Tangible effects of antiretroviral therapy in HIV-1 infected patients
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Chapter 2

"An open randomized controlled trial of zidovudine plus lamivudine versus stavudine plus lamivudine"

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Summary

The objective of this study was to compare the antiretroviral effect and safety of zidovudine (ZDV)-lamivudine (3TC) with that of stavudine (d4T)-3TC. In an open randomized controlled trial antiretroviral therapy-naive patients who had CD4 counts ≥ 200×10^6/l and plasma HIV RNA load ≥ 10 000 copies/ml were randomized to receive ZDV-3TC (200 mg three times daily and 150 mg twice, respectively) or d4T-3TC (40 mg and 150 mg, both twice daily). If the plasma HIV RNA level at week 8 or thereafter was > 500 copies/ml, indinavir was added at the next scheduled visit. Genotypic resistance analysis of the reverse transcriptase gene was performed at week 0 and 12. Results over 24 weeks are reported. Forty-seven patients were treated (24 took ZDV-3TC; 23 took d4T-3TC). Plasma HIV RNA levels decreased from median 4.80 to 3.15 log_{10} copies/ml (ZDV-3TC, P < 0.0001) and from 4.98 to 3.03 log_{10} copies/ml (d4T-3TC, P < 0.0001) after 12 weeks of treatment. Indinavir was added at week 12 in 11 out of 21 patients with ZDV-3TC and 10 out of 22 patients with d4T-3TC. Median virus load at week 24 was 2.41 log_{10} and 2.29 log_{10} copies/ml (P = 0.14), respectively. Seventy-five percent (15 out of 20; ZDV-3TC) and 95% (18 out of 19; d4T-3TC) of patients had a virus load < 500 copies/ml. Genomic evidence for 3TC resistance was found in all patients tested (11/11 ZDV-3TC and 12/12 d4T-3TC). At week 12 CD4^+ cell counts had increased with a median of 110 x 10^6/l in the ZDV-3TC group (baseline, 315 x 10^6/l) and a median of 115 x 10^6/l in the d4T-3TC group (baseline 290 x 10^6/l). At week 24, the median increases were 90 and 120 x 10^6/l, respectively. Overall the increase of CD4 cells was higher in the d4T-3TC group (p= 0.02).

We conclude that d4T-3TC is at least as effective as ZDV-3TC, but 3TC resistance emerged in all patients investigated. The virologic response of the dual nucleoside combination is of short duration. However, after addition of indinavir the virus load could be reduced to < 500 copies/ml in the majority of patients. The increase in CD4 cell count was significantly greater in the d4T-3TC group. To prevent 3TC resistance, the drug should not be used in regimens containing only two nucleosides, irrespective the virus load at baseline.
Introduction

The combination of zidovudine (ZDV) and lamivudine (3TC) is currently considered to be a first choice nucleoside analogue combination in antiretroviral regimens because of drug synergism, non-overlapping toxicity profiles and lack of cross-resistance. The effectiveness of this combination has been proven in large clinical trials in both naive and pre-treated patients [1-6]. Moreover, 3TC may have the ability to prevent and suppress pre-existing resistance to ZDV [7,8]. Although the reversal of ZDV-resistance may allow for the continuation of ZDV in 3TC-containing multidrug regimens [9], it is warranted to study the utility of 3TC in combination with nucleoside analogues other than ZDV. The toxicity and the tolerability profile of ZDV may not allow for its use in every HIV-infected patient [10,11], and it is therefore mandatory to identify potential alternative regimens for such patients.

Its predictable pharmacokinetics, ease of administration, resistance pattern, tolerability and toxicity profile, clinical efficacy [12] and penetration into the cerebrospinal fluid (CSF) [13], make stavudine (d4T) an attractive candidate as an alternative to ZDV in 3TC-containing regimens. The combination of d4T-3TC is already used in daily practice, although a randomized controlled trial of ZDV-3TC with d4T-3TC has not yet been reported.

We investigated whether d4T-3TC was as effective and as safe as ZDV-3TC in improving virological and immunological parameters. Because protease inhibitors became available at the start of the study, patients with a plasma HIV RNA level of \( \geq 500 \) copies/ml after 8 weeks of treatment or thereafter were offered indinavir in addition to the double nucleoside regimen.

Patients and methods

In an open randomized controlled trial, the effectiveness and safety of the combination of ZDV 200 mg three times daily plus 3TC 150 mg twice daily (ZDV-3TC group) versus d4T 40 mg (30 mg if body weight < 60 kg) twice daily plus 3TC 150 mg twice daily (d4T-3TC group) were compared in antiretroviral therapy-naive HIV-1 infected patients.
All patients were participants of the Amsterdam Cohort Study on HIV Infection and AIDS. Inclusion criteria for this study were as follows: CD4$^+$ cell lymphocyte count $\geq 200 \times 10^6$/l, plasma HIV RNA level $\geq 10000$ copies/ml, haemoglobin $\geq 9.0$ g/dl, platelets $\geq 75 \times 10^9$/l, absolute neutrophil count $\geq 1 \times 10^9$/l, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase $\leq 5$ times the upper limit of normal (ULN), plasma creatinine $\leq 1.5$ times ULN and total plasma lipase $\leq 1.4$ ULN. Exclusion criteria were as follows: use of any immune-modulating drugs 1 month prior to initial study drug dosing, acute or ongoing AIDS-defining opportunistic disease, pregnancy or breast feeding, peripheral neuropathy of at least grade 2 (modified World Health Organization criteria), a history of pancreatitis, chronic alcohol use, and previous use of myelosuppressive, neurotoxic, pancreatoxic, hepatotoxic or cytotoxic drugs within 3 months of enrolment. After fulfilling all inclusion and none of the exclusion criteria, the patients were randomized to receive either ZDV-3TC or d4T-3TC. Subsequent visits after the start of therapy were scheduled for weeks 2, 4, and 4-weekly thereafter until week 24. At each visit a complete physical examination was performed and blood was taken for virologic and immunologic assays and for routine safety laboratory parameters.

If the plasma HIV RNA level at week 8 or thereafter was above 500 copies/ml, the protease inhibitor indinavir (800 mg three times daily) was added to the nucleoside combination therapy at the next scheduled visit (week 12). When the plasma HIV RNA level was found to be $\leq 500$ copies/ml, no protease inhibitor was added. However, patients were allowed to start indinavir at week 12 or thereafter on request. This evaluation was repeated every four weeks from week 8 until week 24.

Plasma HIV RNA levels were measured by using a commercial PCR-based assay with a variable lower limit of detection (Amplicor HIV Monitor Test, Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA). For plasma HIV RNA levels below the detection limit this value was set as 500 copies/ml. Syncytium-inducing (SI) capacity of HIV-1 [14] and sequencing of the reverse transcriptase (RT) gene was performed at baseline and at week 24 and 12, respectively in patients with a detectable virus load according to previously described methods [15]. Enumeration of CD4$^+$ and CD8$^+$ T-cell lymphocytes was accomplished by flow cytometry using dual staining techniques. The study was approved by the local medical ethical committee and all patients gave their written informed consent.
Statistical analysis

In the analysis of quantitative data, Student's unpaired t-test was used to compare the study groups with regard to variables with a normal distribution, and the Mann-Whitney U-test was used for variables with a non-normal distribution. A paired t-test was used to test changes from baseline. Data are given as means and SD, medians and the interquartile range (IQR), or as proportions and 95% confidence intervals (CI). Log_{10} transformation was performed on HIV RNA copy number.

The primary measures of antiretroviral drug activity were changes in both HIV RNA levels and CD4^+ cell counts. The efficacy analysis of this study was divided into two parts: (i) overall comparison (with comparison up to week 12 in particular), and (ii) the comparison in which the start of indinavir was taken as timepoint zero. The overall difference between the two groups was tested with an analysis of repeated measures, using the ProcMixed procedure of the SAS software package (version 6.12; SAS Institute, Cary, North Carolina, USA) with adjustment for the baseline value of the parameter tested. Kaplan-Meier plots were made to compare the moment at which the HIV RNA level was above 500 copies/ml amongst the two randomized groups. Although the observation of therapy failure is not a continuous process, the use of Kaplan-Meier plots is a valid method to illustrate therapy failure, since censoring for those who discontinued the study was taken into account.

Results

Patients
The study was designed to include 84 patients, but enrolment had stopped prematurely because of the observed frequency of 3TC resistance, even in patients with a relatively low baseline plasma HIV RNA level. Results of the study after the first 24 weeks of follow-up are presented. Forty-nine patients were randomized, but two patients were excluded because of an inclusion error (one had used ZDV before, and one patient had a viral load below the inclusion threshold). Forty-seven patients have been randomized to receive 3TC either in combination with ZDV (n=24) or d4T (n=23). The baseline characteristics of the 47 patients are presented in Table 1.
Patients from both treatment arms were comparable for age, baseline CD4\(^+\) and CD8\(^+\) lymphocyte count, and plasma HIV RNA level. Two patients withdrew from the study after randomisation but before the start of treatment (one in each study arm). Six patients withdrew during follow-up; three of them wanted to use antiretroviral drugs which were incompatible with the study protocol (at weeks 4, 12, 16, respectively), and three patients (two ZDV-3TC, one d4T-3TC) because of persistent severe side effects (at weeks 10, 21 and 16, respectively). Thirty nine patients (20 ZDV-3TC, 19 d4T-3TC) completed 24 weeks of study. All but one were homosexual men.

**Virological data**

Changes in plasma HIV RNA levels are shown in Fig. 1A for both treatment groups. Median plasma HIV RNA levels at baseline were 4.80 \(\log_{10}\) copies/ml (IQR, 4.44-5.02 \(\log_{10}\) copies/ml) and 4.98 \(\log_{10}\) copies/ml (IQR, 4.32-5.34 \(\log_{10}\) copies/ml) in the ZDV-3TC and d4T-3TC groups, respectively. Within the first 12 weeks, the most profound median plasma HIV RNA reduction was found at week 8 [ZDV-3TC, -1.94 \(\log_{10}\) copies/ml, IQR, -1.48 to -2.19 \(\log_{10}\) copies/ml]; d4T-3TC, -2.11 \(\log_{10}\) copies/ml.

### Table 1: Characteristics at baseline from patients who were randomized to zidovudine (ZDV)-lamivudine (3TC) or stavudine (d4T)-3TC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ZDV-3TC</th>
<th>d4T-3TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Patients</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Median (range) age (years)</td>
<td>38 (24-52)</td>
<td>38 (27-64)</td>
</tr>
<tr>
<td>Median (IQR) weight (kg)</td>
<td>72 (69-78)</td>
<td>75 (71-84)</td>
</tr>
<tr>
<td>Median (IQR) cell count (x 10(^6)/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4(^+)</td>
<td>315 (210-410)</td>
<td>290 (240-440)</td>
</tr>
<tr>
<td>CD8(^+)</td>
<td>960 (730-1230)</td>
<td>1040 (820-1290)</td>
</tr>
<tr>
<td>Median (IQR) plasma HIV-1 RNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\log_{10}) copies/ml</td>
<td>4.80 (4.44-5.02)</td>
<td>4.98 (4.32-5.34)</td>
</tr>
<tr>
<td>HIV phenotype SI/NSI</td>
<td>5/19</td>
<td>3/20</td>
</tr>
</tbody>
</table>

IQR, Interquartile range; SI, syncytium-inducing; NSI, non-syncytium inducing.
A randomized controlled comparison of ZDV-3TC and d4T-3TC

Figure 1

A] Plasma HIV RNA concentration (log₁₀ copies/ml) compared with baseline during 24 weeks of treatment. From week 12, indinavir was added if plasma HIV RNA at week 8 or thereafter was above 500 copies/ml.

B] CD4⁺ cell count (x 10⁹/l) compared with baseline in patients treated with zidovudine - lamivudine (3TC) or stavudine -3TC during 24 weeks of treatment. From week 12, indinavir was added if plasma HIV RNA at week or thereafter was above 500 copies/ml.
IQR, -1.64 to -2.66 log_{10} copies/ml), after which an increase of the plasma HIV RNA concentration was observed in the majority of patients. At week 12, plasma HIV RNA levels had changed from baseline by -1.53 log_{10} copies/ml (IQR, -1.70 to -1.38 log_{10} copies/ml, P<0.0001) and -1.65 log_{10} copies/ml (IQR, -2.09 to -1.24 log_{10} copies/ml; P<0.0001) for ZDV-3TC and d4T-3TC groups, respectively. Plasma HIV RNA levels did not differ statistically significantly between the treatment groups during the first 12 weeks. After 8 and 12 weeks of treatment with ZDV-3TC, the proportion of patients in the ZDV-3TC group with a plasma HIV RNA level below 500 copies/ml was 45% (95% CI, 24-70) and 14% (CI, 3-36), respectively. The proportion of patients in the d4T-3TC group at these timepoints with a plasma HIV RNA level below 500 copies/ml was 55% (95% CI, 32-76) and 32% (95% CI, 14-55), respectively. These values were not statistically significant when both groups were compared.

After 24 weeks, 19 (95%) out of 20 of the patients from the ZDV-3TC and 15 (79%) out of 19 patients from the d4T-3TC group used indinavir (P=0.18; two patients on their own request, 32 because of therapy failure). Of the five patients who did not use a protease inhibitor, three (d4T-3TC) had undetectable virus load. Median HIV RNA levels at week 24 were 2.41 log_{10} copies/ml (IQR, 2.24-2.72 log_{10} copies/ml) in the ZDV-3TC group and 2.29 log_{10} copies/ml (IQR, 2.17-2.45 log_{10} copies/ml) in the d4T-3TC group.

**HIV-1 phenotype and drug resistance**

Analysing the HIV-1 phenotype revealed that nine patients (five ZDV-3TC, four d4T-3TC) had the SI HIV phenotype at baseline, and four of them (three ZDV-3TC, one d4T-3TC) returned to the non-SI (NSI) phenotype after 24 weeks of therapy. The virus load at baseline, 12 and 24 weeks was comparable in patients with the SI and NSI phenotype.

All patients analysed at week 12 (n=23; 12 ZDV-3TC, 11 d4T-3TC) showed the M→V mutation at codon 184 in HIV-1 RT, except for one who showed the M→I mutation at this codon, associated with a >100-fold decreased sensitivity to 3TC. In none of these patients had the mutation been detected at baseline. Moreover, in none of the patients was a mutation associated with ZDV resistance (codons 41, 67, 210, 215 or 219) or d4T resistance (codon 75) found after 12 weeks of treatment. 3TC resistance appeared in patients with a baseline plasma HIV RNA concentration in the
lower quartile (10 000-45 000 copies/ml) as well as in patients who started with a higher baseline plasma HIV RNA level. This nearly universal rapid development of 3TC resistance prompted us to terminate the study prematurely.

**Immunological data**

At baseline, the CD4⁺ lymphocyte count of both treatment groups (median, 315 x 10⁶/l for ZDV-3TC) and 290 x 10⁶/l for d4T-3TC) were comparable. CD4⁺ cells increased statistically significantly (p < 0.001 for both groups) when compared with baseline values after 12 weeks in both treatment arms with a median of 110 x 10⁶/l (IQR, 20-150 x 10⁶/l) in the ZDV-3TC group, and 115 x 10⁶/l (IQR, 50-190 x 10⁶/l) in the d4T-3TC group. The CD4⁺ cell count was consistently higher in patients treated with d4T-3TC than in the ZDV-3TC group (p=0.002; Fig. 1B). At week 24, the CD4⁺ cell counts in both groups were elevated compared with baseline values, and had increased with a median of 90 x 10⁶/l (IQR, 40-145 x 10⁶/l) in the ZDV-3TC group, and 120 x 10⁶/l (IQR, 90-180 x 10⁶/l) in the d4T-3TC group (p = 0.002). The CD8⁺ cell count showed no increase when compared with baseline values and did not differ between the treatment groups. The CD4⁺/CD8⁺ ratio increased significantly in both the d4T-3TC group (from 0.30 at baseline to 0.46 at week 24; p=0.02), and the ZDV-3TC group (from 0.31 to 0.43; p=0.03).

**Protease inhibitor therapy**

Because indinavir could be added from week 12 onwards and data were censored at week 24, a maximum follow-up of 12 weeks for patients who used a triple drug regimen was possible. At week 8, when the decision to add a protease inhibitor could first be made, 11 out of 21 patients from the ZDV-3TC group and 10 out of 22 patients from the d4T-3TC group had a plasma HIV RNA level above 500 copies/ml. Of the 21 patients who were eligible to start the protease inhibitor therapy, 15 started at week 12, five patients preferred to wait and started at week 18, and one patient withdrew from the study. Two patients (one in each treatment group) with a virus load ≤ 500 copies/ml at week 8 also chose to start indinavir at week 12. The proportion of patients on double nucleoside analogue therapy whose virus load was ≥ 500 copies/ml increased to 80% during the study (Fig. 2).
After starting indinavir, a steady decline of plasma HIV RNA concentrations (Fig. 3A) to below 500 copies/ml (Fig. 3B) was observed in most of the patients. HIV RNA levels decreased in the ZDV-3TC group with a median of -1.25 log_{10} copies/ml and in the d4T-3TC group with a median of -1.46 log_{10} copies/ml after 12 weeks of treatment with indinavir. After starting indinavir, no significant change in the total CD4⁺ (Fig. 4), CD8⁺ lymphocyte count, or CD4⁺/CD8⁺ ratio was observed in either treatment arm (data not shown).

**Adverse events**

Three patients withdrew because of serious incapacitating side-effects. One patient did not tolerate ZDV-3TC and stopped definitively on his own request 10 weeks after initiating therapy. Two patients suffered from severe nausea and vomiting after starting indinavir.
A randomized controlled comparison of ZDV-3TC and d4T-3TC

Figure 3
A] Plasma HIV RNA concentration (log_{10} copies/ml) compared with the start of indinavir (week 0).
B] Percentage of patients which had a virus load ≤ 500 copies/ml from the start of indinavir.
Figure 4
CD4+ cell count (× 10^9/l) compared with the start of indinavir (week 0).

Their complaints continued despite symptomatic treatment, so indinavir was replaced by another protease inhibitor. No serious blood abnormalities were observed; however, total bilirubin and triglycerides were slightly increased during indinavir treatment. AIDS diagnoses were not observed. Minor HIV-related events were reported equally in both treatment groups. No signs or symptoms of polyneuropathy developed in any of the patients.

**Discussion**

The main finding of our randomized controlled study was that after 24 weeks of treatment d4T-3TC was at least as effective as ZDV-3TC in reducing virus load and changing the CD4+ cell count. Both combinations were well-tolerated and the frequency of side-effects was comparable in both treatment groups. No randomized controlled trials have been previously published comparing these combinations, which are frequently used in daily practice. Although d4T-3TC resulted in a significantly higher CD4+ cell count than ZDV-3TC, the absolute difference at week
24 was small (median, 30 x 10^6/l). From all nucleoside analogues used in antiretroviral drug regimens, ZDV is often preferred because of a good penetration into the CSF. In a neurological study that we performed in a subset of the same patients described above, we found that d4T and 3TC also had good CSF penetration, and that both combination regimens resulted in a significant lowering of CSF virus load [13].

The second major finding, confirming results reported from other double nucleoside analogue trials, was that in the majority of patients neither treatment combinations resulted in a sustained suppression of plasma HIV RNA [3,4,16-19]. Evidence for 3TC resistance was observed in 100% of the patients examined with a detectable viral load at week 12 before indinavir therapy was added. This percentage is substantial higher when compared with antiretroviral therapy-naive patients who have been treated initially with a protease inhibitor plus ZDV plus 3TC (5%) [20]. The finding that, even in the majority of patients with a low baseline viral load (10 000-45 000 copies/ml), the codon 184 M→V mutation rapidly emerged, has important implications for the proper initiation of antiretroviral therapy. We speculate that this 3TC resistance was at least partly responsible for the viral rebound after eight weeks of treatment.

The third finding of our study was that after addition of indinavir to the nucleoside combination therapy a statistically significant decrease of plasma HIV RNA concentration to a level below 500 copies/ml was observed in most patients. Treatment failures after indinavir addition were not observed during the first 12 weeks. The CD4^+ lymphocyte count had increased significantly with double nucleoside therapy, but no further increase was observed after protease inhibitor therapy was started, despite a further decrease of viraemia. A slow increase of CD4^+ cell counts after the start of a protease inhibitor could be observed in some but not all patients, which indicates that the observation period of 12 weeks might be too short. Other explanations could be that most patients gained their maximal immune reconstitution during the nucleoside combination therapy, or that an increase of CD4^+ cell count after the start of protease inhibition coincided with a decrease due to the non-sustained effect of the nucleoside combination therapy so the total effect resulted in no change of the CD4^+ cell count. As the aim of antiretroviral therapy is to stop viral replication as soon as possible and by as much as possible we suggest that starting with double nucleoside therapy is obsolete because only a small number of
patients will profit from a double nucleoside combination in the long term. Moreover, it is not possible to predict in advance which patients can be treated adequately with only two RT inhibitors.

Patients with relatively low baseline plasma HIV RNA levels (10 000-45 000 copies/ml) are at the same risk of developing 3TC resistance when compared with patients with higher virus load levels. The chance that viral resistance will develop will be minimized when plasma HIV RNA levels are reduced to undetectable levels during the use of double nucleoside therapy in combination with a protease inhibitor or a non-nucleoside RT inhibitor [21].

In summary, we can conclude that d4T-3TC is at least as effective as ZDV-3TC, although both combinations are suboptimal. This is supported by data from another randomized clinical trial [22]. However, addition of a protease inhibitor brings back the HIV RNA levels to undetectable levels in most of the patients. The durability of this response remains to be established.

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