Initiation of HAART in Drug-Naive HIV Type 1 Patients Prevents Viral Breakthrough for a Median Period of 35.5 Months in 60% of the Patients

K. VAN VAERENBERGH,1 T. HARRER,2 J.-C. SCHMIT,3 A. CARBONEZ,4 E. FONTAINE,3 M. KUROWSKI,5 M. GRÜNKE,2 P. LÖW,2 A. RASCU,2 B. SCHMIDT,6 M. SCHMITT,2 I. THOELEN,1 H. WALTER,6 K. VAN LAETHEM,1 M. VAN RANST,1 J. DESMYTER,1 E. DE CLERCQ,1 and A.-M. VANDAMME1

ABSTRACT

The introduction of potent combinations of antiviral drugs is a major breakthrough in the treatment of HIV. We investigated the long-term virologic outcome and the development of resistance after initiating highly active antiretroviral therapy (HAART) in drug-naive patients in daily clinical practice. Twenty-five treatment-naive HIV-1 patients were started on HAART. Fifteen patients responded with a drop in viral load below the limit of detection during 35.5 (interquartile range: 7) months of therapy. In 6 of 10 patients with virologic failure, virus with resistance-related mutations against the received drugs emerged. Compared with responders (R), nonresponding (NR) patients were in a later disease stage at therapy start ($p = 0.0089$) with lower CD4 cell counts at baseline ($p = 0.040$), and a lower proportion of nonresponders showed protease inhibitor (PI) levels above $C_{\text{min}}$ ($p = 0.049$). More NR patients showed secondary PI mutations at baseline ($p = 0.079$), and the CCR2-64I coreceptor polymorphism was absent among NR patients, compared with 38.5% of R patients displaying CCR2-64I ($p = 0.053$), although the differences were not significant. In conclusion, starting HAART in antiretroviral drug-naive HIV-infected patients followed in daily clinical practice prevented viral breakthrough for up to 44 months in 60% of the patients. Virologic failure was associated with the development of resistance-related mutations, a later stage of disease at start of therapy and lower PI drug levels.

INTRODUCTION

During the last decade significant progress has been made in the treatment of HIV. Whereas in the past, monotherapy or sequential therapy of nucleoside analogue reverse transcriptase (RT) inhibitors produced only a temporary and incomplete inhibition of the viral replication,1 the introduction of highly active antiretroviral therapy (HAART) has fundamentally improved the clinical outcome of HIV, at least in the developed countries.2 The long-term success of HAART is based on a complete viral suppression preventing the breakthrough of drug-resistant virus. In clinical trials the majority of patients (up to 80%) receiving HAART maintain an undetectable viral load.3 In real life the initial enthusiasm for HAART has decreased because of the difficulties associated with the complex combination therapies, such as long-term side effects, suboptimal drug potency, the emergence of drug-resistant variants, and the necessity for strict treatment adherence.4 Furthermore, the persistence of viral reservoirs with an extremely long half-life time, even in individuals on combination therapy with an undetectable viral load, precludes the possibility of viral eradication. Instead of hitting hard and early, current therapeutic guide-

1Rega Institute for Medical Research and University Hospitals, Katholieke Universiteit Leuven, Leuven, Belgium.
2Department of Medicine III, University of Erlangen-Nürnberg, Erlangen, Germany.
3Centre Hospitalier de Luxembourg et Laboratoire de Rétrovirologie, CRP-Santé, Luxembourg.
4Universitair Centrum voor Statistiek, Katholieke Universiteit Leuven, Leuven, Belgium.
5HIV-LAB, Auguste-Victoria Krankenhaus, Berlin, Germany.
6Institute of Clinical and Molecular Virology, University of Erlangen-Nürnberg, Erlangen, Germany.
lines advise individualizing the decision to start therapy depending on the patient’s readiness, taking into account that therapy options remain limited after a first HAART failure.4

This study was performed to investigate the success rate in terms of long-term virologic suppression when HAART is started in daily clinical practice in antiviral drug-naive patients and to identify risk factors for virologic failure.

MATERIALS AND METHODS

Patient population and study design

An observational study was performed to investigate the virologic response and the development of genotypic resistance, in relation to baseline viral genotype, protease inhibitor (PI) drug levels, and coreceptor polymorphisms in antiviral drug-naive HIV-1 patients starting HAART. Included were treatment-naive patients from the Department of Medicine III of the University of Erlangen, Germany (n = 18) and the Internal Medicine Service of Centre Hospitalier in Luxembourg, Luxembourg (n = 7) who were started on a three- or four-drug combination between December 1995 and December 1996. Drugs included were ZDV, ddI, ddC, d4T, 3TC, ABC, IDV, RTV, NFV, SQV, NVP, and EFV, started or changed according to the available guidelines. This resulted in a study population of 25 patients who were not enrolled in clinical trials. No patient selection was made, since all patients in the participating centers that initiated HAART outside clinical trial protocols were included, as far as follow-up information was available. At baseline and during follow-up, samples were collected to follow the evolution of the viral load and the CD4 cell count. Genotyping of the protease (PRO) and the reverse transcriptase (RT) gene was performed retrospectively as were drug level monitoring and determinations of host receptor polymorphisms. Clinical data, viral load (bDNA, Chiron, Emeryville, CA, detection limit 500 copies/ml, changed to 50 copies/ml during 1998), and CD4 cell count were provided by the participating centers. The primary endpoint of the study was virologic failure, defined as two consecutive viral load values above the detection limit of the assay used without considering the first 6 months after the start of therapy. For patients with an undetectable viral load during the complete follow-up period, the moment of the most recent viral load determination was considered as the end point.

RNA extraction and cDNA synthesis

Viral RNA was extracted from plasma or serum using Nucli sens (Organon Teknika, the Netherlands), according to the manufacturer’s instructions, followed by reverse transcription into cDNA using the Gene Amp RNA-PCR kit (Applied Biosystems, Belgium) with random hexamers.

Sequencing of the RT and PRO gene

To amplify the PRO gene or the RT gene, an outer polymerase chain reaction (PCR) with primers AV150 and RT2 was followed by a nested PCR with primers RVP5 and RVP3 or M13USP-A35 and M13RSP-NE-(1)35, respectively.5 Sequencing was performed with primers RVP5 and RVP3 for the PRO gene and primers USP, RSP, AV36, and AV44 for the RT gene, using the dye terminator technology (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Biosystems) and analyzed on an ABI Prism 310 (Applied Biosystems).5 The entire PRO gene and the RT gene up to codon 255 were thus obtained.

Coreceptors

Starting from PBMCs, genomic DNA was extracted using the Qiagen blood kit (Qiagen, Hilden, Germany). Characterization of the CCR5 genotype (CCR5-Δ32) was done by PCR as previously described resulting in a 238-bp fragment for the wild-type (wt) allele6 and a 206-bp fragment for an allele with a Δ32 deletion. The presence of CCR2-64I polymorphisms was detected by PCR amplification of a 669-bp region of the CCR2 gene, containing the 64I mutation side, followed by a restriction digest and polyacrylamide gel electrophoresis.8

Plasma drug levels

Drug levels for PIs were determined retrospectively, using liquid chromatography-tandem mass spectrometry on two samples obtained during the study period.9

Statistical analysis

Statistical analysis was assessed using Statistica and SAS software. To analyze differences between groups, the Wilcoxon two-sample test was used for continuous variables and the Fisher exact test for comparing proportions. Potential predictors of virologic response were assessed by logistic regression. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Patient population

Twenty-five HIV-1 patients were enrolled (Table 1). At the moment of starting therapy, half of the patients (n = 13) were in clinical stage A, seven patients had reached stage B, and five patients stage C. The median viral load was 17,500 RNA copies/ml plasma (interquartile range, IQR: 60,390). Baseline viral load values were not available for six patients starting therapy before July 1996. The median baseline CD4 cell count was 310 cells/μl (IQR: 182). The median time between first HIV diagnosis and the start of HAART was 14 months (IQR: 40). Except for one patient who started on a triple nucleoside reverse transcriptase inhibitor (NRTI) therapy, the initial regimen consisted of a PI and either two NRTIs (n = 20) or three NRTIs (n = 4).

Virologic response and characteristics of responders and nonresponders

All patients were followed for a median period of 35.5 months (IQR: 7) before reaching the end point. Fifteen patients responded with a drop in viral load below the limit of detection during the complete study period (median value 37 months; IQR: 8) and were considered responders (R). Ten patients never reached an undetectable viral load (n = 3) or showed a rebound
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All participants</th>
<th>Responders (n = 15)</th>
<th>Nonresponders (n = 10)</th>
<th>p value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (% of total population)</td>
<td>80%</td>
<td>93%</td>
<td>60%</td>
<td>0.12$^b$</td>
</tr>
<tr>
<td>Clinical stage (n)</td>
<td></td>
<td></td>
<td></td>
<td>0.0089$^b$</td>
</tr>
<tr>
<td>A</td>
<td>13</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Viral load (RNA copies/ml)$^d$</td>
<td>17500 (IQR: 60390)(n = 19)</td>
<td>20750 (IQR:34300)(n = 14)</td>
<td>17220 (IQR: 59290)(n = 5)</td>
<td>0.85$^c$</td>
</tr>
<tr>
<td>CD4 cell count (cells/μl)$^d$</td>
<td>310 (IQR: 182)</td>
<td>340 (IQR: 113)</td>
<td>176.5 (IQR: 330)</td>
<td>0.040$^c$</td>
</tr>
<tr>
<td>Time since HIV diagnosis (months)$^d$</td>
<td>14 months (IQR: 40)</td>
<td>14 months (IQR: 42)</td>
<td>18.5 months (IQR: 36)</td>
<td>0.98$^c$</td>
</tr>
<tr>
<td>Baseline genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT mutations$^e$</td>
<td>37.5% (n = 24)</td>
<td>14.3% (n = 14)</td>
<td>40%</td>
<td>1</td>
</tr>
<tr>
<td>RT mutations to first regimen$^f$</td>
<td>8.3% (n = 24)</td>
<td>14.3% (n = 14)</td>
<td>0%</td>
<td>0.49$^b$</td>
</tr>
<tr>
<td>Protease mutations$^g$</td>
<td>75% (n = 24)</td>
<td>50% (n = 14)</td>
<td>90%</td>
<td>0.17</td>
</tr>
<tr>
<td>Coreceptor polymorphism</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR5$^h$</td>
<td>32%</td>
<td>26.5%</td>
<td>40%</td>
<td>0.66$^b$</td>
</tr>
<tr>
<td>CCR2$^h$</td>
<td>22.7%</td>
<td>38.5% (n = 13)</td>
<td>0% (n = 9)</td>
<td>0.053$^b$</td>
</tr>
</tbody>
</table>

$^a$Comparing responders to nonresponders, two-sided p-value <0.05 is considered statistically significant (printed in italics and bold).

$^b$Fisher exact test.

$^c$Mann–Whitney U test.

$^d$Median and interquartile range are given.

$^e$Proportion of patients with resistance-related reverse transcriptase mutations.$^29$

$^f$Proportion of patients with reverse transcriptase mutations related to resistance to the first antiviral regimen.

$^g$Proportion of patients with secondary mutations in PRO.

$^h$Proportion of patients with mutant allele(s).
in viral load after reaching the undetectable viral load level \((n = 7)\), and this after a median time of 30 months (IQR: 22). These 10 patients were considered nonresponders (NR) (Table 2).

Whereas R patients were treated initially with a triple combination therapy, NR patients were treated with a triple or quadruple therapy as first regimen. Quadruple therapy was based on saquinavir (NR \(n = 4\)), while all other PI-including regimens were based on indinavir (R \(n = 6\); NR \(n = 4\)), or ritonavir (R \(n = 8\); NR \(n = 2\)). The NRTI backbone consisted of ZDV and 3TC (R \(n = 10\); NR \(n = 2\)), ZDV and ddC (R \(n = 4\); NR \(n = 3\), or ddI and d4T (NR \(n = 1\)). Saquinavir was combined with ZDV, 3TC, and either ddC (NR \(n = 2\)) or ddI (NR \(n = 2\)). One R patient started on triple NRTI therapy (ZDV, ddC, and 3TC). Treatment changes during the study period were mainly due to drug-related side effects. Six patients (R = 3, NR = 3) during follow-up were changed to a nonnucleoside reverse transcriptase inhibitor (NNRTI)-containing regimen.

At the moment of starting therapy, R and NR patients had a similar viral load, and the time since HIV diagnosis was not significantly different for R (median value 14 months) and NR patients (18.5 months). Compared with R patients NR patients showed a significantly lower CD4 cell count and a more advanced disease (Table 1).

Irrespective of the moment of reaching the end point, when all patients were followed for a period of 36 months, a significantly different viral load outcome and number of consecutive treatment regimens were observed, although both responders and nonresponders showed a favorable evolution in CD4 cells (Table 2). While all responding patients had an undetectable viral load at the end of the study period (below 50 copies/ml), nonresponding patients showed a median viral load of 1147 RNA copies/ml (IQR: 1830) \((p = 0.00018)\) as could be expected considering our definition of responders and nonresponders. R patients had three consecutive treatment regimens (median value, IQR: 3), whereas NR patients had five (IQR: 1) consecutive treatments \((p = 0.028)\). R patients showed a median increase of 263 CD4 cells (IQR: 257) and NR patients showed a median increase of 202 CD4 cells (IQR: 233) \((p = 0.50)\). Univariate logistic regression revealed that virologic failure rate increased with lower CD4 cell count at baseline (odds ratio, OR, 0.07; 95% confidence interval, CI95, 0.006–0.76) and PI drug levels below \(C_{\text{min}}\) (OR 11.25; CI95 1.19–106.12).

**Protease and reverse transcriptase genotype at baseline**

The HIV genotype of the RT and PRO gene before starting antiviral therapy could be analyzed for 14 of the 15 responding patients; for 1 patient no sample was available. Sequences were submitted to GenBank. No major mutations were present in the PRO gene. Eight patients displayed virus with secondary mutations in PRO (L101, M361, S37N, D60E, L63P, A71V, and/or V77I). For three of these patients resistance-related mutations in the RT gene were also observed. One patient displayed virus with the K103R and T215Y mutations. A second patient harbored virus with the M41L and T215S mutations, most probably reflecting the transmission of a ZDV-resistant virus. A third patient displayed virus with the A98S and the T68TN mutations. In two additional patients the virus showed a NNRTI-related change (A98S).

Except for one patient, all NR patients displayed virus with one or more secondary mutations in the PRO gene (M361, S37N, D60E, L63P, A71V, V77I, and/or L89M). For two patients the virus showed only the L63P mutation. In four patients the virus displayed an NNRTI-related change in the RT gene (L74L/A, A98S, K103R, and E138A respectively). The secondary PI mutations were more present at baseline in NR patients, although the difference was not significant (Table 1).

**Accession numbers:** AY063236–AY063287 and AY064490–AY06506.

**Genotypic evolution**

Evolution of genotypic resistance could be followed only for NR patients, since R patients showed an undetectable viral load during the complete study period (Table 3). A second sample was analyzed at the moment of virologic failure or, in case of a low viral load, as soon as amplification was possible. For the three patients who never reached an undetectable viral load (NR5, NR6, and NR7), a second sample was sequenced at, respectively, 15 months, 16 months, and 9 months after start of

<table>
<thead>
<tr>
<th>Table 2. Therapy characteristics of responders compared with nonresponders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Number of consecutive treatment regimensb</td>
</tr>
<tr>
<td>PI drug levels below (C_{\text{min}})d</td>
</tr>
<tr>
<td>Time to end point (months)f</td>
</tr>
<tr>
<td>Virologic outcome (RNA copies/ml)b</td>
</tr>
<tr>
<td>CD4 cell increase (cells/µl)b</td>
</tr>
</tbody>
</table>

\(a\) Two-sided \(p\)-value <0.05 is considered statistically significant (printed in bold and italic); continuous variables are presented as median value and interquartile range (IQR).

\(b\) The number of therapy changes from start of HAART to end of the study period is given (36 months).

\(c\) Mann–Whitney \(U\) test.

\(d\) Proportion of patients with PI level(s) below \(C_{\text{min}}\).

\(e\) Fisher exact test.

\(f\) For responding patients the time from the start of HAART to the end of the study is given; for nonresponding patients the time from starting HAART to the moment of virologic failure is given.

\(g\) The patients who never reached an undetectable viral load (\(n = 3\)) were excluded from this calculation.

\(h\) Viral load and CD4 cell evolution were calculated from the start of HAART to the end of the study period (36 months).
**Table 3. Sequencing Data for Patients with Virologic Failure at the Moment of Starting Therapy and at the Moment of Failure**

<table>
<thead>
<tr>
<th>Patient code</th>
<th>Timea</th>
<th>L10</th>
<th>L24</th>
<th>V32</th>
<th>M36</th>
<th>S37</th>
<th>M46</th>
<th>I47</th>
<th>D60</th>
<th>L63</th>
<th>A71</th>
<th>V77</th>
<th>V82</th>
<th>I84</th>
<th>L89</th>
<th>L74</th>
<th>A98</th>
<th>K103</th>
<th>V106</th>
<th>V108</th>
<th>E138</th>
<th>M184</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR1c</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>I</td>
<td>V/I</td>
<td>I/</td>
<td>I/V</td>
<td>P</td>
<td>T</td>
<td>I</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR3</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR4</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR6</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR7</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR8</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12e</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR9</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR10</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aThe RT gene up to codon 255 and the PRO gene were determined by sequencing starting from viral RNA. Mutations were scored according to Schinazi et al (2000); A, alanine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; R, arginine; S, serine; T, threonine; V, valine.

bSampling time (in months) subsequent to the moment of therapy start is given.
cNR, nonresponding patient.
dThe moment of sampling is indicated; therapy failure occurred 3 months earlier.
eThe month of sampling for this patient was 12 months subsequent to the moment of start of therapy, despite a viral load below the limit of detection (500 copies/ml), while virologic failure occurred at 40 months.
therapy. For one patient (NR8) with virologic failure 40 months after starting therapy, amplification of an earlier sample with an undetectable viral load (DL 500 copies/ml) was successfully performed. The sample was obtained 1 year after therapy initiation. Accession numbers: AY063236–AY063287 and AY064490–AY064506.

For three NR patients (NR3, NR9, and NR10), all of them with secondary PRO mutations and one with the K103R mutation in the RT gene before initiation of treatment, no resistance-related mutations were added except for one secondary PI mutation in one patient. For five patients (NR1, NR4, NR6, NR7, and NR8) the virus had additional resistance-related mutations only in the RT gene (M184V/I, V108I, and a combination of the V106A and the M184V mutations). For one patient (NR2), the virus added primary mutations in the PRO gene (M46I and V82A), and for the last patient the virus showed both RT (M184V) and PRO (M46I, V84I) mutations (NR5). All resistance-related mutations that emerged during the study were acquired due to experience with the respective drugs (3TC for the M184V mutation, ritonavir for M46I and V82A), except for the V106A and V108I mutations. Both NNRTI-related mutations appeared in patients without any NNRTI experience.

PI drug levels

Plasma drug levels for PIs were assessed retrospectively on one sample (n = 2) or on two samples of different time points (n = 16). Samples were obtained from 11 R and 7 NR patients. For the remaining patients (n = 7) no plasma was available for drug level monitoring. Since the samples were obtained at random and not as a specific time point in relation to the moment of drug intake, the $C_{\text{min}}$ (ng/ml ± SD) was considered as the cut-off value for being compliant (saquinavir, nelfinavir, PI levels for ritonavir and indinavir were obtained from the manufacturer). Eleven patients showed PI levels above the $C_{\text{min}}$ at all the time points with drug level monitoring. Three patients had one of two PI levels above the $C_{\text{min}}$. For the last four patients, the PI levels were below the cut-off value. Except for two R patients with only one of two PI levels above the $C_{\text{min}}$, nine R patients showed PI levels above $C_{\text{min}}$. For two of seven NR patients, PI levels were above the $C_{\text{min}}$ at the two occasions. Thus NR patients showed significantly lower PI levels (Table 2).

Coreceptor polymorphism

CCR5 and CCR2 genotypes were analyzed (Table 1). CCR5 genotypes were distributed as follows: 17 patients were wt/wt (68%) and the 8 remaining patients were wt/Δ32 (32%). Four patients with a CCR5 heterozygosity were poorly responding patients. CCR2 coreceptor polymorphism was successfully determined for 22 patients. CCR2 genotypes were distributed as follows: 17 patients were wt/wt (77.3%), 4 patients were wt/64I (18.2%), and the last patient was 64I/64I (4.5%). All patients with a CCR2 heterozygosity and the patient with the CCR2 homozygosity were good responders.

DISCUSSION

In this longitudinal study we wanted to investigate the effectiveness of HAART in terms of virologic suppression and in delaying the emergence of resistant virus and to identify possible risk factors for virologic failure in a daily clinical practice. This study was possible through collaboration with the Internal Medicine Service of Erlangen (Germany) and Luxembourg. Between December 1995 and December 1996, 25 HIV-1 patients who had not received any antiretroviral agent and who were not involved in a clinical study were started on triple or quadruple combination therapy. At that time treating HIV-infected patients with HAART was considered a progressive strategy, since the 1996 guidelines still centered on double NRTI combination therapy. In both clinical centers PIs, and also nevirapine as a first NNRTI, were available since 1995–1996. In these clinical centers, these 25 patients were the first to receive PIs and later on also NNRTIs in addition to the nucleoside analogues in their regimen outside any clinical trial.

In clinical trials HAART has resulted in a sustained drop in viral load in the majority (80–90%) of patients with a follow-up period up to 2 years. It is known, however, that in daily clinical practice viral suppression is achieved in a substantially lower frequency than in clinical trials. In our study HAART was successful in 15 of the 25 patients in shutting down the viral replication below the detection limit of the currently viral load assays for a median period of 35.5 months (responders or R). An incomplete inhibition of the viral replication or a rebound in viral load occurred in 10 NR patients. Despite this poor virologic response in NR patients, the treatment resulted in a favorable CD4 cell evolution. Our observation is similar to the proportion of 66% of treatment-naive patients maintaining an undetectable viral load (DL 400 copies/ml) 30 months after starting HAART in the Swiss Cohort Study.

For the failing patients, no major PI- or NNRTI-related mutation was present at baseline: 90% (9 of 10) patients showed secondary mutations in the PRO gene, in one patient in combination with a K103R mutation in the RT gene, in another patient in combination with a L74I mutation, and in the last patient with an E138A mutation. The L74I, the K103R, and the E138A mutations do not convey resistance to the commercially available NRTI compounds. For the responding patients, baseline samples showed the presence of secondary resistance mutations in PRO in 57% of the patients. Two R patients showed baseline genotypic resistance to ZDV. The protease gene in HIV-1 subtype B is characterized by an extensive polymorphism in PI-naive patients, including the presence of secondary mutations.

In a preliminary report studying therapy failure in naive patients starting HAART, Perno et al. concluded that the presence of secondary PRO mutations at positions 10 and 36 predicted virologic failure, independent of baseline CD4 cell count and viral load and of baseline RT mutations. Although in our study the baseline genotype and especially secondary PI mutations were dissimilar in R and NR patients, these differences did not reach significance.

When we compared R with NR patients, a number of other characteristics were different for both patient groups and may have contributed to therapy outcome. At baseline, R and NR patients had a similar viral load and a similar time lapse since HIV diagnosis. NR patients were, however, in a later phase of disease, reflected by the clinical stage and the CD4 cell counts. This observation confirms the predictive value for a worse treatment outcome of both baseline CD4 cell count and the presence of AIDS at baseline, as was reported previously.
Another factor that was analyzed was the prevalence of two coreceptor polymorphisms (CCR5-Δ32 and CCR2-64I). Homozygotes for a 32-bp deletion in the gene for chemokine receptor CCR5 are almost completely protected against infection with HIV-1 through the formation of a truncated receptor preventing viral entry. Both heterozygosity of CCR5-Δ32 and the presence of CCR2-64I are associated with a delayed progression to AIDS. Where CCR5-Δ32 heterozygosity was present both in R and NR patients (26.5% and 40%, respectively), the presence of CCR2-64I in R patients (38.5%) as compared with 0% in NR group was striking.

The complete viral suppression obtained by HAART is crucial for the prevention of the development of resistant virus. In 7 of the 10 patients included in this study, incomplete viral inhibition resulted in the development of virus with resistance-related mutations. For three NR patients, no resistance-related mutations were added except for one secondary PI mutation in one patient. Six failing patients developed resistance-related mutations in the RT gene, for one patient in combination with primary mutations in the PRO gene. One additional patient developed primary mutations in the PRO gene. As in patients treated with NRTI combinations including 3TC, we observed a high prevalence of the 3TC-related mutation at position 184 (in five of the nine failing patients who were receiving 3TC). The mutations that emerged in the failing patients were related to the drugs they were receiving, except for the one patient who developed a V108I mutation without NNRTI. Another patient developed an NNRTI-related V106A mutation, in addition to the M184V mutation, although this patient was also NNRTI-naive. A possible explanation for these mutations, as well as for the changes at baseline or during follow-up (L74L/I, A98S, K103R, E138A/G) in patients without any NNRTI, is the occurrence of NNRTI mutations as polymorphisms, which we and others observed before in antiretroviral drug-naive HIV patients.

Despite the fact that the responding patients maintained an undetectable viral load during the complete study period, we were able to amplify virus for one of them 6 months after starting ZDV, 3TC, and IDV. This patient was one of the two patients with ZDV-related mutations at baseline, while receiving ZDV in the first regimen. The ZDV-related mutations, M41I and T215S reverted to wt sequences, whereas an M184V mutation was acquired. Since the detection limit of the viral load assay in that time period was only 500 RNA copies/ml, viral loads below this detection limit could not be interpreted as guaranteeing complete inhibition of the viral replication.

Recently, a pivotal role has highlighted the necessity of strict therapy compliance to inhibit viral replication. Noncompliance has been linked to viral resistance and drug failure. Because of the retrospective character of the present study, we were able to get only an indirect estimate of therapy compliance by performing plasma drug level monitoring for the PIs included in the therapy. was considered as the cut-off value for being compliant. The proportion of R patients with PI levels below the cut-off value was significantly lower compared with NR patients. Suboptimal PI plasma levels have also been associated with therapy failure in previous studies.

In conclusion, starting HAART in antiretroviral-naïve HIV-infected patients could prevent viral breakthrough for a median period of 35.5 months in daily clinical practice in 60% of the patients and resulted in a positive CD4 cell evolution for both responders and nonresponders. In 6 of 10 patients, virologic failure was associated with resistance mutations related to the drugs received, most frequently the 3TC-related M184V mutation. Despite the small number of patients who could be included in the present study, we identified the following two risk factors significantly associated with virologic failure: a lower CD4 cell count at the moment of starting therapy and the lower PI drug levels. Thus, in agreement with the new trend to “Hit HIV-1 hard, but only when necessary” our data confirm that virologic suppression is feasible when HAART is initiated in patients with CD4 cell counts of 350 cells/µl, provided drug levels are sufficiently high.

ACKNOWLEDGMENTS

We thank Kristel Declercq and Bart Maes for excellent technical assistance. This work was supported in part by the Biomedical Research Program of the European Commission (EC BIOMED2 Grant BMH4-CT-95-1634), the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO Grant G.0104.98), the Geconcerteerde Onderzoeksacties van de Vlaamse Gemeenschap (GOA 00/12), the Fondation Recherche sur le SIDA, Luxembourg, the DFG (SFB 466, T. Harrer), and the Bayerische Staatsministerium für Kultus, Erziehung und Wissenschaft (T. Harrer).

REFERENCES


Address reprint requests to:
Anne-Mieke Vandamme
Rega Institute for Medical Research
and University Hospitals
Katholieke Universiteit Leuven
Minderbroedersstraat 10
B-3000 Leuven, Belgium

E-mail: annemie.vandamme@uz.kuleuven.ac.be
This article has been cited by:

1. Isidore Sieleunou, Mohamadou Souleymanou, Anne-Marie Schönenberger, Joris Menten, Marleen Boelaert. 2009. Determinants of survival in AIDS patients on antiretroviral therapy in a rural centre in the Far-North Province, Cameroon. *Tropical Medicine & International Health* **14**:1, 36-43. [CrossRef]

2. R. Scott Braithwaite, Steven Shechter, Chung-Chou H. Chang, Andrew Schaefer, Mark S. Roberts. 2007. Estimating the Rate of Accumulating Drug Resistance Mutations in the HIV Genome. *Value in Health* **10**:3, 204-213. [CrossRef]


