Tangible effects of antiretroviral therapy in HIV-1 infected patients
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Chapter 4

“Durable HIV-1-suppression with indinavir after failing lamivudine containing double nucleoside therapy: a randomized controlled trial”

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Submitted
Summary

We previously found in a randomised controlled trial comparing lamivudine with either zidovudine or stavudine, in antiretroviral naive infected patients (CD4+ cell count ≥ 200 cells/µL and a plasma HIV-1 RNA level ≥ 10,000 copies (c)/mL), that indinavir had to be added from week 12 onward in the majority of patients because of virologic failure. At that moment lamivudine resistance was virtually universal. Nevertheless, 24-week data showed HIV-1 suppression of plasma below 500 copies/mL in 95%. At week 12 adequate concentrations of lamivudine, zidovudine and stavudine had been found in cerebrospinal fluid (CSF) and in 21/21 CSF samples HIV-RNA was below 500 c/mL.

The objective of this study was to assess the durability of the antiretroviral effect of intensification with indinavir after 72 weeks follow-up using an ultrasensitive HIV-1 RNA assay. In addition, we now report ultrasensitive HIV-1 RNA results in CSF at week 12 and 48 and CSF concentrations of indinavir at week 48.

Forty-seven patients were enrolled, of whom 33 completed a follow-up of 72 weeks. Indinavir was added in 93% (42/45) of the patients. Only one discontinuation was due to virologic failure. At week 72, the median plasma HIV-1 RNA levels in the zidovudine + lamivudine group had decreased from 4.80 log_{10} c/mL to below 500 c/mL in all and below 50 c/mL in 86.6% of the patients. In the stavudine + lamivudine group the plasma HIV-1 RNA decreased from 4.98 log_{10} c/mL at baseline to below 500 c/mL in all and below 50 c/mL in 66.7% of the patients. On an intention to treat base these figures were 54.2% and 52.2%, respectively, for the 50 c/mL assay. The median CD4+ cell count increased from 315 cells/µL with 150 cells in the zidovudine + lamivudine arm and from 290 cells/µL with 310 cells/µL in the stavudine + lamivudine arm (p =0.0001). In the zidovudine + lamivudine group 9/10 and 5/5 and in the stavudine + lamivudine group 11/11 and 6/6 had a CSF HIV-1 RNA level below 50 c/mL at week 12 and 48, respectively. The CSF indinavir concentration ranged from 50-170 ng/mL.

The long term HIV-1 suppression observed in this study is remarkable, as adding a single antiretroviral agent to a failing regimen goes against current notions of adequate therapy. Lamivudine resistance may have contributed to this phenomenon.
Introduction

Triple drug regimens containing two nucleoside analogue reverse transcriptase inhibitors plus either a protease inhibitor, or a non-nucleoside reverse transcriptase inhibitor for treatment of patients infected with human immunodeficiency virus (HIV-1) have proven to be superior to double nucleoside therapy alone both in antiretroviral naive and in pre-treated patients [1-5]. This benefit refers to the increase of CD4+ lymphocytes, the reduction of HIV-1 viremia, and the decrease of the frequency of AIDS defining events and mortality. These improved results may be attributable to prevention of drug resistance by the superior potency of these triple regimens, which is supported by the fact that better results are achieved in antiretroviral naive than in pre-treated patients [6,7]. Based on these results current clinical guidelines advice the use of triple regimens for first-line treatment of HIV-1-infection [8].

Results of our randomised controlled trial comparing zidovudine plus lamivudine versus stavudine plus lamivudine, with subsequent addition of indinavir, showed that the plasma HIV-1 RNA level decreased below 500 c/mL in 95% (33/35) of the patients after 24 weeks of follow-up [9]. This virologic response is similar to that obtained with regimens in which therapy has been initiated with three drugs simultaneously [4,10-14]. This result was unexpected, because of the high proportion of patients in whom viral resistance to lamivudine had developed prior to the addition of indinavir. Therefore it became especially interesting to investigate the durability of effect of these putative suboptimal regimens. We now report the data on 72 weeks follow-up of the patients included in this comparative trial. Moreover, using standard PCR HIV-1 RNA assays with a lower detection limit ranging from 200-500 c/mL may not disclose the entire message regarding suppression of viral replication. It is becoming clear that more durable viral suppression may be obtained if plasma HIV-1 RNA levels are suppressed to an even lower level than 200-500 c/mL [4,15,16]. Therefore, an ultrasensitive HIV-1 RNA assay was used to measure HIV-1 RNA copies in plasma at week 48 and 72.

In the same trial we previously found that lamivudine, zidovudine and stavudine penetrated well into the cerebrospinal fluid (CSF) and HIV-1 RNA copies were effectively depressed below 500 c/mL in CSF [17]. Now, to assess the maintenance and effectiveness of response in the CSF we show CSF HIV-1 RNA at week 12 and 48.
by the use of an ultrasensitive assay. Moreover, concentrations of indinavir are reported in the CSF at week 48.

**Patients and methods**

Patients and design

The trial design and the 24 week results have been reported previously [9,17]. In short, the effectiveness and safety of the combination of lamivudine with either zidovudine or stavudine were compared in antiretroviral therapy-naive HIV-1 infected patients. Major inclusion criteria were a CD4$^+$ cell count $\geq$ 200 cells/$\mu$L and a plasma HIV-1 RNA level $\geq$ 10,000 copies/mL. Until week 12 patients were treated with the reverse transcriptase inhibitor combination only. If the plasma HIV-1 RNA level at week 8 or thereafter was $> 500$ c/mL, the protease inhibitor indinavir 800 mg t.i.d. could be added to the reverse transcriptase inhibitor combination at the next scheduled visit (week 12) or thereafter. When the plasma HIV-1 RNA level was found to be $\leq 500$ c/mL patients were allowed to start indinavir at week 12 or thereafter on request. All eligible individuals were asked to participate in a neurological substudy to assess HIV-1 RNA levels and plasma drug concentrations in CSF. In this substudy a lumbar puncture was performed on day 0 and after 12 and 48 weeks of treatment. Subjects who could not tolerate the full dose of the study drug regimen for more than a month were withdrawn.

As patients remained allocated to their randomised treatment regimen after the protocolled end of study (week 48) and the scheduled follow-up after week 48 did not differ from the period before, we were able to report data of 72 weeks of follow-up using an ultrasensitive PCR HIV-1-1 RNA assay.

Laboratory methods

Plasma and CSF HIV-1 RNA levels were measured with a commercial PCR-based assay with a variable lower limit of detection. (Amplicor HIV-1 Monitor Test, Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA). Blood and CSF samples were stored at -70°C. Measurements of HIV-1 RNA levels in CSF were validated by reconstruction experiments (personal communication, SJ). If plasma HIV-1 RNA levels from week 48 and 72 or CSF samples from week 12 and 48 were below the
Sustained plasma HIV-1 RNA suppression despite 3TC-resistance

detection limit we re-analysed these samples using a commercial ultrasensitive PCR-based assay with a variable lower limit of detection. (Amplicor HIV-1 Monitor Test (Ultrasensitive Protocol Adaptation), Roche Diagnostic Systems Inc., Branchburg, New Jersey, USA) [18].

Failure of therapy was defined as a plasma HIV-1 RNA level never dropping below 500 c/mL or increasing from the nadir to above 500 c/mL, at two consecutive moments after indinavir had been added to the double nucleoside combination.

Enumeration of CD4⁺ and CD8⁺ T-cell lymphocytes was accomplished by flow cytometry using dual staining techniques. Determination of in vitro T-cell proliferation induced by monoclonal antibodies (mAb) to CD3 in the presence of mAb to CD28 was performed according to previously described methods [19]. Proliferative capacity was calculated back to counts per minute in 1000 CD3⁺ T-cells and results are expressed as percentages of the median of the responses of five healthy controls investigated.

Lamivudine, zidovudine, stavudine and indinavir concentrations in plasma and CSF were analysed as described previously [20-23]. To adjust for the time interval between blood sampling and drug intake, plasma indinavir concentrations were expressed as a ratio of the measured patient plasma indinavir concentration and a reference concentration for the same time interval between sampling and the last drug intake. Reference concentrations were derived from a population of 15 patients in whom a full (8 hr) pharmacokinetic curve was recorded after ingestion of 800 mg of indinavir.

The protocol was approved by the Institutional Review Board, and informed consent was obtained from all participants.

Data analysis

Outcome measures were the effect of the treatment regimens on plasma HIV-1 RNA levels, CD4⁺ and CD8⁺ cell counts, the results of the in vitro T-cell proliferation assay, and the frequency of side effects at week 72. Additional outcome measures were the HIV-1 RNA level in the CSF at week 12 and 48, and the concentration of indinavir in the CSF at week 48. Data are given as means and standard deviations (SD), as medians and interquartile ranges (IQR,) or as proportions with 95%
confidence intervals. Log_{10} transformation was performed on HIV-1 RNA copy numbers. When plasma HIV-1 RNA levels were below the lower limit of detection, this limit was used in descriptive analyses. For statistical analyses of proportions, values below the detection limit were set at 500 c/mL or 50 c/mL when the standard assay or the ultrasensitive assay was used, respectively. Proportions of patients with a plasma HIV-1 RNA level below 500 c/mL or below 50 c/mL were compared with the chi-square test with Yates correction and Fisher’s exact test (for expected frequencies of less than five). Of the patients who had a follow-up of 72 weeks, the proportion of individuals with a plasma HIV-1 RNA level above 500 c/mL at week 48 was compared with the proportion at week 72 using the McNemar’s test. The same analysis was performed regarding the 50 c/mL limit. For the intention to treat analysis drop-out patients were considered as failures. The overall differences in CD4⁺, CD8⁺ cell counts and their percentages between the two groups were tested with an analysis of repeated measures, using the proc mixed 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. The correlation between CSF and plasma indinavir concentrations was analysed by calculating the Pearson correlation coefficient.

Results

Patients and follow-up
Forty-seven patients were randomised (Figure 1). Baseline characteristics are shown in Table 1. Two patients did not receive therapy after randomisation on their own request. During follow-up another 12 patients (eight zidovudine + lamivudine, four stavudine + lamivudine) dropped out. A virologic breakthrough (after 48 weeks) was the reason to drop out in only one patient. Eight dropped out due to side effects (five zidovudine + lamivudine, three stavudine + lamivudine), mostly persistent nausea and vomiting (seven) or nephrolithiasis (one). Three patients preferred an alternative antiretroviral therapy, although there was no virologic failure. Patients who dropped out of the study were comparable regarding baseline plasma HIV-1 RNA and T-cell subset counts with patients who continued.
Within 72 weeks 93% (42/45) of the patients started indinavir (39 because of a viral load increasing above 500 c/mL, and three patients did so on their own request although their plasma HIV-1 RNA levels were below 500 c/mL). Of these 42 patients 39 (93%) started indinavir before or at week 24 (median week 16).
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Table 1: Baseline characteristics. *Values represent median and interquartile ranges.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>zidovudine + lamivudine</th>
<th>stavudine + lamivudine</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of patients</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>age years * (range)</td>
<td>38 (24-52)</td>
<td>38 (27-64)</td>
</tr>
<tr>
<td>weight * kg</td>
<td>72 (69-78)</td>
<td>75 (71-84)</td>
</tr>
<tr>
<td>CD4+ cells/µL *</td>
<td>315 (210-410)</td>
<td>290 (240-440)</td>
</tr>
<tr>
<td>CD8+ cells/µL *</td>
<td>960 (730-1230)</td>
<td>1040 (820-1290)</td>
</tr>
<tr>
<td>plasma HIV-1-1 RNA* log_{10} copies/mL</td>
<td>4.80 (4.44-5.02)</td>
<td>4.98 (4.32-5.34)</td>
</tr>
<tr>
<td>T-cell proliferation to CD3 + CD28 mAb</td>
<td>34% (13%-71%)</td>
<td>29% (13%-67%)</td>
</tr>
</tbody>
</table>

Virologic data

Median plasma HIV-1 RNA levels at baseline were 4.80 log_{10} c/mL (IQR, 4.44-5.02 log_{10} c/mL) and 4.98 (IQR, 4.32-5.34 log_{10} c/mL) in the zidovudine + lamivudine and stavudine + lamivudine group, respectively. Plasma HIV-1 RNA levels decreased significantly (p < 0.0001) during follow-up (Figure 2). The plasma HIV-1 RNA reduction was not significantly different between both treatment groups. As previously reported, before the addition of indinavir the M184V mutation indicating full lamivudine resistance was found in 23/23 (100%) of patients tested [17]. Nevertheless, at week 48, 91.6% percent (33/36) of all patients had a plasma HIV-1 RNA level below 500 c/mL. When tested with the ultrasensitive assay at this timepoint, 83.3% of all patients (30/36) showed a plasma HIV-1 RNA level < 50 c/mL (15/18 zidovudine + lamivudine and 15/18 stavudine + lamivudine). At week 72 100% had a plasma HIV-1 RNA level below 500 c/mL. Of these patients, 86.6% (13/15, zidovudine + lamivudine) and 66.7% (12/18, stavudine + lamivudine) had less than 50 c/mL (p = 0.24; 54.2% and 52.2% on an intention to treat base, respectively, Table 2).

For patients who completed a follow-up of 72 weeks, the proportion of patients with less than 50 c/mL at week 48 and 72 were compared (Table 2). The difference was not statistically significant (p = 0.125). The same analysis was performed regarding the 500 c/mL limit and again no significant difference was found (p = 1.0).
Sustained plasma HIV-1 RNA suppression despite 3TC-resistance

Figure 2: Median (IQR,) plasma HIV-1 RNA levels. Samples at week 48 and 72 are measured by using an ultrasensitive assay. Dashed lines represents a plasma HIV-1 RNA level of 500 c/mL and 50 c/mL, respectively.

Immunologic data

The number and percentage of CD4$^+$ cells increased significantly in both treatment arms ($p < 0.0001$, Figure 3A+B). There was a median increase of 150 cells/$\mu$L (IQR, 100-240 cells/$\mu$L, baseline 315 cells/$\mu$L) and 310 cells/$\mu$L (IQR, 190-400 cells/$\mu$L, baseline 290 cells/$\mu$L) in the zidovudine + lamivudine and the stavudine + lamivudine group, respectively. The increase in CD4$^+$ cells was significantly higher in patients treated with stavudine + lamivudine than in patients treated with zidovudine + lamivudine ($p < 0.0001$, Figure 3A). The CD4$^+$ cells increased biphasically: A rapid increase before and a slow continuous increment after the addition of indinavir. There was no significant difference between the CD4$^+$ cell percentages in both treatment arms ($p = 0.76$). The lymphocyte count did not differ significantly between both treatment groups. In the patients treated with zidovudine + lamivudine a lower haemoglobin level was found ($p < 0.05$). The CD8$^+$ cell count and percentage decreased during treatment ($p < 0.0001$, Figure 3C+D). The course of the absolute CD8$^+$ cell count was also biphasic: A period without significant change before, followed by a decrease after the addition of indinavir.
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Table 2: Proportion of patients with plasma and CSF levels below 500c/mL and 50 c/mL.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>zidovudine lamivudine</th>
<th>+ stavudine lamivudine</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLASMA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 48</td>
<td>N=36</td>
<td>N=18</td>
<td>N=18</td>
<td></td>
</tr>
<tr>
<td>&lt;500 c/mL</td>
<td>91.6% (33/36) *</td>
<td>83.3% (15/18)</td>
<td>100% (18/18)</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;50 c/mL</td>
<td>83.3% (30/36)†</td>
<td>83.3% (15/18)</td>
<td>83.3% (15/18)</td>
<td>NS</td>
</tr>
<tr>
<td>week 72</td>
<td>N=33</td>
<td>N=15</td>
<td>N=18</td>
<td></td>
</tr>
<tr>
<td>&lt;500 c/mL</td>
<td>100% (33/33) *</td>
<td>100% (15/15)</td>
<td>100% (18/18)</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;50 c/mL</td>
<td>75.8% (25/33)†</td>
<td>86.6% (13/15)</td>
<td>66.7 (12/18)</td>
<td>NS</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>week 12</td>
<td>N=21</td>
<td>N=10</td>
<td>N=11</td>
<td></td>
</tr>
<tr>
<td>&lt;500 c/mL</td>
<td>100% (21/21)</td>
<td>100% (10/10)</td>
<td>100% (11/11)</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;50 c/mL</td>
<td>95.2% (20/21)</td>
<td>90% (9/10)</td>
<td>100% (11/11)</td>
<td>NS</td>
</tr>
<tr>
<td>week 48</td>
<td>N=11</td>
<td>N=5</td>
<td>N=6</td>
<td></td>
</tr>
<tr>
<td>&lt;500 c/mL</td>
<td>100% (11/11)</td>
<td>100% (5/5)</td>
<td>100% (6/6)</td>
<td>NS</td>
</tr>
<tr>
<td>&lt;50 c/mL</td>
<td>100% (11/11)</td>
<td>100% (5/5)</td>
<td>100% (6/6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* All patients who completed 72 weeks of follow-up were compared for the proportion with a plasma HIV-1 RNA below 500 c/ml (p=1.000, McNemar’s test).
† All patients who completed 72 weeks of follow-up were compared for the proportion with a plasma HIV-1 RNA below 50 c/ml (p = 0.125, McNemar’s test).

At week 72, in the zidovudine + lamivudine group the number of CD8⁺ cells had decreased by a median of 260 cells/µL (IQR, 480 to +200 cells/µL from baseline (960 cells/µL) and in the stavudine + lamivudine group with 90 cells/µL (IQR, 190 to +80) from baseline (1065 cells/µL). The course of the CD8⁺ cell count, and the CD8⁺ cell percentage did not differ significantly between both treatment groups (p = 0.09 and p = 0.10, respectively). The CD4⁺/CD8⁺ ratio had increased from 28.7 to 49.3% and from 30.5 to 58.1% in the zidovudine + lamivudine and the stavudine + lamivudine group, respectively (p = 0.17). The in vitro T-cell proliferation after stimulation with CD3-28 mAb, improved significantly after 4 weeks of treatment, but did not differ between both treatment groups (Figure 3E). Normal values, however, were not reached in the majority of the patients.
Figure 3A + B:
CD4+ cell count (median + IQR.) as absolute numbers (A) and as percentage (B) (zidovudine + lamivudine vs. stavudine + lamivudine p <0.0001).
Figure 3C + D + E:

CD8+ cell count (median + IQR) as absolute numbers (C) and as percentage (D) (zidovudine + lamivudine vs. stavudine + lamivudine p = 0.09). Proliferative responses in vitro per 1000 CD3+ cells after stimulation with CD3 plus CD28 mAb, as percentage of five healthy donors (E) (zidovudine + lamivudine vs. stavudine + lamivudine NS)
Cerebrospinal fluid HIV-1 RNA findings

Twenty-eight (11 zidovudine + lamivudine, 17 stavudine + lamivudine), 22 (10 zidovudine + lamivudine, 12 stavudine + lamivudine) and 11 (5 zidovudine + lamivudine, 6 stavudine + lamivudine) patients underwent a lumbar puncture at day 0, week 12, and week 48, respectively. All of these patients were neurologically asymptomatic. At week 12, in one patient the second puncture failed and five individuals had dropped out of the neurological substudy. Four of these individuals refused a second puncture and one patient dropped out of the main study at week 4.

At week 48, eight other patients refused a third lumbar puncture and three more patients had discontinued the main study. The patient with the plasma virologic failure refused a third lumbar puncture. The patients who left the neurological substudy were comparable for baseline characteristics with patients who continued.

At week 12 and week 48, CSF HIV-1 RNA levels were below 500 c/mL in all of the patients examined (Figure 4). Using the ultrasensitive assay 90% (9/10) and 100% (5/5) in the zidovudine + lamivudine group and 100% (12/12) and 100% (6/6) of the patients in the stavudine + lamivudine group had a CSF HIV-1 RNA level below 50 c/mL at week 12 and 48, respectively.

In the one patient with a CSF HIV-1 RNA level above 50 c/mL at week 12 by ultrasensitive assay (76 c/mL), a third lumbar puncture was performed at week 48. The virus load had decreased to below 50 c/mL.

![Figure 4](image_url)

Cerebrospinal fluid HIV-1 RNA levels (Box & Whisker plot) at baseline, week 12 and after 48 weeks by using an ultrasensitive assay. If values were below the limit of quantification this limit was used to figure the graphs.
Cerebrospinal fluid pharmacokinetic findings

We previously reported adequate cerebrospinal fluid (CSF) concentrations of lamivudine, zidovudine and stavudine at week 12. The median CSF concentration was for lamivudine 59.5 (IQR, 50.0-82.5), for zidovudine 58.1 (41.2-60.2) and for stavudine 53.0 ng/mL (IQR, 39.2-80.0). These concentrations were comparable with those found at week 12 [17]. At week 48 a median indinavir CSF concentration of 90 ng/mL was found (range 50-170 ng/mL, 5-7½ hours after last drug intake). These indinavir concentrations appeared to be adequate, as the IC_{95} ranges reported in the literature are 17-63 ng/mL [24-26]. The median plasma indinavir concentration was 250 ng/mL. We found no correlation between the CSF indinavir concentration and the standardised plasma ratio (the measured plasma indinavir concentration divided by the reference concentration at the same sampling interval of normal individuals ($r^2 = 0.18$, $p = 0.29$; Figure 5).

![Figure 5: CSF indinavir concentration (ng/mL) versus the ratio of plasma indinavir concentration observed/standard plasma concentration at the same timepoint of a reference group in whom a full pharmacokinetic curve has been determined.](image-url)
Sustained plasma HIV-1 RNA suppression despite 3TC-resistance

Discussion

We found that after 72 weeks of follow-up zidovudine + lamivudine and stavudine + lamivudine with subsequent addition of indinavir were effective and comparable in reducing plasma HIV-1 RNA levels. In an on treatment analysis, the plasma HIV-1 RNA level had been suppressed below 50 c/mL in 75.8% of all patients (in an intention to treat analysis this percentage was 53.1% (95% confidence interval 38-68%)), although lamivudine resistance was present in all patients tested when indinavir was added. Surprisingly these results are highly comparable to those obtained with concomitant initiation of triple regimens in antiretroviral naive patients [4,10-14].

If the traditional view is taken that due to lamivudine resistance the triple drug regimens in our study were in fact double drug regimens (respectively zidovudine + indinavir and stavudine + indinavir) these results are in marked contrast with the findings of induction-maintenance trials, where it was found that two drug combinations were inferior in maintaining viral suppression to prolonged induction therapy with three or four drugs [24-26]. In the Trilege trial, naive patients with a successful induction therapy with indinavir + zidovudine + lamivudine for three months, were randomised to the maintenance regimens of zidovudine + lamivudine, zidovudine + indinavir or the triple regimen. Six months after the randomisation, 72% of the zidovudine + indinavir treated patients had a plasma HIV-1 RNA level below 500 c/mL compared with 90% in the group treated with the triple regimen (p < 0.01). After 24 weeks 87% of our patients had a plasma HIV-1 RNA level below 500 c/mL. This finding suggests that our triple regimen was not simply cut down to a double regimen when 3TC resistance had developed.

A first explanation for the relatively high proportion of patients with plasma HIV-1 RNA levels below 50 c/mL in our study may be that our patients had a relatively high CD4+ cell count, and that they had a low viral load at the moment of addition of indinavir (median 1478 c/mL), although this seems in contrast with the outcome of the Trilege trial. A second explanation may be that lamivudine resistance decreases viral fitness and processivity [27-30] and it is possible that this "non-fit-virus" also has a lower replication capacity in vivo compared with the wild type variant. Subsequent addition
of indinavir to zidovudine or stavudine may then be sufficient to suppress viral replication.

Thirdly, enhanced fidelity of lamivudine resistant HIV-1 reverse transcriptase has been found in vitro to delay the development of resistance to other antiretroviral drugs like zidovudine, nevirapine, delavirdine, and saquinavir [31]. We surmise that these mechanisms may have balanced the fact that lamivudine resistance reduced the effectiveness of the triple combination. A last mechanism that may have contributed is an increased cytotoxic T-lymphocyte response to the lamivudine resistant virus [32]. Similar findings have recently been reported from the Avanti 2 and Avanti 3 trials where addition of indinavir respectively nelfinavir to zidovudine plus lamivudine led to comparable virologic results as concomitant initiation of triple therapy [33,34]. As in our study, these patients were antiretroviral naive when treatment was started. In contrast, in zidovudine pre-exposed patients who had been treated with zidovudine + lamivudine for at least 24 weeks before subsequent addition of indinavir, a significantly lower percentage of the patients with plasma HIV-1 RNA levels below 50 c/mL was observed compared with the patients who have been concomitantly treated with the triple regimen (30% versus 66% after 100 weeks of follow-up, respectively) [35].

The increase in the CD4\(^+\) cell count was significantly higher in the stavudine + lamivudine group when compared with the zidovudine + lamivudine group. The clinical significance of this finding is unsure and can only be assessed by a clinical endpoint study, although as found in the meta-analysis study of Hughes et al, higher CD4\(^+\) cell counts per se are related with a lower incidence of AIDS and mortality [36]. The higher CD4\(^+\) cell count might be explained by a subtle bone marrow suppressive effect of zidovudine as indicated by a lower haemoglobin concentration in the zidovudine + lamivudine group. Beside a quantitative improvement of immunity also a qualitative response was observed in both treatment groups early after initiation of therapy. T-cell capacity to proliferate after stimulation with both CD3 and CD28 mAb could be partially restored, but not to normal values in most of the patients. One explanation could be that the follow-up may still be too short to demonstrate a full immunologic recovery, or alternatively, an irreparable damage may have already been present before initiating antiretroviral therapy. Another explanation could be that our drug regimen was not fully suppressive [37].
For optimal treatment of HIV-1 infection it is of paramount importance to target all infected compartments. Few data are available about the concentration of protease inhibitors in the CSF. Apart from the good CSF penetration of lamivudine, zidovudine and stavudine, which we already reported for the initial phase of the trial [17], we now demonstrate that the protease inhibitor indinavir also penetrated in the CSF above the IC_{95}. Other protease inhibitors like saquinavir and ritonavir do not seem to reach sufficiently high concentrations in the CSF and are not able to clear HIV-1 RNA from the CSF in most of the cases [38,39]. It is possible that the high CSF penetration contributed to the persistent low CSF HIV-1 RNA levels. We found no correlation between plasma and CSF indinavir concentrations (Figure 5). Two patients with the highest plasma indinavir concentration actually showed the lowest indinavir concentrations in the CSF. Little is known about the transport of indinavir into the CSF or the brain. Activity of the P-glycoprotein multi-drug transporter [40], co-medication or other interindividual variable factors may play a role.

In conclusion, we found a surprisingly durable and profound viral load suppression both in plasma and CSF of patients who have been treated with zidovudine + lamivudine or stavudine + lamivudine and subsequent addition of indinavir, despite lamivudine resistance. The CD4\(^+\) cell response was significantly higher in the stavudine + lamivudine group when compared with the zidovudine + lamivudine group. In addition we demonstrated that indinavir reached concentrations above IC_{95} into the CSF, and may be added to the armamentarium of drugs treating HIV-1 in the CNS.

As an afterword it may be remarked that enrolment to this study was prematurely terminated because of the near universal appearance of lamivudine-resistance. Looking at the present results, this might not have been necessary.

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