Predictive Value of Provirus Load and DNA Human Immunodeficiency Virus Genotype for Successful Abacavir-Based Simplified Therapy

Isabelle Pellegrin, Anne Caumont, Isabelle Garrigue, Patrick Merel, Marie-Hélène Schrive, Hervé Fleury, Michel Dupon, Jean-Luc Pellegrin, and Jean-Marie Ragnaud

Departments of Virology and Internal Medicine and Infectious Diseases, Bordeaux University Hospital, Bordeaux, France

Of 75 human immunodeficiency virus (HIV) type 1–infected patients successfully responding to 2 nucleoside reverse-transcriptase inhibitors (NRTIs) plus 1 protease inhibitor (PI), 55 started a simplified abacavir (ABC)–based triple NRTI regimen. Influences of DNA load and DNA reverse-transcriptase (RT) mutations on virological responses were assessed at month 6 after initiation of therapy. Baseline heterogeneity was observed: peripheral blood mononuclear cell (PBMC) genotyping showed 31% RT mutations with 1–5 NRTI-related mutations, 78% protease mutations had 1–5 PI-related mutations; and HIV-1–DNA levels were 1.8–3.5 log copies/10^6 PBMC. Outcomes for 49 patients on a regimen of 2 NRTIs plus ABC were as follows: 22 successes, 10 blips (“blip” defined as intermittent plasma HIV-1 RNA levels between 50 and 100 copies/mL and a return to an undetectable level), and 17 failures, whereas, for patients continuing on a regimen of 2 NRTIs plus 1 PI, there were 19 successes and 1 blip. Previous treatment regimens, baseline provirus level, and PBMC genotype predicted virological outcome.

The current generation of protease inhibitors (PIs) has been associated with both short- and long-term toxicities [1, 2]. Moreover, the impact of high daily pill burden means that some individuals are unable to comply to such combination regimens for extended periods of time. Consequently, there has been widespread interest among patients and physicians to substitute other classes of antiretroviral drugs in patients treated successfully with PI-containing highly active antiretroviral therapy (HAART). Previously simplified maintenance therapy replaced the PI with nonnucleoside reverse-transcriptase inhibitors (NNRTIs), such as efavirenz or nevirapine [1, 3, 4], and resulted in sustained virus suppression. The use of abacavir (ABC) in combination with 2 NRTIs is another alternative that is receiving much attention. Triple therapy with ABC plus zidovudine plus lamivudine has been shown to have antiviral activity comparable with that of indinavir plus zidovudine plus lamivudine in antiretroviral-naive patients [5, 6]. In previous induction and/or maintenance studies, virological failure occurred in 22%–31% of the patients during maintenance [7–9]. The predictors of virological failure in those studies were patients having higher human immunodeficiency virus (HIV)–1 RNA plasma levels and zidovudine-resistant mutations at baseline. Clumeck et al. [10] showed continued virological suppression, significant regression of lipid abnormalities, and easier dosing after switching from 2 NRTIs plus 1 PI to 2 NRTIs plus ABC for patients with prolonged suppression of plasma HIV RNA levels.
plasma HIV-1 RNA levels remained.

Opravil et al. [11–13] showed that having previously taken 1 or 2 NRTIs before HAART and having archived reverse-transcriptase (RT) mutations in HIV-1 DNA at baseline were predictors of failure under simplified ABC-based triple-NRTI regimens. Martinez et al. [14] reported concordant data in the randomized Spanish NEFA study, which compared the switch to nevirapine or efavirenz or ABC; treatment experience with monotherapy or dual therapy with an NRTI explained 82% of virological rebounders in the ABC group. Switching to a triple NRTI–based PI-sparing regimen may potentially place the patient at increased risk for virological failure; therefore, other potentially predictive virological parameters should be assessed to improve the definition of the best candidates for simplified treatment. Therefore, proviral HIV-1 DNA quantification might be a helpful marker, since proviral DNA persists in peripheral blood mononuclear cells (PBMC) and lymphoid tissues, even after prolonged successful HAART [15–19]. The objective of this study was to assess the impact of previous antiretroviral therapy (ART), HIV-1 proviral DNA levels, and RT mutations detected in PBMC from patients successfully treated with a 2 NRTI plus 1 PI regimen on virological responses to a 2 NRTI plus ABC regimen.

**SUBJECTS AND METHODS**

**Study population.** Seventy-five ART-experienced HIV-1–infected adults were enrolled in a prospective nonrandomized cohort from September 2000 to February 2001 and were followed-up until September 2001. At the start of the study (month 0), all patients had been receiving a triple-therapy regimen, 2 NRTIs plus 1 PI, for at least 6 months and had plasma HIV-1 RNA levels <50 copies/mL for at least 6 months. Patients either continued their current regimen (PI group) or switched to the simplified regimen, consisting of the same 2 NRTIs plus ABC (ABC group), to overcome PI-associated problems (e.g., lipid abnormalities or clinical lipodystrophy).

All patients were monitored at months 0, 1, 3, and 6. When plasma HIV-1 RNA levels remained <50 copies/mL throughout the 6-month study period, patients were considered to have virological success. Patients whose plasma HIV-1 RNA levels increased to >50 copies/mL during follow-up were considered to have virological failures. A “blip” was defined as an intermittent plasma viremia with plasma HIV-1 RNA levels between 50 and 1000 copies/mL and a return to an undetectable level. Blood samples were drawn into EDTA at each clinical follow-up visit for measurement of CD4+ cell counts, HIV-1–RNA quantitation, and preparation and storage at −80°C of 106 PBMC aliquots before use for quantification of proviral DNA and analyses of its RT and protease genotypes. Provirus loads and genotypes were determined at month 0, at the time of failure or blip, or at month 6 for patients responding successfully to treatment. Patients with blips were followed until month 12, to assess for the predictive value of blip for virological outcome at 1 year.

**HIV-1–RNA quantitation.** Plasma HIV-1–RNA levels were determined by use of the branched-DNA signal amplification method (Quantiplex HIV-1 RNA 3.0 assay; Bayer), according to the manufacturer’s procedure, with a lower detection limit of 50 copies/mL.

**Quantification of proviral DNA with a real-time polymerase chain reaction (PCR) assay.** PBMC were isolated by standard Ficoll-hypaque density gradient centrifugation in aliquots of 10^6 PBMC and then were frozen at −80°C until use. DNA was extracted from patients’ PBMC by use of the High Pure Viral Nucleic Acid Kit (Roche Diagnostics), according to the manufacturer’s recommendations. DNA was quantified spectrophotometrically to determine the input level of each DNA sample, so as to normalize variations caused by differences in the PBMC count or DNA extraction. DNA also was extracted from the T lymphoblastoid cell line 8E5 [20], which contains a single proviral HIV–lymphadenopathy-associated virus genome/cell. A series of 4 log10 dilutions corresponding to 5 × 10^4 to 5 × 10^7 HIV-1 DNA copies (5 × 10^4 to 5 × 10^7 8E5 cells) were included in each experiment to generate an external standard curve and were considered to be the quantitative standard (QS) to determine the number of copies per clinical sample.

To perform real-time quantitative PCR, 500 ng of extracted DNA (HIV–DNA template or DNA standard from 8E5) were added to the PCR mixture (total volume, 20 μL) containing 4 mM MgCl₂, 0.5 μM of each primer (long terminal repeat [LTR] 152 and LTR 131), 0.25 μM specific probe LTR1, 0.5 U of Uracil D-Glycosylase, and 2 μL of LightCycler (LC)–Fast Start DNA master mix hybridization probes kit (Roche Molecular Biochemicals). Sequences of the primers for the HIV-1 LTR gene were as follows: forward primer, LTR152 (5′-GCC TCA ATA AAG CCT GCC GTC TTG A-3′); and reverse primer, LTR 131 (5′-GCC GCC ACT GCT AGA GAT TT-3′), which amplify a 131-bp fragment in a region of low variability in the B subtype HIV-1 LTR gene. The specific probe labeled at the 5′ end with the reporter dye, 6-carboxy fluorescein (FAM), and at the 3′ end with the quencher dye, N,N,N′,N′-tetramethyl-6-carboxy-rhodamine (TAMRA), was synthesized by MWG Biotech. Its sequence is as follows: LTR1 (5′-FAM-AAG TAG TGT GTC CCC GTC TGT TRT KTG ACT-TAMRA-3′). The LC PCR protocol was as follows: denaturation at 95°C for 10 min (1 cycle); a 2-step PCR consisting of 10 s at 95°C and 30 s at 60°C (45 cycles); and 40°C for 1 min (1 cycle). The entire cycling process was <45 min. Fluorescence was monitored once during each cycle at the end of the synthesis phase. A negative (no template) control was included in each PCR run. Amplification, data acquisition, and analysis were performed using the LC software program (vers. 3.5). For data analysis with the channel

HIV Provirus Load and Genotype on Triple NRTI Regimen • JID 2003:187 (1 January) • 39
setting F1/F2, the second derivative maximum mode was chosen with baseline adjustment set in the arithmetic mode. For each run, a standard curve was generated from 8E5 cells ranging from 5 × 10^4 to 5 × 10^6 copies (in triplicate). Intra- and inter-assay reproducibilities also were analyzed by use of independent extractions of 8E5 cell DNA (in 11 laboratories from the French National Agency for AIDS Research [ANRS] workgroup; data not shown). The HIV-1 provirus load was expressed as the number of HIV-1–DNA copies/10^6 PBMC (threshold, 5 copies/500 ng DNA; i.e., 65 [1.8 log_{10} copies/10^6 PBMC]. HIV-1 provirus loads ranging from 1 to 4 copies/10^6 PBMC were expressed as <5 copies/500 ng DNA (i.e., <1.8 log_{10} copies/10^6 PBMC).

**RT and protease sequence analyses.** Proximal DNA extracted from all patients’ PBMC (as described above) at baseline was analyzed for RT and protease genotypes by use of the CEQ2000 DNA sequencer (Beckman Coulter). When virological failure or blips were observed during follow-up, plasma HIV-1 RNA levels and PBMC proviral DNA were sequenced. For patients considered to be virological successes, PBMC proviral DNA was sequenced at month 6. RT gene (nt 1–230) and the entire protease gene were sequenced as described elsewhere [21] (HIV-1 DNA was sequenced without the RT-PCR step).

We also used the ANRS genotype-resistance guidelines [22] to evaluate the ABC-sensitivity of HIV-1 isolates (i.e., isolates were considered to be resistant to ABC when the RT gene had the Q151M mutation, an insertion at codon 69 alone, or ≥6 mutations among the following: M41L, K65R, D67N, K70R, L74V, Y115F, M184V/I, L210W, T215Y/F, and K219Q/E). The presence of 4 or 5 RT mutations among the latter also was considered to confer partial resistance to ABC.

**Statistical analyses.** The data were analyzed by use of SAS version 8.2 software. ABC and PI groups were compared by use of Fisher’s exact test for qualitative variables and by Wilcoxon’s test for quantitative variables. Failure, blip, and success together with blips. The method of determining the breakpoint value for proviral DNA was achieved by use of the most discriminant Fisher’s exact test statistic value. P < .05 was considered to be statistically significant.

**RESULTS**

**Patients.** Of the 75 patients enrolled in the study between September 2000 and February 2001, 20 continued their current 2 NRTIs plus 1 PI regimen, and 55 retained their current 2 NRTI combination but replaced the PI with ABC. Baseline characteristics of patients are summarized in table 1. Six patients were withdrawn from the ABC group between month 1 and month 3 because of hypersensitivity to reactions (n = 2), pregnancy (n = 2), or were lost to follow-up (n = 2). The remaining 49 patients in the ABC group and 20 in the PI group completed 6 months of follow-up.

**PBMC proviral DNA: baseline virus load and RT and protease genotypes.** Results of baseline PBMC proviral DNA load and RT and protease sequences are presented in table 2. Considering the entire population at baseline, 61 (81.33%) of 75 patients had detectable proviral loads with a median [25th, 75th percentile] of 2.4 [2.0, 2.8] log_{10} copies/10^6 PBMC, whereas the other 14 patients had virus loads <1.8 log_{10} copies/10^6 PBMC. Peripheral provirus load level was significantly associated with previous monotherapy or dual therapy (P = .04), number of previous regimens (P = .02), and number of baseline DNA protease mutations (P = .02), whereas no correlation was found with the duration of virus suppression on HAART or CD4+ cell counts.

In these 75 patients successfully treated on 2 NRTIs plus 1 PI regimen, we observed 1–5 NRTI-related resistance mutations in 23 (31%) DNA-RT sequences and 2–8 mutations in 32 (43%) DNA protease sequences. Primary resistance mutations in the protease gene were observed in 3 (4%) patients. Number of RT or RT plus protease mutations in baseline proviral DNA was not correlated with the number of previous regimens or with previous monotherapy or dual therapy. Importantly, mutated DNA sequences were obtained in 16 of 45 RT mutations and 11 of 45 protease mutations from patients on first-line successful HAART.

**Virologic outcome in patients who continued a PI-containing regimen.** Nineteen (95%) of 20 patients in the PI group were considered to be virological successes at month 6. Two patients had previously received NRTI as monotherapy or dual therapy, and 13 of 19 patients were on first-line successful HAART. Median CD4+ cell counts did not vary significantly between baseline and month 6 (from 495 cells/μL [range, 302–671 cells/μL] to 508 cells/μL [range, 413–660 cells/μL]). In these 19 patients, median proviral DNA load remained stable at 2.2 [1.8, 2.5] copies/10^6 PBMC between baseline and month 6. Fourteen patients (74%) had baseline PBMC RT genotypes expressing wild-type viruses, whereas 5 (26%) of 19 patients had only 1 or 2 RT resistance mutations. At month 6, the PBMC RT genotype was determined again: 2–2 additional mutations emerged in 3 patients, whereas baseline RT mutations were no longer detected in 3 other patients.
Table 1. Patient characteristics at inclusion.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All patients (n = 75)</th>
<th>ABC group (n = 55)</th>
<th>PI group (n = 20)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in yearsb</td>
<td>42 [37, 50]</td>
<td>42 [38, 49]</td>
<td>42 [36, 52]</td>
<td>.86</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>63:12</td>
<td>47:8</td>
<td>16:4</td>
<td>.72</td>
</tr>
<tr>
<td>CD4+ cells count, cells/µLb</td>
<td>477 [332, 676]</td>
<td>477 [332, 686]</td>
<td>482 [327, 657]</td>
<td>.54</td>
</tr>
<tr>
<td>Duration of virus suppression in monthsb</td>
<td>38 [27, 44]</td>
<td>38 [28, 44]</td>
<td>39 [21, 46]</td>
<td>.87</td>
</tr>
<tr>
<td>Previous regimens, 1/2/3/4c</td>
<td>45/20/5/1</td>
<td>32/15/4/1</td>
<td>13/5/1/0</td>
<td>1</td>
</tr>
<tr>
<td>Previous drugs, 3/4/5/6d</td>
<td>41/26/2/6</td>
<td>28/20/1/6</td>
<td>13/6/1/0</td>
<td>.3</td>
</tr>
<tr>
<td>Previous monotherapy or dual therapy</td>
<td>11 (14.7)</td>
<td>9 (16.3)</td>
<td>2 (10)</td>
<td>.72</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. ABC, abacavir; NRTI, nucleoside reverse-transcriptase inhibitors; PI, protease inhibitors.

a P values were determined by use of Fisher’s exact test (qualitative variable) or Wilcoxon’s test (quantitative variable).

b Age in years, CD4+ cell counts, and months of virus suppression (<50 copies/mL) on PI-containing regimens are expressed as median [25th, 75th percentiles].

c 1/2/3/4, no. of patients with 1, 2, 3, or 5 previous line(s) of treatment.

d 3/4/5/6, no. of patients with 3, 4, 5, or 6 different drugs previously prescribed.

One patient in the PI group experienced blips at month 1 and month 6 (61 and 76 copies/mL, respectively) and subsequently achieved a plasma virus load <50 copies/mL at month 12 (3 time points); no mutation was detected in his DNA RT sequence.

Virological outcome in 49 patients who switched to ABC-containing regimen according to virological parameters. At month 6, of the 49 on-treatment patients, 22 (45%) were considered to have virological success, 17 (35%) were considered to virological failure, and 10 (20%) had blips. Results are presented in table 3.

Baseline proviral DNA loads were significantly higher in patients considered to have virological failures and blips than in patients with virological successes (P = .00012). We determined the breakpoint value of proviral DNA for virological failure; the most discriminating DNA threshold was estimated to be 2.7 log_{10} copies/10^6 PBMC. Of the 19 patients with high proviral DNA (≥2.7 log copies/10^6 PBMC), 10 (53%) were considered to have virological failure, 7 (37%) exhibited blips, and 2 (10%) patients were considered to have virological success. Of the 30 patients with low proviral DNA (<2.7 log_{10} copies/10^6 PBMC), 7 (23%) were considered to have virological failure, 3 (10%) had blips, and 20 (67%) were considered to have virological success. Median proviral DNA load values determined at month 0 and at follow-up for all patients did not change significantly: ΔDNA values (median [25th, 75th percentiles]; 0 [−0.2, +0.3], −0.2 [−0.7, +0.4], and 0 [−0.2, +0.3] for virological successes, failures, and blips, respectively.

Concerning the 22 patients considered to be virological successes, ABC was prescribed as the second ART regimen for most of them (81%), and only 1 patient had previously received monotherapy with zidovudine with the K219Q-archived RT mutation in HIV-1 DNA at baseline. Median CD4+ cell counts did not vary significantly between baseline and month 6. Eighteen (82%) of 22 had baseline PBMC RT genotypes expressing wild-type viruses, whereas 4 (18%) had only 1 or 2 RT resistance mutations. At month 6, PBMC RT genotypes were available for 18 of 22 sequences. Similar RT patterns were observed at baseline and month 6 in 15 of 18 sequences; 1–5 additional mutations had emerged in 3 others, 2 of whom had wild-type RT patterns at baseline. At month 12, these 3 patients remained as virological successes.

Seventeen patients were considered to have virological failure (7 at month 1, 9 at month 3, and 1 at month 6). Of these 17 patients, treatment experience with monotherapy or dual therapy with NRTI was found in 6 (35.3%), of whom 4 shared 1–5 archived RT mutations in PBMC DNA at baseline. Among 9 (53%) of 17 patients, ABC was prescribed as the third or later ART regimen. The median plasma virus load at the time of the
first detected rebound was 1182 copies/mL (range, 346–3209 copies/mL), with a median CD4+ cell count decrease of −43 cells/μL (range, −187 to 59 cells/μL). RT and protease sequencing and virus loads determination were performed at baseline (in PBMC) and at the time of failure (in PBMC and plasma). Of these 17 patients with virological failure, 8 (47%) had baseline isolates expressing wild-type RT, whereas 9 (53%) originally carried PBMC isolates with 1–5 RT mutations associated with NRTI resistance. At virological failure, a wide range of resistance-conferring RT mutations was detected in 15 of 17 patients in the RT gene isolated from plasma (3–7 mutations/sequence) and from PBMC DNA (2–5 mutations/sequence). In most DNA sequences, mutated and wild-type viruses were present in a mixed population. As early as month 1 after the switch, the multidrug-resistant Q151M mutation was observed in PBMC DNA and plasma for 2 patients. Two patients experienced virological failure but without emergence of mutations in either plasma or PBMC; lack of adherence to therapy was proven for one and suspected for the other.

The switch to ABC resulted in a high rate of HIV-1–RNA blips during the first 6 months, since blips occurred in 10 (20%) of 49 patients, of whom 2 had initially received monotherapy or dual therapy with an NRTI. The median plasma virus load at the first blip was 104 copies/mL (range, 74–167 copies/mL), with a median decrease of −18 [−41; +76] CD4+ cells/μL between baseline and the first blip. At baseline, 8 of 10 patients had wild-type RT genotypes in PBMC DNA, and 2 patients had 3–4 NRTI-related mutations. Genotypic resistance was evaluated at the time of the first blip; 2–3 additional mutations conferring resistance had emerged in previously wild-type DNA RT gene of 2 patients with low level intermittent viremia (130 and 167 copies/mL, respectively). In 9 patients with low-level viremia, no plasma RNA sequences were obtained. In the patient with the higher level of intermittent viremia (543 copies/mL), 4 mutations were detected in the plasma RT gene without detected mutation in PBMC DNA. This latter patient experienced treatment failure 6 months after the blip.

**DISCUSSION**

The results of our study demonstrate that, for patients previously achieving success with PI-based HAART, replacing the PI
Table 3. Abilities of baseline virological parameters to predict virological outcome after switching from 2 nonnucleoside reverse-transcriptase inhibitors (NRTIs) plus 1 protease inhibitor (PI) to 2 NRTIs plus abacavir (ABC).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Virologic outcome in ABC group (n = 49)</th>
<th>Statistical analyses</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Success (n = 22)</td>
<td>Failure (n = 17)</td>
<td>Blip (n = 10)</td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td>Success, failure, blip</td>
<td>Success vs. failure</td>
<td>Success vs. failure + blip</td>
<td>Success vs. failure</td>
<td></td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>3.19</td>
<td>3.14</td>
<td>1.9</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Age in years(^b)</td>
<td>40 [36, 46]</td>
<td>42 [39, 49]</td>
<td>45 [42, 54]</td>
<td>.42</td>
<td>.45</td>
</tr>
<tr>
<td>Baseline CD4(^+) cell count, cells/(\mu)L(^b)</td>
<td>522 [339, 740]</td>
<td>578 [331, 686]</td>
<td>393 [325, 616]</td>
<td>.42</td>
<td>.65</td>
</tr>
<tr>
<td>Baseline DNA proviral load(^d)</td>
<td>2.2 [2.0, 2.6]</td>
<td>2.8 [2.4, 3.2]</td>
<td>2.9 [2.3, 3.1]</td>
<td>.0012</td>
<td>.0023</td>
</tr>
<tr>
<td>Duration of virus suppression, months(^d)</td>
<td>36 [29, 40]</td>
<td>38 [28, 44]</td>
<td></td>
<td>.88</td>
<td>.68</td>
</tr>
<tr>
<td>Treatment history(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Previous ART regimen</td>
<td>18 (82)</td>
<td>8 (47)</td>
<td>6 (60)</td>
<td>.06(^d)</td>
<td>.04</td>
</tr>
<tr>
<td>&lt;3 Previous drugs</td>
<td>15 (68)</td>
<td>8 (47)</td>
<td>4 (40)</td>
<td>.28(^d)</td>
<td>.21</td>
</tr>
<tr>
<td>Previous monotherapy and/or dual therapy</td>
<td>1 (4.5)</td>
<td>6 (35.3)</td>
<td>2 (20)</td>
<td>.04</td>
<td>.03</td>
</tr>
<tr>
<td>Baseline PBMC DNA sequences</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT mutations/sequence(^f)</td>
<td>0.21 (0–2)</td>
<td>1.2 (0–5)</td>
<td>0.5 (0–3)</td>
<td>.03</td>
<td>.0087</td>
</tr>
<tr>
<td>ABC-resistance mutations(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT mutations</td>
<td>18 (82)</td>
<td>8 (47)</td>
<td>8 (80)</td>
<td>.06(^d)</td>
<td>.04</td>
</tr>
<tr>
<td>1–3</td>
<td>4 (18)</td>
<td>7 (41)</td>
<td>2 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–5</td>
<td>0</td>
<td>2 (12)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR mutations/sequence(^f)</td>
<td>1.3 (0–5)</td>
<td>2.1 (0–8)</td>
<td>1.5 (0–4)</td>
<td>.23</td>
<td>.095</td>
</tr>
<tr>
<td>WT or 1 secondary mutation(^c)</td>
<td>14 (64)</td>
<td>6 (35)</td>
<td>7 (70)</td>
<td>.12(^d)</td>
<td>.11</td>
</tr>
<tr>
<td>1 Primary or 2–8 mutations(^c)</td>
<td>8 (36)</td>
<td>11 (65)</td>
<td>3 (30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RT + PR mutations(^f)</td>
<td>1.5 (0–5)</td>
<td>3.3 (0–8)</td>
<td>2 (1–5)</td>
<td>.01</td>
<td>.003</td>
</tr>
</tbody>
</table>

**NOTE.** ART, antiretroviral therapy; Blip, intermittent plasma human immunodeficiency virus type 1 RNA levels between 50 and 100 copies/mL, and a return to an undetectable level; CI, confidence interval; OR, odds ratio; PBMC, peripheral blood mononuclear cells; PR, protease; WT, wild type. Sex: women (reference class) vs. men; no. of previous lines of treatment: <1 (reference class) vs. >1 line of treatment; baseline RT mutations: :=1 mutation (reference class) vs. wild type. Proviral DNA load is expressed as log\(_{10}\) copies/10\(^6\) PBMC.

\(^a\) P values were determined by means of Wilcoxon’s test.

\(^b\) Data are median [25th, 75th percentiles].

\(^c\) Data are no. (%) of patients.

\(^d\) 1 vs. >1 previous line of treatment.

\(^e\) <3 vs. >3 drugs previously prescribed.

\(^f\) Data are mean no. (range).
with ABC does not always represent an effective treatment option. We showed that treatment failure and blips were experienced by 45% and 20% of patients on simplified therapy, respectively. Sustained suppression of the plasma virus load also probably depends on previous ART regimens, number of PBMC DNA RT mutations associated with NRTI resistance, and proviral DNA loads before treatment simplification.

The study by Clumeck et al. [10] showed that PI replacement by ABC in triple therapy after prolonged suppression of plasma HIV-1–RNA levels was a successful treatment option for patients who did not have a history of single or dual ART; treatment failures occurred at similar rates in the ABC (9%) and PI groups (11%). Data on baseline DNA mutations were available only for the patients with virological failure, and, in most cases, no mutations were detected. Opravil et al. [11–13] reported concordant results: treatment with zidovudine as part of a single or dual NRTI regimen before the initiation of HAART and archived RT resistance in HIV-1 DNA at baseline were significant predictors of failure after the switch to simplified therapy combining ABC plus combivir. Virological failure was more frequent in this simplified-regimen group than in the continuation group (15% vs. 6%). The CNA30017 trial [11] and the TRIZAL-AZL 300002 trial [23], which included patients with no history of treatment failure on HAART, compared outcomes after switching from HAART to 2 NRTIs plus ABC [11] or to trizivir [23] versus continued HAART. For these patients with only 1 line of successful HAART before switching, virological failure after treatment occurred in only 3%–5% patients in the simplified-regimen group. In the present study, 16.3% of patients in the ABC group had started treatment on single or dual ART, and ABC was prescribed as a third- to fifth-line of therapy for 36% of patients, which could explain the high rate of virological failure observed in our population. Indeed, 67% of patients who started with monotherapy or dual therapy and 75% of patients with history of virological failure since starting HAART failed after the switch. Moreover, we defined failure as a plasma virus load >50 copies/mL versus a less stringent definition of >400 copies/mL.

In patients receiving HAART, plasma viremia is rapidly suppressed to undetectable levels [24–30], even though low-level replication continues. For this reason, proviral DNA could be an informative marker to explore viral reservoirs and to assess the long-term impact of treatment [31]. Our results show that PBMC provirus loads are heterogeneous on HAART, which translates into prolonged undetectable plasma loads: 81.3% patients had detectable proviral DNA loads at baseline (1.8–3.5 log_{10} copies/10^6 PBMC). Baseline DNA level was significantly associated with previous monotherapy or dual therapy, number of previous lines of treatment, number of baseline DNA mutations, and virological outcome at month 6. Defining a proviral DNA threshold for use in clinical practice is difficult; nevertheless, our findings showed that patients with proviral DNA ≥2.7 log_{10} copies/10^6 PBMC are more likely to experience virological failure than those who had a proviral load <2.7 log_{10} copies/10^6 PBMC. DNA levels did not vary significantly between baseline and follow-up samples, regardless of the virological outcome. The proviral DNA load probably reflects individual parameters, because host genetic factors and response to treatment probably are involved in the constitution of the pool of HIV-1–infected cells [32, 33]. These findings support proviral DNA quantification before switching to a simplified regimen with the aim of maintaining virus replication suppression.

In this study, patients with long-term sustained suppression of plasma HIV RNA levels were found to have a virological heterogeneity of baseline PBMC HIV-1 genotypes. Thirty-one percent of RT genotypes had 1–5 NRTI-resistance mutations, 78% of protease genotypes had 1–4 secondary mutations, and 4% had 1–2 primary mutations. These results showed that, even in patients achieving first-line HAART success, mutations related to this successful HAART had been selected and archived in 36% of PBMC proviral DNA sequences. The rapidity of first rebound (between month 1 and month 3 for the majority of our patients) and the above mentioned genotype-profile changes suggest that archived virus resistant to NRTIs can quickly become the dominant circulating virus population if the simplified regimen is not fully suppressive. Virus strains representing <10%–20% of the total virus population could have been missed by the sequencing, and some studies have shown the compartmentalization of resistant virus populations in lymph nodes under effective HAART [34]. Nevertheless, considering that higher number of DNA-RT mutations was significantly associated with failure in the simplified ABC group, it could be relevant to assess the PBMC genotype pattern before switching, even in patients on first-line successful HAART.

Finally, during prolonged follow-up of patients on HAART with sustained suppression of plasma HIV-1–RNA loads <50 copies/mL, blips (50–1000 copies/mL) were observed in up to 40% of the patients [35–38], but their clinical implications are unclear. Easterbrook et al. [38] reported that patients with intermittent viremia (≥400 copies/mL) are 3 times more likely than those who have undetectable virus loads to experience sustained viral rebound and to have an impaired CD4 cell count increase, whereas, in studies of patients with episodes of lower-level intermittent viremia, between 50 and 400 copies/mL, blips did not always preclude successful inhibition of virus replication [36, 39]. As a consequence of continuous replication during suboptimal suppression, the replicating viruses might acquire drug-resistance mutations [38–40]. In the present study, low-level blips occurred in 10 (20%) patients on simplified treatment. Additional resistance mutations in the PBMC DNA or the plasma RT gene were found in 3 patients at their first blip, despite the low-level blips. During a 12–month follow-up period, virological
failure occurred in the only patient with intermittent viremia >500 copies/mL. More research is warranted to identify the threshold value of blips predictive of virological failure and the long-term consequences of such lapses.

We showed the virological heterogeneity of baseline PBMC provirus genotype and proviral DNA levels in a population of patients with sustained long-term suppression of plasma HIV-RNA loads to <50 copies/mL. We conclude that switching to a triple NRTI-based PI-sparing regimen may not be as effective in patients who began treatment on suboptimal ART, have archived mult_mutuated viruses in PBMC, and who have higher proviral DNA loads. The clinical management implications of our findings include the potential interest of intracellular virological analyses.

**Acknowledgements**

We gratefully acknowledge the French Human Immunodeficiency Virus Quantification Group for helpful collaboration; François Montestruc and Jérôme Rapion for statistical analysis; Hélène Duffoire and Danièle Jacquemart for excellent technical assistance; and Janet Jacobson for editing our English.

**References**


24. Burgard M, Izopet J, Dumon B, et al. HIV RNA and HIV DNA in HIV Provirus Load and Genotype on Triple NRTI Regimen • JID 2003;187 (1 January) • 45


