Anatomical and cellular reservoirs for HIV-1 during potent antiretroviral therapy
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Citation for published version (APA):
CHAPTER 8

Improved long-term suppression of HIV-1 replication with a triple-class multidrug regimen compared to standard of care antiretroviral therapy

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Submitted for publication
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Abstract

Background Treatment of antiretroviral naive HIV-1 infected patients with standard of care antiretroviral therapy results in the majority of patients in plasma HIV-1 RNA levels (pVL) below the limit of quantification (LLQ) of standard assays, for a period of at least three years. In a large percentage of such patients, more sensitive assays provide evidence for residual viral replication.

Methods Thirty control patients who using strict criteria had not experienced virological failure during 3 years of standard therapy were compared with 10 patients who initiated therapy with a five-drug regimen consisting of three different classes of antiretroviral drugs (alternative multidrug regimen). Plasma obtained at week 48 and at three timepoints around week 144 was retested in a modified ultrasensitive assay with an LLQ of 5 copies/mL.

Results At week 48, pVL could be quantified in 10 of 24 control patients (41.7%), whereas in all 10 patients treated with the alternative multidrug regimen pVL was <5 copies/mL (P=0.017). Around week 144 pVL could be quantified at 0 of 3 timepoints in 12 controls, at 1 of 3 timepoints in 14 controls, and at 2 of 3 timepoints in 4 controls. In only 1 of 7 patients of the alternative multidrug regimen still on therapy pVL was quantifiable, at 1 of 3 timepoints (P=0.036). A low baseline CD4+ T cell count was predictive of quantifiable pVL in the control patients, but not in the alternative multidrug patients.

Conclusion The use of an alternative multidrug regimen resulted in a stronger long-term suppression of pVL compared to clinically successful treatment with standard therapy.
Introduction

Treatment of HIV-1 infected patients with triple-drug combination therapy can result in profound suppression of viral replication. In most therapy naive patients plasma HIV-1 RNA levels (pVL) drop below the detection limit of currently used assays, and this can be sustained for a period of at least three years.\textsuperscript{1-3} However, a reservoir of resting memory CD4\textsuperscript{+} T cells harbouring replication competent virus has been demonstrated to persist during sustained suppression of pVL.\textsuperscript{4-7} The half-life of this cellular reservoir has been estimated to be very long and it therefore represents a major barrier for eradication of HIV from a patient by antiretrovirals alone.\textsuperscript{8,9} Furthermore, an undetectable pVL using standard assays should not be interpreted as evidence of complete suppression of viral replication. In a large percentage of patients with undetectable pVL using standard assays, more sensitive assays show evidence for ongoing low-level viral replication. A low pVL (1-20 copies/mL), expression of viral RNA in lymphoid tissue, and unintegrated circular forms of viral DNA or HIV-1 mRNA in peripheral blood mononuclear cells (PBMC) could still be detected in many of these patients despite prolonged therapy.\textsuperscript{9-22} Ongoing low-level viral replication below the limit of quantification (LLQ) of standard assays is worrisome, as it may allow for the selection of resistance mutations with the fear of subsequent therapeutic failure.\textsuperscript{9,21,23,24} Indeed, during potent antiretroviral therapy successfully suppressing plasma HIV-1 RNA to below 50 copies/mL, new drug resistance mutations were demonstrated in minority populations of viruses cultured from blood cells.\textsuperscript{23} Ongoing low-level replication also precludes eradication of HIV from a patient. To prevent viral resistance and in any attempt at viral eradication, an important question is whether intensification of antiretroviral therapy might reduce this residual viral replication.

We have demonstrated earlier that an alternative multidrug regimen consisting of three different classes of antiretroviral drugs resulted in a more rapid initial pVL decline than a standard of care regimen consisting of two different classes, which suggested that the initial viral suppression by standard of care therapy can be improved upon.\textsuperscript{25} In this study we explored, using a modified ultrasensitive HIV-1 RNA assay, whether this multidrug regimen also results in stronger suppression of viral replication compared to standard of care therapy during prolonged treatment.
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Patients and Methods

Patients

Alternative multidrug regimen group

Ten chronically infected HIV-1 patients, aged 31-60 years, started a five-drug regimen with the nucleoside analogue reverse transcriptase inhibitors (NRTIs) zidovudine (ZDV), lamivudine (3TC) and abacavir (ABC), the non-nucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP), and the protease inhibitor (PI) indinavir (IDV) between January 1997 and February 1998. One patient had previously received antiretroviral therapy: ZDV for 16.5 months and 3TC for 10.5 months, both of which were stopped 14 days before enrolment, and didanosine (ddl) for 6 months, which had been stopped one year before enrolment. This subject received a similar five-drug regimen as the others, except that stavudine (d4T) was substituted for ZDV and ddl was substituted for 3TC. In case of toxicity, the drug regimen was changed in individual patients but always consisted of antiretroviral drugs from 3 different classes during the 144 weeks of follow-up. Due to toxicity, three patients (002, 006 and 015) decided to stop therapy after 73, 118 and 87 weeks of treatment, respectively. At the moment of discontinuation of therapy these three patients had a pVL below 5 copies/mL, and they were only included in the analysis at week 48. The study was approved by the Medical Ethics Committee of our hospital and informed consent was obtained from all patients.

Control group

Control patients were selected from the HIV-1 positive patient population at the outpatient clinic of the Academic Medical Center, Amsterdam, The Netherlands. The selection criteria were chronic HIV-1 infection, antiretroviral therapy naive at the start of a standard potent antiretroviral regimen, start of antiretroviral therapy between July 1996 and February 1998, and no evidence of virological failure during 144 weeks of follow-up. A standard potent antiretroviral regimen was defined as a combination regimen containing two different classes of antiretroviral drugs: 2 NRTIs plus 1 or 2 PIs, or 1 NRTI plus 2 PIs. Saquinavir (SQV) hard gel formulation as a single PI plus 2 NRTIs was not considered to be a potent antiretroviral regimen. Treatment modifications within the definitions of the standard combination regimen for reasons other than virological failure were allowed. Virological failure during the 144 weeks of follow-up was defined as having one of the following: (I) not reaching pVL below the LLQ of the standard assay used at that moment within 24 weeks after starting antiretroviral therapy, or (II) having a single pVL > 1000 copies/mL or two consecutive pVL measurements between 400
and 1000 copies/mL after having had a pVL below the LLQ. If the last pVL in the 144 week period was between 400 and 1000 copies/mL, the patient was excluded if no subsequent measurement was available. Each control patient had to have enough frozen plasma available at three different time points after three years of therapy (week 144, and the first available time-point before and after week 144) for re-testing in the modified NucliSens HIV-1 QT assay with a LLQ of 5 copies/mL.²⁵

Plasma HIV-1 RNA quantification

Alternative multidrug regimen group
pVL in EDTA plasma was measured every eight weeks using the NucliSens HIV-1 QT assay (Organon Teknika, Boxtel, The Netherlands) with 0.2 mL plasma input, resulting in a LLQ of 400 copies/mL. During the first 16 weeks of antiretroviral therapy pVL was measured more frequently. When pVL decreased to below 400 copies/mL, an initial input volume in the assay of 2 mL plasma was used combined with an ultrasensitive protocol adaptation, resulting in a LLQ of 5 copies/mL.²⁵

Control group
pVL in EDTA plasma was routinely measured every 12 weeks. After the start of antiretroviral therapy, and following a change in regimen, measurements were done more frequently for 12 weeks. Before August 1999 pVL was measured using standard HIV-1 RNA assays with a plasma input of 0.1 or 0.2 mL. The NASBA HIV-1 QT assay (Organon Teknika, Boxtel, The Netherlands) had a LLQ of 1000 copies/mL, and the NucliSens HIV-1 QT assay and Ampli­cor HIV Monitor (Roche Diagnostic Systems Inc., Branchburg, NJ, USA) had a LLQ of 400 copies/mL. After August 1999 ultrasensitive HIV-1 RNA assays were used, either the NucliSens HIV-1 QT assay with 2 mL plasma input and a LLQ of 40 copies/mL or Quantiplex bDNA 3.0 (Bayer Corporation, Tarrytown, NY, USA) with 1 mL plasma input and a LLQ of 50 copies/mL. At the moment the first patient reached week 48, the assays with a LLQ of 400 copies/mL were in use. Plasma of the patients fulfilling the selection criteria, obtained at 48 and 144 weeks after the start of therapy and at the first available time-point before and after week 144, was re-tested using the NucliSens HIV-1 QT assay with 2 mL plasma input combined with the ultrasensitive protocol adaptation, resulting in a LLQ of 5 copies/mL.
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Statistical Analysis
Data were analysed with SAS version 8.0 (SAS Institute, Cary, NC). Differences between groups were considered significant at P<0.05. All reported P-values are two-sided.

Group comparisons of baseline CD4+ T cell counts and pVL were made with the Wilcoxon Rank Sum Test. The primary objective of the study was to compare low level viremia in both groups during prolonged therapy. We therefore compared differences between the alternative multidrug regimen group and the control group in pVL, as assessed by ultrasensitive testing (LLQ 5 copies/mL), at week 48, and at week 144 and the first available time-point before and after week 144.

The number of patients in the two groups with a quantifiable pVL at week 48 was compared with the Fisher's Exact test. The number of timepoints with a quantifiable pVL per patient at and around week 144 (ranging from 0 of 3 to 3 of 3 measurements per patient) was compared using the Cochran-Armitage Trend Test.

We compared controls with no quantifiable pVL at and around week 144 with controls with ≥ 1 sample with quantifiable pVL at and around week 144 for the CD4+ T cell count at baseline and week 144, and the pVL at baseline, using the Wilcoxon Rank Sum Test.

Results

Thirty control patients were selected from 111 HIV-1 positive antiretroviral naive patients who initiated a standard potent antiretroviral regimen between July 1996 and February 1998. Of these 111 patients, 101 had an initial virological response as evidenced by an undetectable pVL within 24 weeks after starting therapy. After 48 weeks, 66 of 111 patients (60%) and after 144 weeks, 54 of 111 patients (49%) had not experienced virological failure according to our criteria. Of these 54 patients 24 had switched to an NNRTI containing regimen; 30 patients were still on a standard potent PI containing regimen. The initial regimens used in the 30 selected control patients are described in Table 1. Baseline CD4+ T cell count and pVL were not significantly different for the multidrug regimen group and the control group (Table 1).
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Table 1 Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Standard of care (n=30)</th>
<th>Alternative multidrug regimen (n=7)</th>
<th>P-value</th>
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</thead>
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<tr>
<td><strong>CD4+ T cell count</strong></td>
<td>100 (40-250)</td>
<td>130 (30-260)</td>
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<tr>
<td>(cells/mm³)*</td>
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<td><strong>Plasma HIV-1 RNA</strong></td>
<td>4.6 (4.2-5.4)</td>
<td>4.9 (4.8-5.1)</td>
<td>0.34</td>
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<tr>
<td>(log₁₀ copies/mL)*</td>
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<tr>
<td><strong>ARVT</strong></td>
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<td></td>
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<tr>
<td>(no of patients)</td>
<td></td>
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<tr>
<td>ZDV/3TC/IDV</td>
<td>(10)</td>
<td>ZDV/3TC/ABC/NVP/IDV</td>
<td>(6)</td>
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<td>d4T/ddI/ABC/NVP/IDV</td>
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</tr>
<tr>
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<tr>
<td>d4T/ddI/SQV/RTV</td>
<td>(1)</td>
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*median (interquartile range); ARVT, antiretroviral therapy; ZDV, zidovudine; 3TC, lamivudine; d4T, stavudine; ddI, didanosine; ABC, abacavir; NVP, nevirapine; IDV, indinavir; RTV, ritonavir; SQV, saquinavir; NFV, nelfinavir

At week 48, one of the 30 control patients had a quantifiable pVL (550 copies/mL) using the then available standard assay. Frozen plasma samples were available for re-testing in the modified ultrasensitive assay (LLQ 5 copies/mL) in 23 of the other 29 control patients; 9 of these contained quantifiable pVL. Thus, pVL could be quantified (range: 25 to 550 copies/mL) in 10 of 24 control patients (41.7%), whereas in all ten patients treated with the alternative multidrug regimen pVL was below 5 copies/mL (Fisher's Exact test, P=0.017).

At and around week 144, we did three measurements per patient. Of the 3 x 30 measurements in control patients, seven samples already contained quantifiable pVL (range 79-453 copies/mL) using standard assays with an LLQ of 40, 50 or 400 copies/mL. Five of the 30 patients had switched to an NNRTI between week 144 and the first pVL measurement after week 144. In one patient, two samples were not available for re-testing because this patient had been enrolled in another study. One of the samples yielded an invalid test result in the modified ultrasensitive assay despite repeated testing. These eight measurements were not censored, but considered to be undetectable for the purpose of this study.
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Figure 1 Number of samples with quantifiable and unquantifiable plasma HIV-1 RNA using the modified ultrasensitive HIV-1 RNA assay at and around week 144, in 7 patients treated with an alternative multidrug regimen and in 30 patients treated with a standard antiretroviral regimen. At and around week 144 three measurements per patient were performed. LLQ, lower limit of quantification of the modified ultrasensitive HIV-1 RNA assay (5 copies/mL).

At and around week 144, pVL could be quantified either in the standard assays or in the modified ultrasensitive assay (LLQ 5 copies/mL) at 0 of 3 time points in 12 control patients, at 1 of 3 time points in 14 control patients, and at 2 of 3 time points in 4 control patients. The measured viremia varied between 13–453 copies/mL. In 6 of 7 patients of the alternative multidrug regimen pVL was below 5 copies/mL at all 3 time points around week 144, and in one patient a pVL of 21 copies/mL could be quantified at 1 of 3 time points. The number of samples with quantifiable pVL per patient differed significantly between the two groups (P=0.036, Cochran-Armitage Trend Test).
Of the 10 control patients with a quantifiable pVL at week 48, pVL could be quantified at 0 of 3 time points at and around week 144 in 2 patients, at 1 of 3 time points in 5 patients, and at 2 of 3 time points in 3 patients. Of the 14 control patients with a unquantifiable pVL at week 48, pVL could be quantified at 0 of 3 time points in 7 patients, and at 1 of 3 time points in 7 patients (P=0.029, Cochran-Armitage Trend Test).

Control patients were further divided into a subgroup of 12 patients with no quantifiable pVL at and around week 144, and a subgroup of 18 patients with at least one quantifiable pVL at that moment. The median baseline CD4+ T cell count of the 18 control patients with at least one quantifiable measurement was lower than that of the other control subgroup (65 cells/mm³, IQR 30-240 cells/mm³ and 150 cells/mm³, IQR 100-370 cells/mm³, respectively; P=0.026, Wilcoxon Rank Sum Test). Both subgroups were comparable regarding baseline HIV-1 RNA levels, CD4+ T cell count at week 144, and the drugs used in the standard regimen at baseline and at week 144 (data not shown).

**Discussion**

This study demonstrates that the use of a multidrug regimen consisting of three different classes of antiretroviral drugs resulted in a stronger suppression of pVL compared to clinically successful treatment with standard potent antiretroviral therapy.

Although this study was not designed as a comparative study from the start of therapy, we feel justified to draw conclusions from this comparison. The control group was a selection consisting of the most successfully treated patients in our outpatient clinic, who during three years of potent standard antiretroviral therapy had not experienced virological failure using strict criteria. This makes suboptimal compliance with the medication not a likely explanation for the difference in viral suppression between the two groups. Furthermore, we also identified patients who showed virological failure according to our strict criteria between weeks 48 and 144. These patients were excluded from our analyses, but their virological failure might very well have been due to ongoing low-level viral replication despite good adherence to their standard potent antiretroviral therapy.

At and around week 144, we did three measurements per patient. Testing plasma samples with a concentration of HIV-1 RNA below the LLQ of a modified ultrasensitive assay can still yield a positive test result. For instance,
using an ultrasensitive assay with a LLQ of 3 copies/mL, the chance of detecting HIV-1 RNA in plasma samples containing 10, 5, 2.5, and 1.25 copies/mL was 100, 100, 91 and 55%, respectively. Therefore, the chance of detecting pVL below the LLQ can be increased by repeated testing of the same blood sample. We preferred measurements of blood samples obtained at different timepoints instead of repeated measurements of the same sample, as by doing so we also allowed for the detection of a possible day-to-day variation in pVL.

The fact that we were able to demonstrate a significantly higher level of plasma viremia in these highly selected control patients is indicative for the presence of ongoing low-level viral replication in the majority of patients using so called potent antiretroviral therapy despite undetectable pVL using standard assays. The clinical implication of ongoing viral replication is first of all the risk of continued viral evolution and the selection of resistance mutations, which has been described to occur even in patients with undetectable pVL. Ongoing low-level viral replication might in the long run lead to therapeutic failure. A decrease over time in the percentage of patients with pVL below 50 copies/mL, from 75% after one year to 65% after three years of triple therapy, has recently been described. One possible explanation for this late virological breakthrough may be the development of viral drug resistance during ongoing low-level viral replication. Although it is currently not known what degree of virological suppression is necessary to ensure a long-term response, it has been demonstrated that the risk of virological failure was substantially reduced for chronically infected patients whose plasma HIV-1 RNA nadir was below 20 copies/mL.

Further, the presence of a cellular reservoir consisting of resting memory CD4+ T lymphocytes harbouring replication-competent virus despite prolonged potent antiretroviral therapy represents a major barrier for eradication of HIV. In treated patients with undetectable pVL the mean half-life of this pool of latently infected memory CD4+ lymphocytes has been estimated to be 6 to 43.9 months, leading to a theoretical time for eradication of HIV by antiretrovirals alone of 10-60 years. The difference in decay rate has been attributed to differences in suppression of pVL, since individuals who experienced episodes of plasma viremia had a slower decay rate of their cellular reservoir. So, the persistence of the cellular reservoir during prolonged treatment is not only determined by its intrinsic slow decay characteristics, but also by continuing replenishment in case of active viral replication. In line with this is the observed more rapid decay of the cellular reservoir fol-
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lowing treatment intensification with ABC ± efavirenz in five patients compared to five other patients whose therapy was not intensified.30

The question is whether the improved suppression of viral replication should be explained by more drugs or by more classes of drugs used. A possible mechanism for the improved suppression of viral replication using the alternative multidrug regimen is that the presence of more reverse transcriptase inhibitors results in stronger inhibition of the reverse transcription of HIV-1 RNA. Furthermore, sub-optimal penetration of antiretroviral drugs into anatomical (e.g. the brain and male genital tract) and cellular sites might result in only partial suppression of viral replication in these sites in the case of standard of care therapy. In the multidrug regimen, the drugs were selected because of their known good penetration into these anatomical sites.26,31-33

When assuming that combination therapy does not completely block infection of new cells, but rather results in a decrease of the amplitude of ongoing HIV infection cycles, the use of more antiretroviral drugs could further reduce the size and frequency of these local bursts of viral replication.34

A quantifiable pVL at week 48 was predictive of residual viral replication around week 144. Also, a low baseline CD4+ T cell count was predictive for residual replication, despite comparable CD4+ T cell counts at week 144 for patients with and without quantifiable low-level viral replication. The relation between a high baseline CD4+ T cell count and stronger suppression of viral replication during standard antiretroviral therapy has been demonstrated earlier.13,35 An explanation might be that in chronically HIV-1 infected patients with advanced immunosuppression the capacity to restore a functional HIV-1 specific cytotoxic T-lymphocyte response is diminished.36 Administration of a multidrug regimen may compensate for this diminished HIV-1 specific immunity in such advanced patients.

In conclusion, the use of a multidrug regimen consisting of three different classes of antiretroviral drugs resulted in a further reduction of pVL compared to treatment with standard potent antiretroviral therapy. This provides evidence that ongoing low-level viral replication during standard therapy is at least partially due to limited potency of the currently used drug regimens. It is evident that further long-term follow-up studies are required to answer the important question whether the advantages of improved viral suppression by this multidrug regimen outweigh the disadvantages of additional toxicity and costs of using more antiretroviral agents.
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Acknowledgements

The authors thank Glaxo-Wellcome for providing abacavir and Boehringer-Ingelheim for providing nevirapine. We thank P. Reiss, F. Miedema, H. Schuitemaker, and G-J Weverling for helpful discussions, and S.N. Blank, S.A. Danner, J.K.M. Eeftinck Schattenkerk, M.H. Godfried, S. Jansen (RN), J.T.M. van der Meer, M. Nievaard (RN), D.W. Notermans, T. van der Poll, M.H.E. Reijers, and A. Verbon for patient care. The study is financially supported by a private foundation, which does not wish to be named.
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