of the cohort or the patients with undetectable HIV-1 RNA.

Genotypic resistance

Genotypic resistance was measured in the 14 patients with detectable plasma viral load at month 12. Median (range) of plasma viral load of these 14 patients were 2492 (764–5948). Genotypic resistance to nevirapine was noted in seven patients; five patients did not show genotypic resistance to any antiretroviral drug and RNA could not be amplified in two patients. The two mutations observed were K103N and Y181C in four patients and K103N in three patients.

Effects of treatment on CD4+ cell counts

The mean rise for the patients who received twice-daily treatment with didanosine and nevirapine was 132 ± 79 × 10⁶ cells/l, and that for the patients who

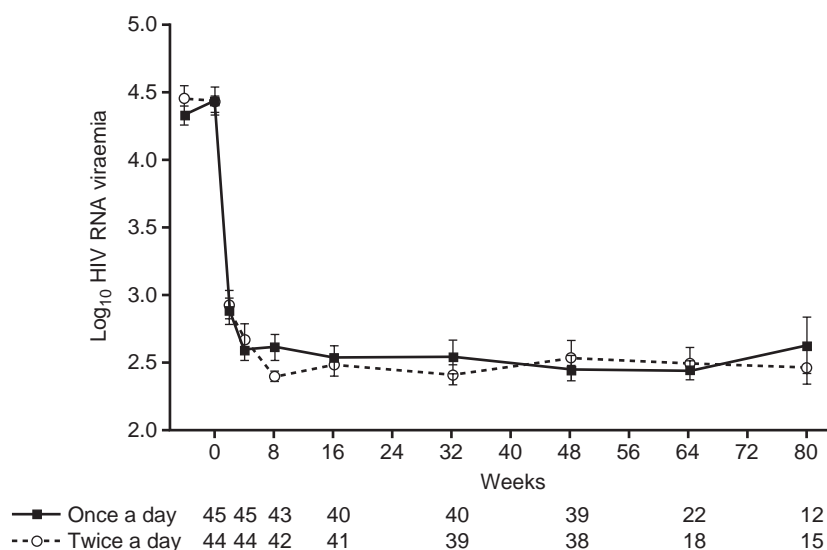


Fig. 1. Reductions in viral load for the patients receiving stavudine plus didanosine (once-daily) plus nevirapine (once-daily) and stavudine plus didanosine (twice-daily) plus nevirapine (twice-daily).

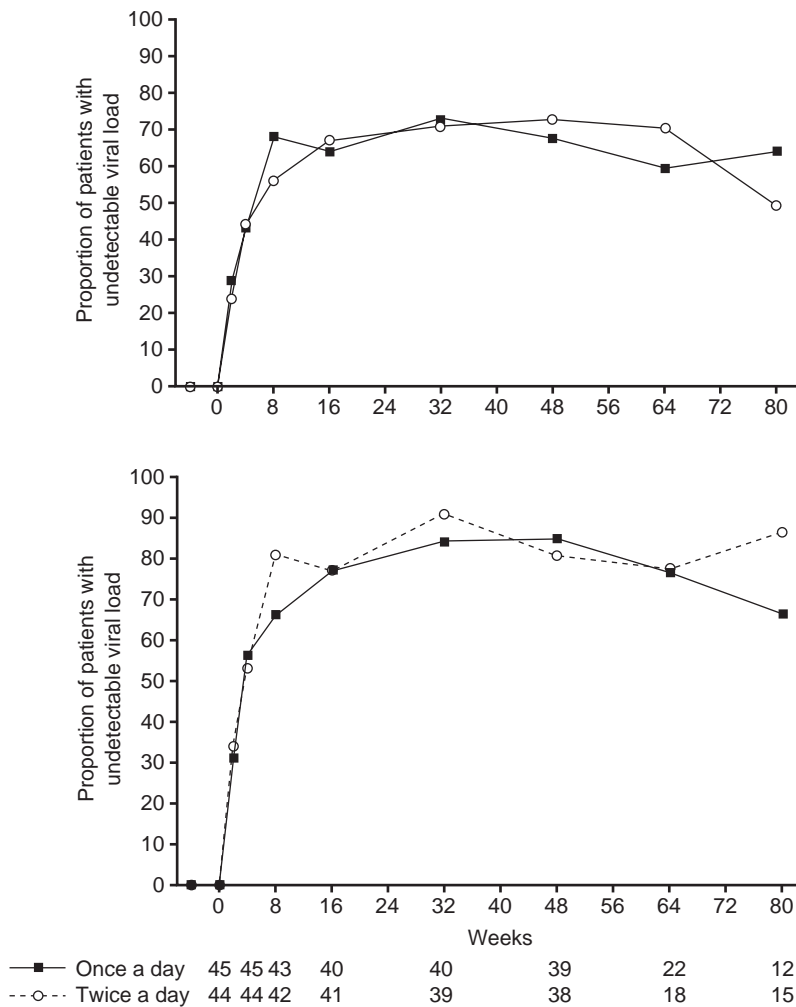


Fig. 2. Cumulative percentages of patients in the intent-to-treat (top) and on-treatment (bottom) populations achieving viral loads erapy: stavudine plus didanosine (once-daily) plus nevirapine (once-daily) and stavudine plus didanosine (twice-daily) plus nevirapine (twice-daily).

received these drugs once daily was $154 \pm 53 \times 10^6$ cells/l ($P = 0.7$, ITT population) (Fig. 3).

T-lymphocyte subsets

Results for the two treatment arms were combined for the analysis of T-lymphocyte subsets. These data were evaluated in a subset of 11 patients recruited at the coordinating center. The baseline data of these patients were not statistically different from the rest of the patients (data not shown) The results of this analysis are summarized in Table 2. This analysis demonstrated that 12 months of treatment with stavudine plus didanosine and nevirapine had no significant effect on the percentage of naive CD4+ cells, memory CD4+ and CD8+ cells, and CD8+CD28+ cells. There was a significant decline in the percentage of CD8+CD38+ cells ($P = 0.001$) and a significant increase in naive CD8+ cells ($P = 0.02$).

Proliferative responses

Lymphocytes from all 11 of the patients evaluated showed a strong response to phytohaemagglutinin at baseline that did not change over 12 months of therapy. The mean SI was 35.7 at baseline and 36.3 at the end of 12 months ($P = 0.96$). There was no significant increase in the SI for the response to p24 antigen (0.91 at baseline versus 2.32 at 12 months, $P = 0.10$). There was a significant rise in the response to gp160 (0.91 versus 1.49, $P = 0.027$), but this change was not considered to be significant because the SI after treatment was not greater than 3.

Safety

There were no significant differences in the occurrence of adverse events in patients treated with once-daily didanosine and nevirapine versus those who received these agents twice per day (Table 3). A total of nine adverse events were reported by the patients who received once-daily treatment versus seven for the

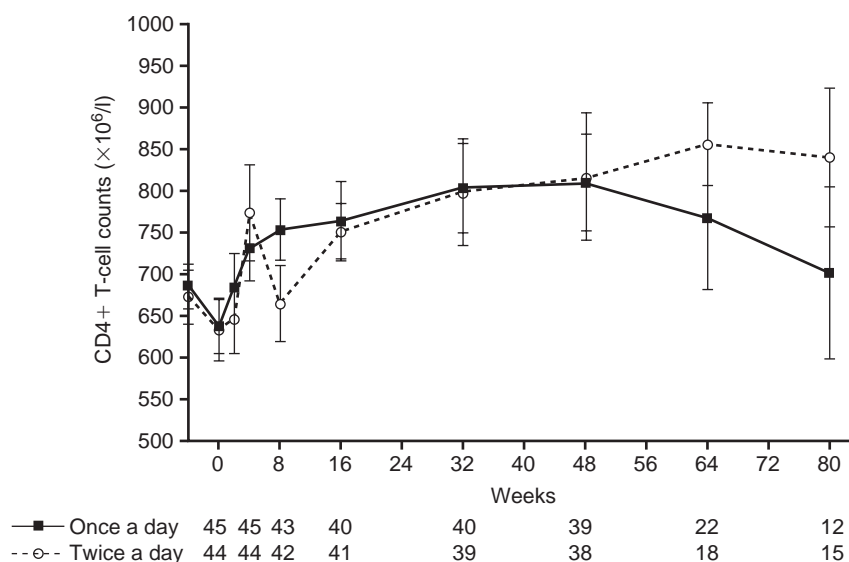


Fig. 3. Mean changes in CD4+ cell counts. Antiretroviral therapy: stavudine plus didanosine (once-daily) plus nevirapine (once-daily) and stavudine plus didanosine (twice-daily) plus nevirapine (twice-daily). CD4+ T-cell counts over a period of 12 months were analysed by an area-under-the-curve (AUC) measurement that incorporated the baseline value. The AUC was compared among the different groups with a *t*-test; *P* = 0.7.

Table 2. Effects of treatment on T-lymphocyte subsets. Results for the two treatment arms were combined for the analysis of T-lymphocyte subsets. These data were evaluated in a subset of 11 patients recruited at coordinating centre.

Cell-type	Percentage at baseline	Percentage at 12 months
Naive CD4+	48.3	34.9
Memory CD4+	34.5	48.5
Naive CD8+	48.2	55.6 ^a
Memory CD8+	36.5	29.9
CD8+CD28+	47.6	55.0
CD8+CD38+	72.8	50.9 ^a

^a *P* < 0.05 versus baseline.

patients assigned to twice-daily dosing. Four patients who received once-daily didanosine and nevirapine discontinued treatment because of adverse events (skin rash and fever in two patients, pancreatitis in one patient, and lipodystrophy in one patient, who had lipid atrophy mainly in limbs) versus three patients who received twice-daily dosing (skin rash and fever in all three patients). Two patients in the twice-daily regimen and none in the once-daily regimen had increases in the transaminase level, but the treatment did not need to be discontinued for this reason.

Discussion

The results presented in the preceding section provide new information about antiretroviral therapy with two nucleosides and a NNRTI. They suggest that treatment

Table 3. Adverse events.

Adverse event	Twice-daily ddl and NVP		Once-daily ddl and NVP	
	n	%	n	%
Skin rash/fever ^a	4	9	4	9
Pancreatitis ^a	0		1	2
Lipodystrophy ^a	0		1	2
Digestive intolerance	1	2	1	2
Hepatitis	2	4	0	
Jaundice			1	2
Polyneuropathy			1	2
Total	7	16	9	20

^a Characteristics severe enough to indicate discontinuing therapy. Skin rash/fever was the indication to discontinue therapy in two cases in twice-daily arm and three cases in once-daily arm. ddl, didanosine; NVP, nevirapine.

with combinations of twice-daily stavudine with either once- or twice-daily didanosine and nevirapine might be equally effective in reducing viral loads and increasing CD4+ cell counts when used as initial antiretroviral therapy in patients with HIV infection in its early stages. Safety results show further that both treatment regimens are well tolerated, with very few patients discontinuing because of adverse events. These results are important because they add significantly to the relatively small amount of data concerned with the efficacy of antiretroviral therapy in patients in relatively early stages of HIV infection. The present findings are consistent with those of previous studies [19,20,23] that have demonstrated the efficacy of initial treatment with triple-drug regimens in these patients.

The effectiveness of the combination of stavudine, didanosine, and nevirapine in the present study is consistent with the results from previous small-scale clinical trials. Raffi *et al.* [24] administered stavudine and nevirapine twice daily and didanosine once daily at the doses used in the present study to 60 antiretroviral-naïve patients with CD4+ cell counts $> 200 \times 10^6$ cells/l and baseline viral loads > 5000 copies/ml. After 8 weeks of treatment, the mean reduction in viral load was 2.2 log and 75% of patients had HIV RNA < 500 copies/ml. After 4 weeks, the mean increase in CD4+ cell count was 158×10^6 cells/l. Gatell *et al.* [9] evaluated the effectiveness of stavudine plus didanosine and nevirapine, with the latter two drugs dosed once daily as in the present study, in previously untreated patients with HIV infection. After 24 weeks of treatment, 88% of the individuals receiving this combination had viral loads < 500 copies/ml and 81% had < 50 copies/ml. These values were similar to those achieved in patients treated with the combination of stavudine, didanosine, and lamivudine (88 and 76%, respectively) or with stavudine, didanosine, and indinavir (87 and 81%). Pell *et al.* [25] have shown further that the combination of stavudine, didanosine, and nevirapine is effective in patients who have failed treatment with combinations that included PIs. They evaluated stavudine plus didanosine and nevirapine in 36 patients who had a mean of 49 weeks of prior treatment with regimens that included a PI. After 24 weeks, these patients experienced a mean reduction in viral load of 1.1 log copies/ml. The average CD4+ cell count for these patients increased by 42×10^6 cells/l after 6 weeks. These investigators concluded that 'stepping down' to a more convenient, but still effective, treatment regimen may be very attractive to many patients.

The fact that once-daily administration of didanosine and nevirapine in the present study was as effective as twice-daily delivery of these two drugs is also consistent with previous results for each of these agents. Reynes *et al.* [11] used an open design to evaluate the antiretroviral activity and safety of combination therapy with twice-daily stavudine and once-daily didanosine in antiretroviral-naïve patients. Follow-up of 48 weeks indicated that this combination resulted in a sustained > 1.5 log copies/ml decrease in viral load. In addition, four of five patients evaluated at 48 weeks had HIV RNA < 50 copies/ml. As noted in the introduction, the effectiveness of once-daily treatment with didanosine is consistent with the long intracellular half-life of its active triphosphate metabolite [10].

Nevirapine has a long (> 24 h) half-life in plasma [16,17], and in vitro studies have demonstrated that plasma levels of this drug achieved with once-daily doses of 400 mg were 251-fold higher than the 95% inhibitory concentration for wild-type HIV [11]. Ne-

virapine has been used effectively with once-daily dosing as part of a number of different antiretroviral regimens [26].

One additional aspect of the present results that deserves mention is the effect of antiretroviral therapy on T-lymphocyte subsets. Our findings indicated that the combination of stavudine, didanosine, and nevirapine significantly reduced numbers of CD8+CD38+ cells while increasing the number of naïve CD8+ cells. Increased CD8+CD38+ cells are commonly observed in untreated or ineffectively treated patients with HIV infection and their numbers are positively correlated with plasma viraemia [27–27]. The number of these cells is reduced with effective antiretroviral therapy [23,30]. The statistically significant reduction in CD8+CD38+ cells observed in patients treated with the combination of stavudine, didanosine, and nevirapine documents the effectiveness of this combination for immune system restoration in patients with HIV infection. Similar results have been observed with other highly active antiretroviral regimens [31]. It is important to emphasize that this restoration is not likely to completely restore HIV-1-specific T-helper responses in patients with chronic HIV-1 infection [32]. Different therapeutic strategies must be developed to achieve this goal.

The effectiveness and safety of the combination of stavudine, didanosine, and nevirapine in the present study is also consistent with the fact that there are no clinically significant pharmacokinetic interactions among these three drugs. Available results indicate that there are no clinically significant pharmacokinetic interactions between nevirapine and either stavudine or didanosine [33], and Kline *et al.* [34] reported no significant pharmacokinetic interactions between stavudine and didanosine. In this regard, it is interesting to note that Zhou *et al.* [33] reported that co-administration of didanosine and nevirapine results in a one-third reduction in the bioavailability of zidovudine. In our cohort no patients had to discontinue treatment because of clinical hepatitis.

Although the present results provide strong support for the combination of stavudine plus didanosine and nevirapine in patients with HIV-1 infection, it must be noted that nevirapine therapy can select for resistance-conferring mutations such as K103N. Importantly, previous results have shown that this mutation is also associated with resistance to other NNRTI including efavirenz [35]. In considering the treatment regimen used in this study, it is important to note that development of resistance is rare in viral isolates from patients treated with the combination of stavudine and didanosine [23]. Adherence to therapy is important for reducing the occurrence of resistance-conferring mutations (see below).

Although one-half of the patients evaluated still had detectable HIV RNA in lymphoid tissue, the combination of two nucleosides and a NNRTI employed in the present study was very effective in reducing HIV RNA levels in blood. While previous studies have documented the effectiveness of PI-containing regimens in reducing viral load in lymphoid tissue [19,23,36–38], there is less information about the efficacy of regimens that do not contain this class of antiretroviral agents [39,40]. In fact, although PI-sparing and PI-containing regimens may display similar virological activity in plasma, there are no comparative data in lymphoid tissue. Our findings suggest that similar optimal responses at the plasma level are not always equivalent at the lymphoid tissue level and suggest consideration of the viral load response in lymphoid tissue as another virological end-point necessary to compare the efficacy of future clinical trials comparing PI-sparing and PI-containing regimens.

Results obtained in large-scale clinical trials have made clear that multi-drug therapy is the best currently available approach for the achievement of long-term viral suppression in patients with HIV infection [1–3]. Although most initial treatment regimens include a PI, drugs in this class have also been shown to be effective in salvage regimens for patients who fail initial treatment [41,42]. Curry *et al.* [43] noted further that prior treatment with nevirapine-containing combinations does not impair the effectiveness of these regimens. Thus, the use of two nucleosides and a NNRTI, as in the present study, may allow reservation of PIs for salvage therapy.

The combination of once-daily didanosine and nevirapine with twice-daily stavudine in the present study also provides a convenient dosing regimen that has the potential to significantly enhance patient adherence. As noted in the introduction, compliance with complex antiretroviral treatment regimens is relatively poor [5], with 40 to 60% of patients failing to take prescribed medications. It has been recently noted that non-adherence increases markedly as the number of drugs included in the antiviral treatment rises [5]. Compliance has been identified as having a significant effect on the virologic efficacy of antiretroviral therapy [5]. The drawback of this simplified combination is that stavudine has been administered twice daily to our patients. However, from our data it is possible to conclude that both didanosine and nevirapine could probably be dosed once daily and therefore could be used as part of a future once daily HAART regimen when a third once-daily dose drug is available.

The results summarized in this paper suggest that combination antiretroviral therapy might be simplified by once-daily administration of didanosine and nevir-

apine along with twice-daily stavudine. This combination is effective in reducing viral loads and increasing CD4+ cell counts and is well tolerated by patients with HIV infection.

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