Prevalence of genotypic and phenotypic HIV-1 drug-resistant strains among patients who have rebound in viral load while receiving antiretroviral therapy in the UNAIDS–Drug Access Initiative in Abidjan, Côte d'Ivoire

Christiane Adjé-Touré[a], Bilé Celestin[a], Debra Hanson[b], Thierry H. Roels[a,b], Kurt Hertogs[c], Brendan Larder[d], Fabien Diomande[a], Martine Peeters[e], Serge Eholié[f], Eve Lackritz[b], Terence Chorba[a,b] and John N. Nkengasong[a,b]

Objective: To determine the prevalence of genotypic and phenotypic antiretroviral (ARV) drug-resistant HIV-1 strains among patients with viral load rebound while receiving ARV therapy in Abidjan, Côte d'Ivoire.

Methods: Between August 1998 and April 2000, we selected all patients (n = 241) who had received ARV drug therapy for at least 6 months in the UNAIDS–Drug Access Initiative (DAI), in Abidjan. We analyzed for genotypic and phenotypic drug resistance among 97 (40%) of the 241 patients who had a rebound in plasma viral load, defined as an initial decrease of > 0.5 $\log_{10}$ copies/ml followed by a subsequent increase of > 0.25 $\log_{10}$ copies/ml.

Results: Of the viruses isolated from the 97 patients, 86 (88.7%) had usable sequences and 68 (79%) of the 86 patients had genotypic resistance to at least one reverse transcriptase inhibitor (RTI) or protease inhibitor (PI). Resistant mutations were found for zidovudine in 50 (78%) of 64 patients who had received the drug, 11 (68.7%) of 16 patients on lamivudine, for nevirapine in two (2%), for indinavir in one (1%), and for ritonavir in one (1%). Phenotypic resistance to at least one nucleoside RTI was seen in 45 (56%) of the 80 patients tested, to non-nucleoside RTIs in eight (10%), and to PIs in one (1.3%). Multivariate regression analysis showed factors associated with resistance to be initial treatment with dual therapy ($P = 0.04$) compared with highly active antiretroviral therapy, and maximal initial viral load response ($P = 0.006$).

Conclusion: Our results demonstrate a high prevalence of ARV drug resistance associated with dual ARV therapy. These results indicate the limited role for dual ARV therapy.

AIDS 2003, **17** (suppl 3):S23–S29

Keywords: Africa, antiretroviral, drug resistance, HIV-1, viral load rebound

From the [a]Projet RETRO-CI, Abidjan, Côte d'Ivoire, the [b]Division of HIV/AIDS Prevention, National Center for STD, HIV, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, USA, [c]VIRCO NV, Mechelen, Belgium, [d]VIRCO, Cambridge, UK, the [e]Laboratoire Retrovirus, IRD, Montpellier, France, and the [f]Infectious Disease Clinic, University Hospital, Treichville, Abidjan, Côte d'Ivoire.

Correspondence and requests for reprints to Dr John N. Nkengasong, Laboratory of Virology, 01 BP 1712, Projet RETRO-CI, Abidjan, Côte d'Ivoire. Tel: +225 21 25 41 89; fax: +225 21 24 29 69; e-mail: jcn5@cdc.gov

Introduction

In developed countries, highly active antiretroviral therapy (HAART) has been shown to suppress viral replication and dramatically alter the rate of disease progression for persons infected with the human immunodeficiency virus (HIV) [1]. Because of the recent decrease in prices of antiretroviral (ARV) drugs, several countries in Africa have started pilot programmes aimed at making ARV drugs accessible to HIV-infected patients. Information about patterns and factors that favor the occurrence of ARV drug resistance as provided by these pilot programmes may help expand access of ARV to more patients in different countries. Systematically expanding ARV therapy programmes in Africa based on lessons learned from pilot programmes may improve the appropriate prescription and use of ARV drugs thereby reducing the incidence of acquired treatment-based drug resistance. Additionally, more insights may be gained into drug-resistant mutation profiles of persons infected with HIV-1 non-B subtypes. Several factors may influence the occurrence of ARV drug resistance in these pilot programmes: lack of viral suppression and rebound in viral load, poor adherence to therapy, sub-optimal drug potency, and inappropriate drug exposure.

When the UNAIDS–Drug Access Initiative (DAI) started in Côte d'Ivoire, patients seeking care had high viral loads (median, 5.5 $\log_{10}$ copies/ml) and low CD4 counts (median $< 150 \times 10^6$ cells/l) [2]; however, because of the high cost of drugs, only two drug regimens were prescribed for most patients. Even though officially HAART is standard of care in Côte d'Ivoire [3]. In this study, we report on the prevalence of genotypic and phenotypic ARV drug-resistant HIV-1 strains among patients with viral load rebound while receiving ARV therapy in the UNAIDS–DAI, Abidjan, Côte d'Ivoire.

Methods

UNAIDS–DAI

The UNAIDS–DAI was started in Côte d'Ivoire in 1998 and aimed to provide ARV therapy and other AIDS-related therapies at reduced cost to persons infected with HIV. Patients accessing the UNAIDS–DAI were screened for biomedical eligibility and eligibility for public financial subsidies. Social workers collected sociodemographic information from each patient, and physicians conducted physical examinations, assessed the patients' past medical history and current ARV, and completed a questionnaire that asked among other things, about adherence to therapy, at enrollment. Blood was collected at each clinic visit (at baseline, 1 month after initiation of therapy, and every 3 months thereafter) and adherence to therapy was assessed at each visit of the patient. Projet RETRO-CI laboratories carried out all laboratory testing. Patients were considered to be following a HAART regimen if they received a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PIs), and dual therapy if they were receiving two NRTIs only.

Patients

Between August 1998 and April 2000, we selected all HIV-1 drug-naive patients who had received ARV drugs for at least 6 months in the UNAIDS–DAI, and then looked for ARV drug resistance among those who had a rebound in plasma viral load. Rebound in viral load was defined as an initial decrease of > 0.5 $\log_{10}$ copies/ml with a subsequent increase of > 0.25 $\log_{10}$ copies/ml compared with prior plasma viral load. To determine whether drug resistance was present at baseline, we sequenced HIV DNA from a random subset of specimens.

Because of the potential effects of receiving no prior ARV on the development of drug resistance, only patients receiving no prior use of ARV were included in this study. Patients enrolled in the UNAIDS–DAI consented to the use of information from their medical chart and samples for surveillance of ARV drug resistance.

Laboratory testing

Blood samples were collected into Vacutainer CPT tubes (Becton Dickinson, San Jose, California, USA) from all patients enrolled in the UNAIDS–DAI. Within 4 h of blood collection, plasma was separated from cells by centrifugation at 200 ***g***, aliquoted, and stored at –70°C. HIV antibody status was determined using an enzyme-linked immunosorbent assay (ELISA)-based parallel testing algorithm [4]. HIV type-specific serodiagnosis was done using a combination of monospecific ELISAs as described previously [5].

CD4+ cell counts were determined by three-color flow cytometric measurements using FACScan (Becton Dickinson) on fresh peripheral whole blood within 4 h of collection. Aliquots of cells were stained with commercially available monoclonal antibodies. The Tritest kit and Multiset software (Becton Dickinson) were used for analysis.

Genotypic resistance

For sequencing of the *pol* gene, we extracted HIV-1 RNA from plasma by the Qiagen method (Qiamp Viral RNA Mini Kit; Qiagen, Valencia, California, USA). The RNA was used in polymerase chain reaction (PCR) amplification of 1.6-kilobase pairs of the *pol* gene by specific primers. We sequenced 200 ng of purified complementary DNA using the TrueGene™ HIV-1 genotyping assay (version 2.5; Visible Genetics, Toronto, Ontario, Canada) [6]. Mutations were classified as either

primarily or secondarily associated with ARV drug resistance, according to the consensus statement on ARV drug-resistance of Stanford HIV Reverse Transcriptase and Protease Sequence Database [7].

Phylogenetic and sequence analysis

Genetic subtypes were determined using phylogenetic tree analysis. The new nucleotide sequences and sequences of reference strains representing different genetic subtypes in the protease and reverse transcriptase genes were aligned using the CLUSTAL W program. Phylogenetic trees were generated using the neighbor joining method, and reliability of branching orders was assessed by bootstrap using the CLUSTAL W program.

Phenotyping

Phenotypic resistance was analyzed using a recombinant virus assay technology (Antivirogram; VIRCO NV, Mechelen, Belgium) as described previously [8,9]. Resistance was expressed as an increase in mean inhibitory concentration [IC_{50} (μM)] of a particular drug when tested with patient-derived recombinant virus isolates, relative to the mean IC_{50} (μM) of the same drug when tested with a reference wild-type virus isolate. Phenotypic resistance to any particular drug was classified as susceptible (< 4-fold reduction), intermediate (4- to 10-fold reduction), or high level resistance (> 10-fold reduction). The drugs tested were NRTIs: zidovudine (ZDV), lamivudine (3TC), stavudine (D4T), didanosine (ddI), zalcitabine (ddC), and abacavir (ABC); NNRTIs: nevirapine (NVP), delavirdine (DLV), and efavirenz (EFV); and PIs: indinavir (IDV), ritonavir (RTV), saquinavir (SQV), and nelfinavir (NFV).

Statistical analysis

We used a logistic regression model to study factors associated with development of ARV resistance. Covariates considered in the model included gender, age < 35 years, base-10 logarithm transformation ($\log_{10}$) of viral load and CD4+ cell count < 50×10^6 cells/l at initiation of ARV therapy, maximal virologic and immunologic response to initiation of ARV therapy, regimen (HAART versus dual therapy two-drug therapy), switch in ARV regimen at any time prior to resistance testing, and missed pills or interruptions in ARV therapy prior to resistance testing. Maximal virologic response was defined as [$\log_{10}$(viral load at ARV initiation) − $\log_{10}$(viral load nadir)] / (days between measurements) × 30.

Results

Characteristics of study population

Of the 241 HIV-1-infected patients who had not been receiving ARV at entry and had received ARV drug therapy for at least 6 months in the UNAIDS–DAI, 97 (40%) had a rebound in plasma viral load. Of the viruses from these 97 patients, six (6%) were negative by PCR testing (median viral load was 3.0 $\log_{10}$ copies/ml), five (5%) had bad quality DNA sequences, and 86 (89%) had analyzable sequences. Thus, for the 86 patients evaluated in this analysis, baseline median [interquartile range (IQR)] age was 38 years (IQR, 31–43), CD4+ cell count was 150×10^6 cells/l (IQR, 66–311), and viral load was 5.0 $\log_{10}$ copies/ml (IQR, 4.0–6.0) (Table 1). Of these patients, 33 (38%) were women and 10% were infected with subtype A viruses, 89% were infected with the HIV-1 A/G-recombinant viruses and 1% was infected with subtype G virus. Bootstrap analysis of reverse transcriptase and protease sequences did not show any distinct clusters of viruses, thus excluding any possibility of contamination.

Table 1. Baseline characteristics of 86 patients with HIV-1 genotypic resistance to antiretroviral drugs.

Variable	
Age (years)	38 (31–43)
CD4+ cell count (× 10^6 cells/l)	150 (66–311)
Viral load ($\log_{10}$ copies/ml)	5.0 (4.0–6.0)
Dual therapy	73 (85)
HAART	13 (15)
NRTI-containing regimens	
ddI	66 (77)
ZDV	64 (74)
D4T	19 (22)
3TC	16 (19)
ddC	4 (5)
NNRTI-containing regimens	
NVP	1 (1)
PI-containing regimens	
IDV	7 (8)
SQV	4 (5)
NFV	2 (2)

Values are median [interquartile range (IQR)] or number (%). HAART, highly active antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ZDV, zidovudine; ddI, didanosine; D4T, stavudine; 3TC, lamivudine; ddC, zalcitabine; NVP, nevirapine; IDV, indinavir; SQV, saquinavir; NFV, nelfinavir.

Of the 86 patients included in this analysis, 73 (84%) had been prescribed two-drug therapy and 13 (15%) patients had been prescribed triple-drug regimens containing PIs or NNRTIs (Table 1). The median duration of therapy was 8 months (IQR, 6–10). Thirteen patients (15%) switched ARV drug regimen. Missed dose was reported by 43 (50%), and 34 (40%) had interrupted therapy for 1 or more days prior to ARV resistance testing. The average total number of pills missed between clinic visits among those 43 patients was six; the average number of days of interrupted ARV therapy between visits among the 34 patients who stopped therapy intermittently was 36. Median reduction in maximal viral load response to

therapy was 1.05 $\log_{10}$ change per 30 days. Median CD4+ cell count increased from ARV initiation was 82 × 10^6 cells/l. Of 144 patients without rebound in viral load, median viral load was 5.45 $\log_{10}$ copies/ml (IQR, 4.6–5.7), with a median reduction in maximal viral load response to therapy of 1.96 $\log_{10}$ change per 30 days. Median CD4+ cell count was 115 × 10^6 cells/l (IQR, 20–303), median age was 37 years (IQR, 31–43) and median duration of therapy was 12.5 months (IQR, 9.5–15), 62.5% were prescribed dual therapy.

Genotypic resistance

Of the 86 patients with rebound in viral load, 68 (79%) had genotypic resistance to at least one reverse transcriptase inhibitor (RTI) or PI.

Resistance to NRTIs

Of the 64 patients receiving ZDV-containing regimens, 50 (78%) of their viruses had primary resistance mutation and of 16 patients receiving 3TC-containing therapy, 11 (68.7%) had viruses with primary resistance mutations. Three patients had the M184V mutation but had received only ZDV + ddI at baseline. The 118I mutation was found in the virus from one patient who was receiving ZDV and 3TC. In another patient who had received ZDV and ddI, we found the 44D and 118I mutations. PCR amplification of RNA for the determination of phenotypic resistance was not successful in plasma of six (7%) of the 86 patients. Of the 80 patients with successful PCR amplicons, viruses from 45 (56%) patients had phenotypic resistance to at least one NRTI. One patient with phenotypic resistance to ddC, D4T, and ABC did not have documented evidence of receiving these drugs prior to phenotypic resistance testing (Fig. 1).

Resistance to NNRTIs

Only one patient had recorded medical history of NNRTI use. Two (2.5%) patients had viruses that were genotypically resistant to NVP. One patient's virus had the G190A mutation, and one had both the G190A and the K103N mutation (Table 2). Of the 80 patients tested, eight (10%) had phenotypic resistance: six with intermediate and two with high levels of resistance to NVP, EFV, and DLV. Interestingly, only one patient had received NVP and none had received EFV and DLV (Fig. 1).

Resistance to protease inhibitors

Of the 86 patients, 13 (15%) had received HAART; 12 of these were on PI-containing regimens. Of the 12 patients receiving PIs, one had viruses with high level of phenotypic resistance with the presence of M46I and L90M mutations. The M36I mutation was the most frequent secondary mutation (Table 2).

Correlation between genotypic, phenotypic resistance and drug used

In the 80 patients who had both phenotypic and genotypic drug resistance, genotypic and phenotypic resistance to NRTI drugs was concordant in 44 (75%) of 59 patients who had phenotypic drug resistance results to ZDV and 14 (93%) of the 15 patients with 3TC phenotypic resistance. None of the four patients with genotypic resistance to DDI had phenotypic resistance. With regard to NNRTIs, genotypic and phenotypic resistance results were concordant in the one patient who received NVP. Interestingly we found seven patients' viruses with genotypic and phenotypic resistance that was not related to the drugs that they had used. Of these seven patients, six were receiving ddI + D4T at baseline and had developed ZDV-specific genotypic resistance mutation. The remaining patient had received ZDV + 3TC and had developed genotypic and phenotypic resistance to RTV, SQV and NFV.

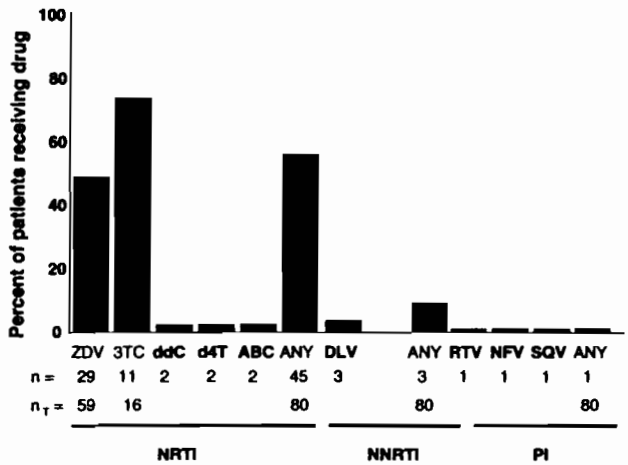

Fig. 1. Prevalence of phenotypic antiretroviral drug resistance among the 80 patients evaluated. White bars indicate the percentage of patients with viruses exhibiting an intermediate 4- to 10-fold reduced susceptibility, and black bars indicate patients whose viruses had high level (> 10-fold) reduced susceptibility to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs). n, number of patient with phenotypic resistant viruses, n_T, total number of patients receiving the drug. ANY, phenotypic resistance to at least one of the drugs within a drug class; ZDV, zidovudine; 3TC, lamivudine; D4T, stavudine; ddI, didanosine; ddC, zalcitabine; ABC, abacavir; NVP, nevirapine; DLV, delavirdine; EFV, efavirenz; IDV, indinavir; RTV, ritonavir; SQV, saquinavir; NFV, nelfinavir. The drugs highlighted in bold are those in which we found phenotypic resistance although the patients were not receiving the drugs and the percentage of resistance was calculated using 80 (the number of sample tested) as denominator.

Cross-resistance patterns

We observed some cross-resistance patterns: for instance, in one patient, ZDV-specific mutations (T215Y, L210W, and K70E) and the 118I mutation were observed. This patient's virus had phenotypic resistance to ZDV

Table 2. Distribution of resistance mutations in the reverse transcriptase and protease region of HIV-1 among 86 patients with rebound in viral load.

Mutations (drugs)	n (%) patients
Primary mutations to NRTIs	
T215Y/F (ZDV)	48 (56)
K70R (ZDV)	30 (35)
M184V (3TC)	13 (15)
K65R (ddI, ddC, ABC)	4 (5)
Q151M (MNR)	2 (2)
V75T (D4T)	1 (1)
44D (3TC)	1 (1)
118RT (3TC)	1 (1)
Secondary mutations to NRTIs	
D67N (ZDV)	30 (35)
L214F (ZDV\3TC)	38 (44)
M41L (ZDV)	11 (13)
L210W (ZDV)	6 (7)
F77L (MNR)	3 (4)
K219E/Q (ZDV)	2 (2)
F116Y (MNR)	2 (2)
Primary mutations to NNRTIs	
G190A (NVP\EFV)	2 (2)
K103N (DLV\NVP\EFV)	1 (1)
Primary mutations to PI	
M46I (IDV)	1 (1)
L90M (SQV)	1 (1)
I84V	1 (1)
Secondary mutations to PI	
M36I (SQV\RTV\IDV\NFV)	50 (58)
L63P (SQV\RTV\IDV)	9 (11)
L10I\R\V (SQV\IDV\APV)	7 (8)
V32I (RTV\IDV)	1 (1)
A71V (SQV\RTV\IDV)	1 (1)

NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors, ZDV, zidovudine; ddI, didanosine; D4T, stavudine; 3TC, lamivudine; ddC, zalcitabine; NVP, nevirapine; IDV, indinavir; SQV, saquinavir; NFV, nelfinavir; DLV, delavirdine; EFV, efavirenz; RTV, ritonavir; MNR, multi nucleoside resistant mutation; APV, amprenavir.

(52-fold reduction), 3TC (17-fold reduction), ddC (8-fold reduction), D4T (15-fold reduction), and ABC (17-fold reduction). The same patient's virus had the I84V, M46I, A71V and L90M mutations with correspondingly high levels of phenotypic resistance to RTV, NFV, and SQV but not to IDV. This patient had received dual therapy (ZDV + 3TC) for 7 months. The viruses from two patients had the Q151M mutation that confers multinucleoside drug resistance in association with the F116Y and F77L mutations. These two patients had been on dual therapy (ZDV + ddI) for 7 and 12 months, respectively.

Table 3. Logistic regression analysis of factors associated with ARV drug resistance among the 86 patients.

Variable	Adjusted odds ratio	95% CI	*P*-value
Male sex	1.5	0.3–7.0	0.62
Age ≥ 35 years	2.3	0.6–9.9	0.25
Use of dual therapy regimens	**9.7**	**1.2–81.6**	**0.04**
Switch in therapy	9.4	0.4–208.4	0.16
Skipped pills	0.7	0.1–6.2	0.72
Interrupt therapy ≥ 1 day	0.3	0.1–2.9	0.29
Baseline viral load (log_{10})	0.6	0.2–2.0	0.38
Maximal viral load response (log_{10} lower)	**2.8**	**1.3–5.9**	**0.006**
Baseline CD4 × cell count < 50 × 10^6 cells/l	1.2	0.3–4.8	0.80

Variables significantly associated with the occurrence of drug resistance are shown in bold. CI, confidence interval.

Drug resistance at baseline

Although all 86 patients were reportedly ARV-naive, we determined whether drug-resistant viruses were present at baseline by sequencing a randomly selected subset of 20 of the viruses from the 86 patients. None of the samples had primary drug-resistance mutations. However, a high prevalence of secondary mutations was observed: 20 (100%) of the 20 viruses had the M36I mutation, 19 (95%) had the K20I/V mutation for protease and 16 (84%) and six (31.6%) had the L214F and the R211K, respectively, for reverse transcriptase.

Factors associated with development of ARV drug resistance

Several factors were evaluated in a logistic regression model to determine their ability to predict the development of ARV drug resistance in patients with rebound in viral load. ARV drug resistance was significantly associated with use of dual therapy regimens) and maximal viral load (log_{10} copies/ml) response to therapy (Table 3). There was insufficient evidence to conclude differences in occurrence of drug resistance by baseline viral load, CD4+ cell count, skipped pills, interrupted therapy, age, and sex.

Discussion

Among the drug-naive patients receiving ARV in the UNAIDS–DAI who had a rebound in viral load, a high proportion (79%) harbored HIV-1 strains that were genotypically resistant, and 61% had strains that were phenotypically resistant to at least one of the drugs they had received. ARV drug resistance in these patients was associated with use of dual ARV and with lower initial viral load response to therapy. Our results are remarkably similar to those reported by Lepri *et al.* [10], who found that 76% of 60 patients with viral load rebound had phenotypic drug resistance. However, our study differs

from that of Lepri and coworkers because all our patients were ARV-naive at baseline, whereas 83% of their patients were ARV-experienced, and genotypic drug resistant testing was not done. Consistent with what others have reported, we observed that exposure and genotypic resistant to ZDV and 3TC were most frequent, whereas the prevalence of ARV drug-resistance mutations to NNRTIs, and PIs were low, likely due to their infrequent use. Similar to the findings of Coakley and coauthors [11], we found that six patients receiving ddI + D4T during a mean duration of 7 months had developed ZDV-specific resistant mutations. One limitation of our study is that we cannot conclusively know that resistant viruses caused rebound in viral load since it cannot be ruled out that the rebound in viral load led to the occurrence of resistant viruses.

Another noteworthy aspect of our study was that 21% of patients with rebound in viral load harbored HIV strains that were phenotypically susceptible to all of the drugs that they had received. Thus, it is possible that viral load rebound in these patients was associated with other factors such as lack of efficacy of the dual ARV with which most patients were treated and had inadequate drug adherence. Indeed, minor differences in adherence have been shown to have a major effect on viral load. For instance, a decrease of 10% in adherence has been associated with a doubling of plasma viral load [12]. Other factors that may influence ARV failure are elevated baseline plasma viral load and low CD4 cell counts of the patients. Indeed, persons seeking care from the UNAIDS–DAI generally had very low CD4 cell counts (median values of less than 150×10^6 cells/l) and high viral loads (median, 5.5 $\log_{10}$ copies/ml) [2]. Alternatively, genotypic resistance could have been present below the threshold of detection in this population; however, the high degree of correlation between genotypic and phenotypic ARV drug resistance results makes this possibility less likely, and none of the 20 samples analyzed for drug resistance at baseline had primary drug-resistant mutations.

The focus of the debate on the use of ARV has shifted from whether it should be used in Africa to whether it will lead to high levels of drug resistance when implemented. Our findings have important implications for this debate.

First, our observations that dual therapy regimens were associated with drug resistance among patients with rebound in viral load suggest that the concerns for occurrence of treatment-induced resistant viruses in Africa may be addressed by using only highly effective ARV.

Second, our results highlight the need to expand access to ARV in a systematic way that will reduce inappropriate prescription and use of ARV. This can be ensured by setting up committees in African countries that provide guidelines for locally implementing, monitoring, and evaluating ARV programmes to ensure that patients are prescribed only highly effective regimens. Use of highly effective regimens is becoming more feasible because drug prices have fallen sharply. These measures may minimize a large-scale epidemic of acquired ARV drug-resistant HIV strains that may prohibit future benefit of ARV.

Third, our results show that in patients for whom ARV therapy fails, the predominant virus population may not be resistant to all components of the regimen; thus, not all drugs in a failing regimen may be lost options. Lastly, in areas such as Africa where ARV drug-resistance testing of patients not responding to therapy is not possible, emphasis should be laid on preventing the occurrence of drug resistance because of the fact that the presence of single drug resistant mutations can result in extensive cross-resistance that limits further therapeutic options. Indeed, in this study we found out that viruses from some patients had genotypic and phenotypic resistance that was not related to the drugs they had used. For instance, six patients were receiving ddI + D4T at baseline and developed ZDV-specific genotypic resistance mutations. Furthermore, in one patient, although only ZDV-specific mutations were observed, the patient's virus had phenotypic resistance to ZDV, 3TC, ddC, D4T, and ABC, consistent with what has been described [13] and termed nucleoside-associated mutations (NAM) [14], which are sets of six mutations in the reverse transcriptase that may confer broader cross-resistance to many nucleoside analogs. These mutations include M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E. In fact the influence of ZDV-resistant mutations has been shown to affect other thymidine analogs such as D4T, ABC, and ddI [15,16].

In summary, we have documented a high prevalence of genotypic and phenotypic drug resistance among patients in the UNAIDS–DAI who have a rebound in plasma viral load after 6 months of therapy. This high prevalence is similar to that reported in industrialized countries. Drug resistance was associated with use of less potent ARV therapy, and insufficient initial decrease in viral load. Our findings highlight the need to implement ARV in Africa in a coordinated fashion such that only highly potent ARVs are accepted practices, with enhanced support for good adherence and uninterrupted stock management.

Acknowledgement

The authors thank Gaston Djomand, Marie-Yolande Borget, Mireille Kalou, Jean-Claude Kadio and Chantal Maurice for technical assistance.

References

1. Palella FJ, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, *et al.* **Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection.** *N Engl J Med* 1998; **338**:853–860.
2. Nkengasong J, Borget M-Y, Maurice C, Boateng E, Kalou M, Djomand G, *et al.* **Distribution of HIV-1 plasma RNA viral load and CD4+ T-cell counts among HIV-infected Africans evaluated for antiretroviral therapy.** *J Acquir Immune Defic Syndr* 2001; **28**:99–101.
3. Djomand G, Roels T, Ellerbrock T, Hanson D, Diomande F, Monga B, *et al.* **Virologic and Immunologic outcomes and programmatic challenges of an antiretroviral treatment pilot project in Abidjan, Côte d'Ivoire.** *AIDS* 2003; **17 (Suppl 3)**:S5–S15.
4. Nkengasong J, Maurice C, Koblavi S, Kalou M, Yavo D, Maran M, *et al.* **Evaluation of HIV serial and a parallel serologic testing algorithms in Abidjan, Cote d'Ivoire.** *AIDS* 1999; **13**:109–117.
5. Nkengasong J, Maurice C, Koblavi S, Kalou M, Bile C, Yavo D. **Field evaluation of a combination of monospecific ELISAs for type-specific diagnsosis of HIV-1 and HIV-2 infections in HIV-seropositive persons in Abidjan, Ivory Coast.** *J Clin Microbiol* 1998; **36**:123–127.
6. Adjé C, Cheingsong R, Roels T, Maurice C, Djomand G, Verbiest W, *et al.* **High prevalence of genotypic and phenotypic HIV-1 drug-resistant strains among patients receiving antiretroviral therapy in Abidjan, Côte d'Ivoire.** *J Acquir Immune Defic Syndr* 2001; **26**:501–506.
7. Stanford HIV RT and Protease sequence database. Available at http://hivdbford.edu/hiv/Notes.pl.
8. Hertogs K, de Béthune MP, Miller V, Ivens T, Schel P, Van Cauwenberge A, *et al.* **A rapid method for simultaneous detection of phenotypic resistance to inhibitors of protease and reverse transcriptase in recombinant human immunodeficiency virus type 1 isolates from patients treated with antiretroviral drugs.** *Antimicrob Agents Chemother* 1998; **42**:269–276.
9. Pauwels R, Hertogs K, Kemp S, *et al.* **Comprehensive HIV drug resistance monitoring using rapid, high-throughput phenotypic and genotypic assays with correlative data analysis [abstract 51].** *Antiviral Ther* 1998; **3 (Suppl 1)**:35–36.
10. Cozzi-Lepri AC, Sabin CA, Staszewski S, Hertogs K, Muller A. **Resistance profiles in patients with viral load rebound on potent antiretroviral therapy.** *J infect Dis* 2000; **181**:1143–1147.
11. Coakley EP, Gillis JM, Hammer SM. **Phenotypic and genotypic resistance patterns of HIV-1 isolates derived from individuals treated with didanosine and stavudine.** *AIDS* 2000; **14**:F9–F15.
12. Bangsberg DR, Hecht FM, Charlebois ED, Zolopa AR, Holodniy M, Sheiner L, *et al.* **Adherence to protease inhibitors, HIV-1 viral load, and development of drug resistance in an indigent population.** *AIDS* 2000; **14**:357–366.
13. Harrigan R, Larder B. **Extent of cross-resistance between agents used to treat HIV type 1 infection in clinically derived isolates.** *Antimircob Agents Chemother* 2002; **46**:909–912.
14. Witcomb JM, Paxinos EE, Huang W, Maranta M, Limoli K, Chappey C, *et al.* **The presence of nucleoside analogue mutations (NAMs) is highly correlated with reduced susceptibility to all NRTI.** *Ninth Conference on Retroviruses and Opportunistic Infections.* Seattle, February 2002 [abstract 569].
15. Pellegrin I, Izopet J, Reynes J, Denayrolles M, Montes B, Pellegrin JL, *et al.* **Emergence of zidovudine and multidrug resistance mutations in the HIV-1 RT gene in therapy naïve patients receiving stavudine plus didanosine combination therapy.** *AIDS* 1999; **13**:1705–1709.
16. Miller V, Ait-khaled M, Stone C, Griffin P, Mesogiti D, Cutrell A, *et al.* **HIV-1 RT genotype and susceptibility to RT inhibitors during abacavir monotherpy and combination therapy.** *AIDS* 2000; **14**:163–171.