The evaluation of the HIV/AIDS Drug Access Initiatives in Côte D'Ivoire, Senegal and Uganda: how access to antiretroviral treatment can become feasible in Africa

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AIDS 2003, **17** (suppl 3):S1–S4

Introduction

Effective antiretroviral treatment of AIDS in resource-limited settings, particularly sub-Saharan Africa, has become a bellwether issue for patients, physicians and policy-makers. The immense tragedy and enormous challenge of global AIDS raises fundamental questions in ethics and human rights accompanied by key logistic and technical issues in medicine, immunology, virology, public health, macroeconomics and social development. The successes of science and public health interventions in wealthy nations must be translated into a sustainable response for the 90% of people with HIV and AIDS in resource-limited settings.

In the first two decades of the HIV epidemic, the parallel efforts of activists, scientists and clinicians resulted in a largely successful paradigm that included prevention of HIV infection and treatment of AIDS. In resource-rich countries these two key modalities reduced the growth of the epidemic and mortality, morbidity and health care costs, the latter most dramatically in the late 1990s with widespread (although not universal) access to highly active antiretroviral therapy (HAART). By contrast, for the vast majority of HIV-infected people that live in sub-Saharan Africa, surveillance, education and information and (potential) vaccines strategies were thought to be the only possible modalities to combat the epidemic. Although the efficacy of antiretroviral therapy was evident from results presented at the 11th World Aids Conference in 1996 in Vancouver, access to antiretroviral treatment (ART) was not considered feasible in developing countries by most experts in the field [1–3].

Despite the powerful reluctance to introduce ART in the South, various non-governmental organizations (NGOs) and some governments of developing countries moved quickly to address access to effective treatments for HIV infection. Responding to their demands, the UNAIDS secretariat launched the so-called Drug Access Initiative (DAI) in November 1997. The DAI was designed to explore the feasibility of a '*structured introduction of price-reduced ARV therapy*' and of a '*rational and affordable use*' of ART '*in a range of developing countries*'. Participants in an initial phase of the DAI were Chile, Côte d'Ivoire, Uganda and Vietnam. In these countries, the explicit goal of the DAI was to set up the necessary infrastructure and systems to increase access to HIV-related drugs '*on a small but sustainable scale*' [4]. In sub-Saharan Africa, the projects respectively started in June 1998 in Côte d'Ivoire, with a population of almost 13 million and over 800 000 people infected with HIV, and in August 1998 in Uganda, with 21 million people of whom 1 million were estimated to be living with HIV/AIDS. In parallel, building upon a successful national prevention campaign, and with an HIV prevalence of less than 2% among adults, the Senegalese government introduced its own pilot Initiative for Access to Antiretroviral Drugs (ISAARV) in 1998.

In the context of global scepticism, very real economic and political barriers surrounded even small-scale programs for access to ART in developing countries. Thus, UNAIDS and the Ministries of Health of Côte d'Ivoire, Senegal and Uganda developed programs to carefully evaluate

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David Katzenstein is a recipient of a Doris Duke Distinguished Clinical Scientist Award which provided support.

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these first experiences of organized ARV delivery in Africa. Independent teams of researchers conducted evaluations of these HIV Drug Access Initiatives between 1998 and 2002, benefiting from the technical assistance of the French Agency for AIDS Research (ANRS) in Senegal, of the Centers of Disease Control and Prevention (CDC) in Uganda, and of both agencies in Côte d'Ivoire.

During this 5-year period, the attention of the world and the public health community has become progressively more focused on drug access and equity in HIV treatment [5–7]. The International AIDS Society (IAS) meetings 'Bridging the Gap' in Geneva in 1998, and 'Breaking the Silence' in Durban in 2000, emphasized the growing inequity in access to care and treatment. These events led to the United Nations General Assembly Special Session on AIDS in 2001, culminating in the establishment of a multi-lateral Global Fund to Fight AIDS, Tuberculosis and Malaria at the beginning of 2002. While it is currently estimated that 4 million people are in immediate medical need of ART in sub-Saharan Africa alone, the goal of scaling up access to ART is increasingly shared by governments and international donor organizations. A recent analysis of the national HIV/AIDS plans of 90 developing countries conducted by WHO indicates that about 60% of these countries have now either incorporated ART into their national strategies to fight the epidemic or have defined specific ART coverage targets [8]. In July 2002 at the 14th International AIDS Conference in Barcelona, WHO and other UN organizations committed themselves to the goal of expanding access to ART to 3 million people in the developing world by 2005 [9]. For this special edition of AIDS, the investigators who led the evaluation of the DAIs in Côte d'Ivoire, Senegal and Uganda provide reports on a wide range of topical issues that inform the feasibility and practicalities of scaling up antiretroviral therapies in resource-limited settings, especially in Africa.

Papers in this issue from project RETRO-CI in Côte d'Ivoire (Djomand *et al.*, Koblavi-Dème *et al.*) and from ISAARV in Senegal (Desclaux *et al.*), as well as previous evidence from the DAI in Uganda [10] and Senegal [11], clearly establish that ART can be successful in Africa. Virologic and immunologic outcomes, adverse events, and estimated survival are similar among patients in African DAIs and ART-treated patients in Europe and the USA. Small pilot studies suggest that sustained viral suppression can also be achieved in HIV-2 infected patients which is still largely confined to Africa and Africans (van der Ende *et al.*; Adjé-Touré, Cheingsong *et al.*). Overall, biological and clinical results strongly suggest that physicians involved in the DAIs have expertise and knowledge about HAART, an observation reinforced by the detailed survey carried out in a sample of Ivoirian physicians by Souville *et al*. These pilot programs also suggest that once financial barriers to access to drugs have been overcome, adherence to HAART can be as high among HIV-infected patients in Africa as that generally observed in industrialized countries (Lanièce *et al.*).

The risk of dissemination of resistant viral strains due to suboptimal anti-HIV drug combinations, inadequate prescription and patients' non-adherence with ARV may mitigate the long-term benefit of expanded ART [12]. Data presented here show rates of phenotypic resistance and corresponding genotypic mutations among ART-treated patients in the Ivoirian (Adjé-Touré, Célestin *et al.*), Senegalese (Vergne *et al.*) and Ugandan (Weidle *et al.*) initiatives that are similar, or in fact lower, than similar studies in the North. Thus, implementation of ART with clinical, biological and logistical monitoring can limit the emergence of drug resistance in Africa. Observations of drug-resistant HIV at levels below those in the US and Europe provide evidence to counter arguments for withholding diffusion of ART in developing countries. The additional concern that access to effective treatment may jeopardize prevention efforts, especially by inducing a 'disinhibition effect' on risk taking [13] is addressed by the evaluation of the DAI in Côte d'Ivoire where access to ARV treatment was not associated with an increase in HIV-related risky sexual behaviours (Moatti *et al.*). However, the follow-up period of these studies is rather short and the possibility that drug resistance and risky behaviours will go up quickly, once ARVs are used on a larger scale, cannot be excluded. There remains a clear need for continuous surveillance of resistance at an international level [14], and for operational research about how to assure life-long adherence to taking ARV drugs and to maintaining preventive behaviours in a developing country setting.

The efficiency of government systems, including national health service systems, has gradually declined over the past decades in many African countries. It is obvious that scaling up access to ART will often take place in a context of limited health care resources with little absorption capacity [15]. The evaluation of the DAIs has, however, demonstrated the feasibility of initiating delivery and monitoring of ART in the context of existing health care infrastructures. An additional common lesson emerges from the evaluations: namely that strong public control and support are essential for a successful diffusion of ART. In the African context of scarce resources and the huge unmet demands for HIV care, efficient programs clearly require the delivery of ARV drugs through organized channels. The observations by Vergne *et al.* and Adjé-Touré, Célestin *et al.* provide evidence that drug resistance rates were higher in Senegal and Côte d'Ivoire before the introduction of organized DAIs. The DAIs were based on the commitment of governments to promote access to ART in the public health sector and to regulate their delivery in the whole health

care system (including the private not-for-profit and private sectors). In other countries, such as Burkina-Faso, whose experience is reported here by Nguyen *et al.*, NGOs played a pioneering role for the introduction of ART to the point that the dynamism of the community response resulted in synergies with the public health care system. Strong public health regulation, clinical education and organization in concert with NGO [8], academic and private partnerships are needed to reduce the risk of 'antiretroviral anarchy' [16,17]. Economic and equity barriers which restrict availability of ARV drugs to the most privileged will only fuel diversion to 'black market' sales, irrational prescribing and increase the risk of drug resistance.

Of course, the African DAIs whose evaluation is reported have been limited in size, concerning a few hundreds or thousands of patients in each of the three countries. Of necessity, these pilot ARV delivery programs were initiated in medical centres restricted to the capitals and the most populated urban areas. The process of scaling up access to ART through delivery by centres at regional level is already ongoing in Senegal and Uganda and, although planned, has been delayed in Côte d'Ivoire due to the recent political situation. Additional challenges will face the extension and 'scaling up' of access to ART in more diverse geographic settings. However, evaluation of these urban pilot projects has identified limitations that must be overcome to promote wider access to ART in both urban and rural settings.

Economic sustainability remains a major problem for ART programs in Africa. The three DAIs benefited from significant price reductions for ARVs and other HIV-related drugs. These price decreases were facilitated by the partnership between five UN organizations (UNAIDS, UNFPA, UNICEF, WHO and World Bank) and six pharmaceutical companies introduced in May 2000 following the DAI pilot projects [18]. However, the world-wide campaign to improve access to essential drugs in poor countries [19,20] and the introduction of generic competition have been the driving forces that have decreased the price of HIV drugs. Between 1996 and 2000, prices of ARVs were lower in Côte d'Ivoire where the Public Health Pharmacy introduced a tender mechanism open to all international suppliers, including generic producers, than in Uganda where procurement was restricted to a private not-for-profit company (Medical Access Uganda Ltd.) which represented the interests of international patent-holding companies. In 2001, the Joint Clinical Research Centre in Kampala imported generic drugs, and there was a 20–45% decrease in the cost of the most frequently prescribed combinations in Uganda [21]. In Côte d'Ivoire, Senegal and Uganda the annual cost of HAART is still higher than the average GDP per capita (US$660, 490 and 310, respectively). Scaling up access to ART will drive further reductions in costs of delivery, further reduce drug prices and identify cheaper techniques for clinical and biological monitoring of therapy in resource-limited settings [22]. The paper by Diomandé *et al.* proposes to identify persons eligible for ART with CD4 cell measures alone, which significantly improves the cost-effectiveness of strategies for initiating treatment. However, the paper by Souville *et al.* suggests that consensus may be difficult to achieve through guidelines that are specific to resource-limited settings. Evolution of flexible and effective guidelines for ARV use and their acceptance will require continuous evaluation and educational efforts by national and international public health authorities.

No matter the resources, ART will remain out of reach for the great majority of the HIV-infected population in Africa. In Uganda, the government considered that it did not have the financial capability to provide any subsidy for covering the costs of ARVs, regardless of private health insurance. In Côte d'Ivoire and Senegal, governments adopted the provision of ARVs at a 50–95% subsidized price for persons who meet predefined socio-economic conditions. The Ivoirian DAI has indeed facilitated access to ART for several hundred patients with limited ability to pay, but the majority of HIV-infected patients seeking care faced persisting socio-economic barriers for access to ARVs and basic prophylaxis of opportunistic infections (Msellati *et al.*). A similar 'mixed' result in terms of equity has also been shown in Senegal [23]. Obviously, constraints on government expenditures will prevent the public and private sector from establishing equitable access to ART in most developing countries. The experience of the DAIs nonetheless suggests that a national consensus can be reached in each country about the priorities for subsidizing costs of treatment. Local experiences show that it is possible to directly involve communities of people living with HIV/AIDS, a process that is under way in Uganda between the government and the community-based organization TASO, and in South Africa in private not-for-profit projects supported by Médecins Sans Frontières and Treatment Action Campaign.

Basic informational barriers continue to restrict access to ART. Evaluation of the DAIs show that more appropriate public information and accessible voluntary testing and counselling (VCT) are needed to define HIV/AIDS as '*a public health and infectious disease emergency*' [24]. Policies and practices around HIV prevention and testing with a focus on treatment include broader training and information about ART for health care professionals, beyond the limited number working in a few specialized centres. Lack of adequate information currently remains a major barrier to equitable access to health care. Scaling up access to ART in Africa will require dissemination of information about these treatments and their availability to the population at large.

Accomplishments in bridging the North/South gap in access to ART have, so far, remained modest. It is estimated

that ART was initiated for an additional 70 000 patients during 2002, leading to only 300 000 HIV-infected individuals in developing countries currently receiving ARVs of any kind, nearly one-half of them in Brazil alone [25]. The first funding commitments by the Global Fund made in 2002 will allow a two-fold increase world-wide in the total number of individuals receiving ART in developing countries, and a six-fold increase in Africa. A recent pledge of 15 billion US$ in bilateral assistance for prevention, care and treatment was made by the US government [26]. These ART programs are not limited to experimental projects. There are recent models which suggest that they can also provide significant public health impact by reducing transmission [27]. Although limited, the experience reported here of the evaluation of the first African DAIs may serve as a foundation for future evaluation efforts that will have to be scaled up in parallel to the increase in size of ARV delivery programs in Africa.

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Virologic and immunologic outcomes and programmatic challenges of an antiretroviral treatment pilot project in Abidjan, Côte d'Ivoire

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Background: In Côte d'Ivoire, a pilot project was developed by UNAIDS and the Ministry of Health to improve access to AIDS care, including antiretroviral therapy, for adults and children infected with HIV. This evaluation of the project is the first to provide results of a large number of HIV-infected patients receiving antiretroviral therapy in West Africa.

Methods: We evaluated records of persons who presented for care from August 1998 to August 2000 at six accredited centers in Abidjan. Patients were treated with two nucleoside reverse transcriptase inhibitors (2NRTI) or highly active antiretroviral therapy (HAART).

Results: Of 2878 patients who were screened, 2351 (83%) were HIV-infected and eligible (CD4 T lymphocyte count $< 500 \times 10^6$ cells/l or plasma HIV-RNA level > 10 000 copies/ml) for antiretroviral therapy. Of those who were eligible, 81% were symptomatic, 63% had a CD4 cell count $< 200 \times 10^6$ cells/l, 12% had previously taken antiretroviral drugs, and 56% returned to the clinic for follow-up. Of the patients screened, 768 (27%) were started on antiretroviral therapy, including 450 on HAART, 296 on 2NRTI, and 22 on other regimens. We analyzed data from 480 HIV-1-infected adults, who were naive to therapy, were prescribed HAART or 2NRTI, and had at least one clinic visit after starting therapy. In an intent-to-treat analysis of patients who received HAART, the estimated plasma HIV-1 RNA level was approximately 1.9 $\log_{10}$ copies/ml (80-fold) lower, while estimated CD4 cell count was $> 100 \times 10^6$ cells/l higher than baseline values, after 1 year of therapy. Approximately 25% of adults on 2NRTI and 50% of those on HAART had < 200 copies/ml, after 1 year of therapy. The probability of an adverse event occurring within 6 months after starting therapy was 0.20. The probability of survival for at least 1 year was 0.84 (95% confidence interval, 0.80–0.89).

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Use of trade names is for identification only and does not imply endorsement by the US Centers for Disease Control and Prevention or the US Department of Health and Human Services.

Presented in part at the XIIIth International AIDS Conference, Durban, South Africa, July 2000.

Sponsorship: Funding for laboratory testing and data management was provided by the Centers for Disease Control and Prevention. Funding for drugs was provided by the Côte d'Ivoire Fonds National de Solidarité and the Fonds de Solidarité Thérapeutique International (FSTI).

Conflict of interest: none

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Conclusion: After starting antiretroviral therapy, these HIV-1-infected patients in West Africa had similar virologic and immunologic outcomes, probability of an adverse event, and estimated survival, as patients enrolled in clinical trials in the USA and Europe. However, only one-third of eligible patients received therapy, highlighting the importance of providing adequate education and support for initiating and adhering to therapy in this and similar programmes.

AIDS 2003, **17** (suppl 3):S5–S15

Keywords: Africa, AIDS, antiretroviral treatment, Côte d'Ivoire, highly active antiretroviral therapy, HIV infection

Introduction

Antiretroviral therapy, especially highly active antiretroviral therapy (HAART), reduces the incidence of opportunistic infections and prolongs survival in persons infected with HIV [1–3]. However, the high cost and complexity of administering antiretroviral drugs have severely limited their use in resource-poor settings where the majority of HIV-infected persons live [4–7]. As a result, little information is available about the use of antiretroviral drugs in less-developed countries, including those in sub-Saharan Africa.

In 1998, UNAIDS implemented an HIV Drug Access Initiative (DAI) in Chile, Côte d'Ivoire, Uganda, and Vietnam in partnership with the Ministry of Health of each country [8]. The DAI was a 2-year pilot project designed to develop model programmes for providing antiretroviral drugs in resource-poor countries. The initiative sought to address the challenges of treating patients with antiretroviral therapy in countries where the healthcare infrastructure, drug distribution networks, and medical training were limited. Consequently, the countries participating in the DAI were required to develop the infrastructure and systems necessary to provide antiretroviral drugs on a small but sustainable scale, with the objective of expanding the programme after effective models were developed.

The DAI in Abidjan, Côte d'Ivoire, which was one of the first antiretroviral treatment programmes in Africa, began in August 1998. For this evaluation, we analyzed information collected from the programme during the period August 1998–August 2000 to examine the screening, evaluation, and follow-up of patients and to determine the impact of antiretroviral therapy on patients enrolled in the programme. This information should be helpful for those planning antiretroviral treatment programmes in other resource-poor settings and evaluating clinical outcomes in similar patient populations.

Methods

Background

UNAIDS and the Côte d'Ivoire Ministry of Health implemented the DAI pilot project during the period August 1998–August 2000. The Côte d'Ivoire government established a US$1 million fund to provide subsidies for antiretroviral drugs. In addition, the Fonds de Solidarité Thérapeutique International established by the French government provided funding for subsidies. An advisory committee of medical experts was created to guide the overall programme design, policy, and medical eligibility criteria for initiation of therapy. In collaboration with UNAIDS, the Ministry of Health selected six treatment centers to participate in the programme, based on the medical expertise, patient load, and geographic location of each center. The Public Health Pharmacy, which supplies drugs for public health institutions in Côte d'Ivoire, managed the antiretroviral drugs for the programme. Furthermore, UNAIDS and the Ministry of Health provided training about antiretroviral therapy for care providers participating in the programme.

To implement the DAI, funds for antiretroviral drugs and laboratory tests for patients participating in the programme had to be identified. In August 1998, the cost of a 30-day supply of antiretroviral drugs in Côte d'Ivoire was approximately US$180 for therapy using two nucleoside reverse transcriptase inhibitors (2NRTI) and US$400 for HAART. HAART is a combination of two or more nucleoside reverse transcriptase inhibitors and at least one protease inhibitor, non-nucleoside reverse transcriptase inhibitor, or abacavir. UNAIDS negotiated with pharmaceutical companies to reduce the costs of most of the drugs. An advisory committee of civic leaders was created to determine who should receive subsidies. Patients paid for their drugs and medical care, but subsidies were available to pay for 50 to 95% of the cost of the drugs. However, in 1998 and early 1999, many of the patients participating in the programme could not afford

HAART, even after receiving the subsidies. As a result, at the beginning of the programme, 2NRTI were prescribed for most of the patients. In late 1999 and 2000, when the cost of antiretroviral drugs dropped sharply, almost all of the new patients and many of those who were started on 2NRTI were prescribed HAART.

Projet RETRO-CI, a collaboration of the Ministry of Health and the US Centers for Disease Control and Prevention (CDC) since 1989, performed and funded the laboratory testing for the programme, including CD4 T lymphocyte counts, plasma HIV-1-RNA levels (viral loads), complete blood counts (hemoglobin, white blood cell count, and platelets), and serum chemistries (serum urea, creatinine, bilirubin, alkaline phosphatase, aspartate transaminase, alanine transaminase), and amylase).

Patient selection

Six treatment centers in Abidjan, the largest city of Côte d'Ivoire, were accredited to provide antiretroviral therapy through the DAI. These centers, which were within 30 min driving from the RETRO-CI laboratory, were the Infectious Diseases Ward, University Hospital of Treichville; the Department of Pediatrics, University Hospital of Yopougon; the Outpatient Tuberculosis Clinic of Adjamé; the Military Hospital, Abobo; the USAC Outpatient Clinic for HIV patients, University Hospital of Treichville; and the Montagnier Foundation Clinic for HIV patients, Treichville. Patients came to these centers on their own initiative or were referred by health care providers to learn about the DAI and to be screened for the programme.

Persons who wished to enroll in the DAI were screened to determine if they were eligible for antiretroviral therapy. Screening at each of the six participating treatment centers included answering questions from a standardized interview form, giving a medical history, undergoing a physical examination, and providing a blood sample to determine HIV antibody status, complete blood count, the serum chemistries listed above, and CD4 cell count. Viral loads were determined for patients who tested HIV positive but had CD4 cell counts $> 500 \times 10^6$ cells/l. Persons were eligible for treatment if they: (1) tested HIV positive; (2) had a CD4 cell count $< 500 \times 10^6$ cells/l or a viral load > 10 000 copies/ml; (3) had hematology and serum chemistry results that were normal or were abnormal and of grade 1 or 2 severity [9]; and (4) did not have an acute medical problem such as active tuberculosis.

If a person was eligible for the programme, a standardized, anonymous summary report was submitted to the advisory committee to determine if the person was eligible for a subsidy to help pay for antiretroviral drugs. Eligibility for a subsidy was based on the summary report and selected socio-demographic characteristics, including age, gender, number of dependent children, and income. If the person could afford to pay for their treatment in full or at a subsidized rate antiretroviral therapy was started.

Following initiation of therapy, patients were scheduled for a clinic visit at 1 month and every 3 months thereafter. At initiation of therapy and at each follow-up visit, a standardized interview form was completed, and a blood sample was drawn for a complete blood count, serum chemistries, CD4 cell count, and viral load. The laboratory results obtained at initiation of therapy were considered the baseline results, which were compared with subsequent results to evaluate the response to therapy. The therapy was modified by clinicians for medical or financial reasons.

Laboratory methods

Laboratory evaluation included tests for HIV serology, complete blood count, selected serum chemistries, CD4 cell count, and viral load. Whole blood was collected from programme participants in ethylenediamine tetraacetic acid (K3) tubes (Becton Dickinson, San Jose, California, USA). Within 4 h of obtaining the blood, plasma was separated and aliquoted. HIV antibody status was determined by use of an enzyme-linked immunosorbent assay (ELISA)-based testing algorithm, and a combination of monospecific ELISAs was used for HIV type-specific serodiagnosis, as described previously [10,11]. Viral load was quantified, using the Amplicor HIV-1 Monitor test version 1.5 (Roche Diagnostics Systems, Branchburg, New Jersey, USA), which accurately quantifies HIV-1 subtype A/G recombinant viruses, the predominant subtypes in Côte d'Ivoire [12,13]. The lower limit of detection of this assay is 200 copies/ml. All assays were performed using the methods recommended by the manufacturers. CD4 cell counts were determined within 4 h of obtaining the blood by use of a FACScan flow cytometer (Becton Dickinson).

Statistical analysis

For this study, adults were defined as those who were 13 years of age or older. The analyses to determine the probability of remaining in care and on antiretroviral therapy, response to therapy, adverse events on therapy, and survival on therapy were restricted to adults who were infected with HIV-1 or both HIV-1 and HIV-2.

The probability of HIV-1-infected adults remaining in care and on antiretroviral therapy was estimated by use of the Kaplan–Meier method and was restricted to patients who were antiretroviral drug naive, which was defined as having never taken antiretroviral drugs prior to enrolling in the programme. The last day of the programme evaluation period was 31 August 2000. Patients who started therapy during the 90 days prior to 31 August 2000, and had not yet returned for a follow-up visit, were excluded from this analysis. Patients were

considered lost to follow-up at the date of their last medical visit if they did not have a visit during the 90 days prior to 31 August 2000. Patients who were not prescribed antiretroviral therapy at their last visit were considered to have stopped therapy on that date. Patients who were prescribed antiretroviral therapy at their last medical visit and those who died were considered remaining in care on the date of their last visit or the date of their death, respectively. The proportions of HIV-1-infected, antiretroviral drug-naive adults who changed from 2NRTI to HAART or from HAART to 2NRTI, were also estimated by use of the Kaplan–Meier method, but this analysis included only those who returned for care after initiation of therapy.

The virologic and immunologic responses to antiretroviral therapy in HIV-1-infected, antiretroviral drug-naive adults were estimated by use of intent-to-treat and as-treated analyses. In the intent-to treat analysis, outcomes of patients were analyzed with the treatment group to which they were originally assigned, whether or not they changed therapy. In the as-treated analyses, all follow-up measurements after a patient changed therapy from 2NRTI to HAART, or from HAART to 2NRTI, were excluded. The intent-to-treat and the as-treated analyses were restricted to patients who had viral load and CD4 cell count measurements at baseline and at least one clinic visit after starting therapy. The effect of therapy on viral load ($\log_{10}$-transformed) and CD4 cell count was assessed by computing the change from respective baseline measurements. The baseline measurement was the test result obtained 60 days before starting therapy that was closest to the day of starting therapy. The percentage of antiretroviral drug-naive adults who had viral loads less than 200 copies/ml after starting therapy was also estimated. Regression estimates were calculated at 30 days after starting therapy, then at 90-day intervals. A non-parametric regression smoothing procedure was used to display graphically the estimated change from baseline viral load and CD4 cell count at times since starting therapy [14]. As independent measurements were used to evaluate the responses to therapy, longitudinal methods were employed to re-evaluate and confirm the robustness of the results of the intent-to-treat and as-treated analyses [15].

Complete blood count and serum chemistries were obtained at the screening exam, the initiation of therapy, and each follow-up visit. Laboratory test results were graded by use of the scoring system developed by the Division of AIDS, National Institute of Allergy and Infectious Diseases [9]. The probabilities of grade 3 or 4 adverse events occurring within 6 months of starting therapy were estimated for HIV-1-infected adults who were antiretroviral drug naive and had laboratory results that were normal or were abnormal and of grade 1 or 2 severity at baseline. As the only information known about when an adverse event occurred was the interval between a normal and an abnormal laboratory measurement, a Kaplan–Meier method with interval censoring was used to estimate the probability of adverse events [16]. Patients with normal or grade 1 or 2 abnormal test results were considered to have remained free of an adverse event at the time of their last laboratory measurement. This analysis examined only the first occurrence of each grade 3 or 4 adverse event for each patient included in the analysis. Furthermore, the occurrences of adverse events for each type of laboratory measurement were studied independent of the adverse events for other types of laboratory measurements.

Kaplan–Meier methods were used to estimate the probability of survival for at least 1 year of HIV-1-infected, antiretroviral drug-naive adults on therapy. For this analysis, the follow-up time for decedents with unknown dates of death and patients who were still living ended on the dates of their last medical visits. A Cox proportional hazards model was used to assess factors associated with survival time [17]. The multivariate Cox model included covariates for type of antiretroviral treatment regimen, history of an AIDS-defining condition prior to starting antiretroviral therapy, year of initiation of antiretroviral therapy, 55 years of age or older, baseline CD4 cell counts of $< 50 \times 10^6$ cells/l and $50–199 \times 10^6$ cells/l with a referent of $> 200 \times 10^6$ cells/l, baseline viral loads of 10 000–99 999 copies/ml and > 100 000 copies/ml with a referent of < 10 000 copies/ml, and baseline hemoglobin < 8 g/dl. Plots of the logarithm of the cumulative hazard function were used to validate the proportionality assumption for each covariate.

Results

During August 1998–August 2000, a total of 2878 persons were screened for the programme. The ages of those screened ranged from less than 1 year to 83 years (Table 1). Approximately two-thirds were married, and 85% had some primary schooling. Almost 40% had no monthly income, and about one-fifth had medical insurance. All but 2% tested positive for HIV. In addition, more than half had a CD4 cell count $< 200 \times 10^6$ cells/l, and almost 80% had HIV-related symptoms.

Of those who were screened, 1584 (55%) returned for a follow-up evaluation, 975 (34%) did not return for follow-up, and 319 (11%) had recently been screened and had not yet returned for follow-up (Fig. 1). Those who did not return for follow-up were not statistically different from those who returned for evaluation in most demographic and economic characteristics examined, including age, sex, marital status, history of having some primary schooling, reporting no monthly income, and having medical insurance (Table 1). However, 96% of those who did not return tested positive for HIV, and

Table 1. Demographic, economic, and medical characteristics at the initial screening of persons who were screened for the programme, who returned for follow-up evaluation, and who did not return for follow-up.

Characteristic	Screened for programme (n = 2878)	Returned for evaluation (n = 1584)	Did not return for follow-up[a] (n = 975)	*P* value[b]
Demographic				
Median age (range) (years)	35 (< 1–83)	35 (< 1–75)	35 (< 1–83)	0.26
Female	1418 (49%)	785 (50%)	460 (47%)	0.24
Single	968 (34%)	526 (33%)	342 (35%)	0.33
Without religion	145 (5%)	75(5%)	53 (5%)	0.43
Without primary schooling	426 (15%)	219 (14%)	135 (14%)	0.93
Economic				
Unemployed	950 (33%)	524 (33%)	206 (31%)	< 0.01
No monthly income	1119 (39%)	621 (39%)	370 (38%)	0.53
Monthly income > US$200	690 (24%)	376 (24%)	243 (25%)	0.50
Has medical insurance	603 (21%)	320 (20%)	223 (23%)	0.11
Shared or substandard housing	820 (28%)	431 (27%)	275 (28%)	0.58
Medical				
Tested positive for HIV	2813 (98%)	1575 (99%)	933 (96%)	< 0.01
Viral load < 10 000 copies/ml[c]	126 (33%)	58 (31%)	57 (36%)	0.36
Median CD4 cell count × 10^6 cells/l[d]	172 (53, 362)	182 (55, 372)	149 (46, 324)	0.04
CD4 cell count < 200 × 10^6 cells/l[e]	1525 (54%)	819 (53%)	543 (57%)	0.06
No HIV-related symptoms	630 (22%)	322 (20%)	240 (25%)	0.01
Prior use of prophylactic drugs[f]	1008 (35%)	556 (35%)	323 (33%)	0.31
No prior antiretroviral drug use	2527 (88%)	1441 (91%)	803 (82%)	< 0.01

[a]This category excludes patients who were screened within 90 days of 31 August 2000.
[b]Pearson chi-square statistic (categorical) and Kruskal–Wallis statistic (continuous) were used to test for differences between patients who returned for post-screening evaluation and patients who did not return for follow-up.
[c]Viral load is plasma HIV-RNA level. At the initial screening, 126 (33%) of 380 persons tested for viral load had < 10 000 copies/ml, whereas 58 (31%) of 187 persons, who returned for evaluation, and 57 (36%) of 160 persons, who did not return for follow-up, had a viral load < 10 000 copies/ml.
[d]Median CD4 cell counts were determined for persons tested for CD4 cell level at the initial screening, including 2813 persons who were screened, 1545 who returned for evaluation, and 954 who did not return for follow-up. The two numbers in parentheses are the 25th and 75th percentiles of the interquartile range of the median CD4 cell count.
[e]At the initial screening, 1525 (54%) of 2813 persons had a CD4 cell count < 200 × 10^6 cells/l, whereas, 819 (53%) of 1545 persons, who returned for evaluation, and 543 (57%) of 954 persons, who did not return for follow-up, had a CD4 cll count < 200 × 10^6 cells/l.
[f]Prior use of prophylactic drugs to prevent opportunistic infections.

approximately 57% had a CD4 cell count < 200 × 10^6 cells/l.

At the follow-up evaluation, patients were informed about their eligibility for antiretroviral therapy. Of the patients who returned for follow-up, 1482 (94%) were eligible for therapy (Fig. 1).

A total of 768 patients initiated antiretroviral therapy, including 757 (51%) of those who were eligible and 11 who were initially ineligible (Fig. 1). Of those who started therapy, 707 (92%) were infected with HIV-1, 19 (2%) were HIV-2 infected, and 42 (5%) were infected with both HIV-1 and HIV-2. At initiation of therapy, approximately 56% of those who were prescribed antiretroviral drugs received a subsidy to pay for medications. Patients who started therapy included 450 who were prescribed HAART, 296 who received 2NRTI, and 22 who were prescribed other regimens. Of those starting therapy, 105 (14%) had been on antiretroviral medications prior to enrollment in the programme. Although we did not systematically gather data from those who did not return to receive therapy, anecdotal information supported the assumption that financial constraint interfered with broader access.

After initiating therapy, 605 (79%) of 768 patients had at least one follow-up clinic visit, 73 (10%) did not return for follow-up, and 90 (12%) had recently started therapy and had not yet returned for follow-up (Fig. 1). The 605 patients who returned after initiating therapy, included 490 HIV-1-infected, antiretroviral drug-naive adults, 79 HIV-1-infected, antiretroviral drug-experienced adults, 20 HIV-infected children, and 16 HIV-2-infected adults. The 490 HIV-1-infected, antiretroviral drug-naive adults had a median of three subsequent clinic visits (range,

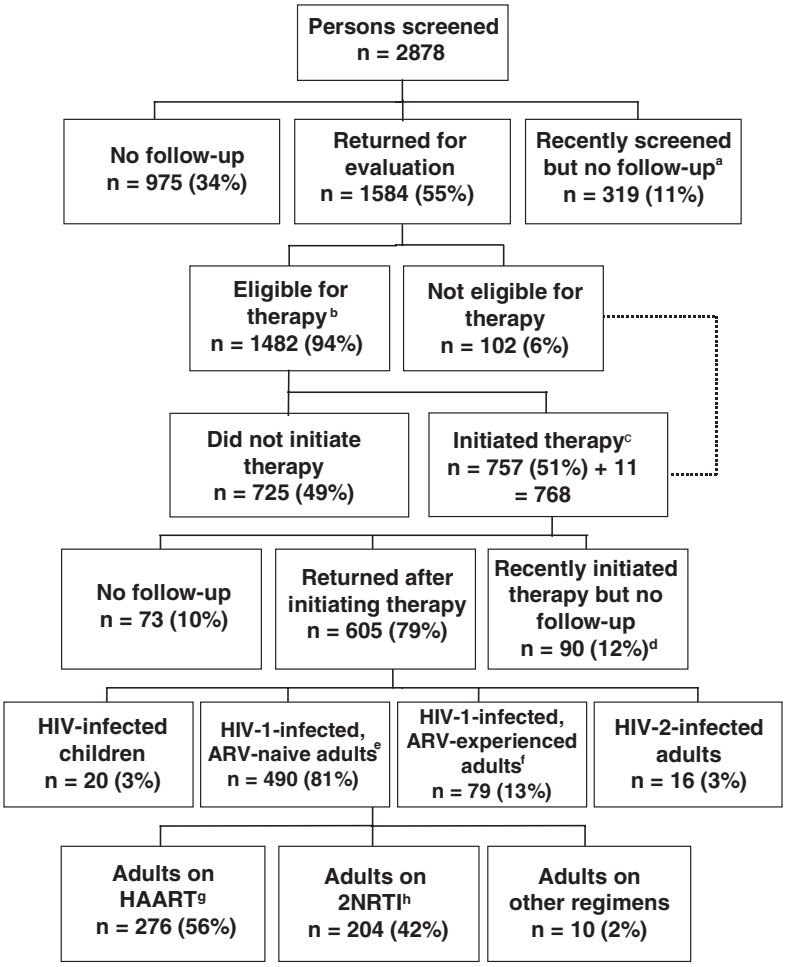

Fig. 1. Persons screened, evaluated, treated, and followed during the programme. [a]This category includes persons who were screened within 90 days of 31 August 2000, but had no subsequent follow-up visit. [b]HIV-infected adults and children were eligible for therapy if they had a CD4 cell count < 500 × 10^6 cells/l or a viral load > 10 000 copies/ml. [c]A total of 768 patients initiated antiretroviral therapy (ARV), including 757 (51%) of those who were eligible and 11 who were initially ineligible. [d]This category includes persons who were started on antiretroviral therapy within 90 days of 31 August 2000, but had no subsequent follow-up visit. [e]HIV-1-infected, ARV-naive adults were defined as those who had not taken antiretroviral drugs prior to enrolling in the programme. [f]HIV-1-infected, antiretroviral-experienced adults were defined as those who had taken antiretroviral drugs prior to enrolling in the programme. [g]HAART is highly active antiretroviral therapy, which is a combination of two or more nucleoside reverse transcriptase inhibitors and at least one protease inhibitor, non-nucleoside reverse transcriptase inhibitor, or abacavir. [h]2NRTI is antiretroviral therapy that includes two nucleoside reverse transcriptase inhibitors.

1–14) during a median follow-up time of 177 days (range, 2–695 days).

Of the 490 adults who were HIV-1-infected, antiretroviral drug naive and returned for care after initiation of therapy, 276 were started on HAART, 204 on 2NRTI, and 10 on other regimens (Fig. 1). Those on HAART received indinavir (136 patients), nelfinavir (65), efavirenz (52), saquinavir (19), abacavir (three), or amprenavir (one), and 2NRTI, including zidovudine/lamivudine (110), lamivudine (92), zidovudine (80), didanosine (62), stavudine (86), and zalcitabine (12). The three most common HAART regimens were indinavir, zidovudine, and lamivudine (38); efavirenz and zidovudine/lamivudine (37); and indinavir and zidovudine/lamivudine (37). Of those who received 2NRTI, 96 were prescribed zidovudine and didanosine; 51, stavudine and didanosine; 18, zidovudine and lamivudine; 11, zidovudine and zalcitabine; nine, zidovudine/lamivudine; and 19, other combinations.

For the 204 patients who began therapy on 2NRTI, the probability of changing to HAART was 0.21 within 6 months and 0.58 within 1 year of starting therapy. Furthermore, for the 276 patients who began therapy on

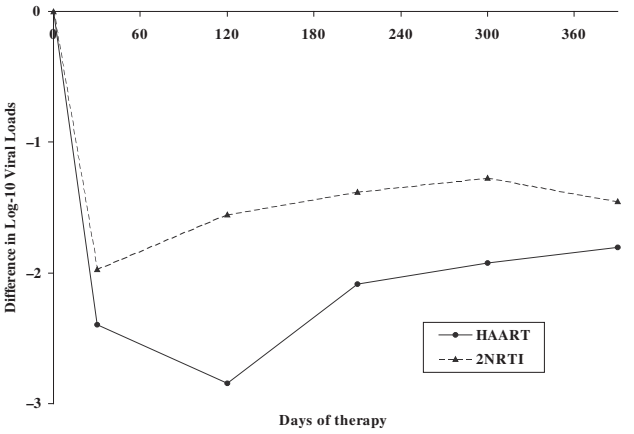

Fig. 2. Estimated differences in $\log_{10}$ viral loads from baseline for adults, who were antiretroviral drug naive, received highly active antiretroviral therapy (HAART) or two nucleoside reverse transcriptase inhibitor (2NRTI), and returned for care after initiation of therapy, using an intent-to-treat analysis[a].

No. of assay measurements at each time interval by type of therapy.

Days	0	1–30	31–120	121–210	211–300	> 300
HAART	236	72	203	107	62	31
2NRTI	166	63	132	115	119	194

[a]The analysis includes 236 (86%) of 276 adults on HAART and 166 (81%) of 204 adults on 2NRTI, respectively, who had viral loads at both baseline and at least one subsequent clinic visit. Regression estimates were calculated at 30 days after initiation of therapy, then at 90-day intervals. The results were similar, using an as-treated analysis.

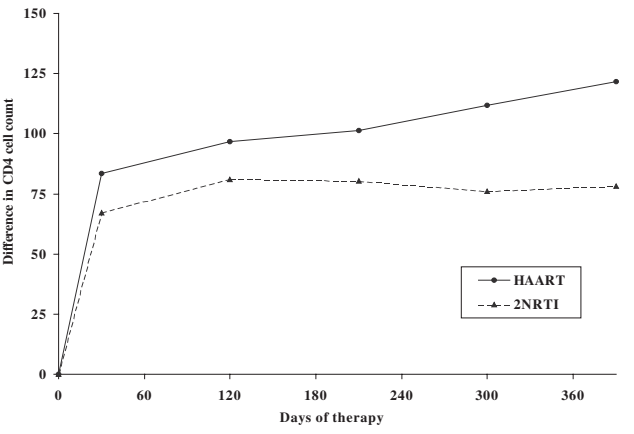

Fig. 3. Estimated differences in CD4 cell counts from baseline for adults, who were antiretroviral drug naive, received highly active antiretroviral therapy (HAART) or two nucleoside reverse transcriptase inhibitor (2NRTI), and returned for care after initiation of therapy, using an intent-to-treat analysis[a].

No. of assay measurements at each time interval by type of therapy.

Days	0	1–30	31–120	121–210	211–300	> 300
HAART	247	76	215	116	65	33
2NRTI	181	66	147	125	131	222

[a]The analysis includes 247 (89%) of 276 adults on HAART and 181 (88%) of 204 adults on 2NRTI, respectively; who had CD4 cell counts at both baseline and at least one subsequent clinic visit. Regression estimates were calculated at 30 days after initiation of therapy, then at 90-day intervals. The results were similar, using an as-treated analysis.

HAART, the probability of changing to 2NRTI was 0.11 within 6 months and 0.20 within 1 year of starting therapy.

Of the 768 patients who initiated therapy, 621 were adults who were infected with HIV-1 or both HIV-1 and HIV-2 and were antiretroviral drug naive. Of these, 74 recently initiated therapy and had not yet returned for follow-up. The probability of the other 547 adults remaining in care and on antiretroviral therapy was 0.76 [95% confidence interval (CI)], 0.72–0.80] at 6 months and 0.62 (95% CI, 0.57–0.68) at 1 year of follow-up.

A total of 480 HIV-1-infected adults were antiretroviral drug naive, received 2NRTI or HAART, and returned for care (Fig. 1). Of these adults, 60% were male, and the median age was 37 years (range: 14–69 years). In addition, 177 (37%) had baseline CD4 cell counts $< 50 \times 10^6$ cells/l; 154 (32%) had 50–199 $\times 10^6$ cells/l; 79 (16%) had 200–349 $\times 10^6$ cells/l; 29 (6%), 350–499 $\times 10^6$ cells/l; and 19 (4%), $\geq 500 \times 10^6$ cells/l, whereas 22 (6%) were missing baseline CD4 cell counts. Furthermore, 33 (7%) of 480 had viral loads < 10 000 copies/ml, 64 (13%) had 10 000–99 999 copies/ml, and 324 (68%), ≥ 100 000 copies/ml, whereas 59 (12%) were missing baseline viral loads. Furthermore, prior to initiation of therapy 285 (59%) had clinical AIDS, as defined by the 1993 CDC Revised Classification System for HIV infection [18].

For the adults who were antiretroviral drug naive, received HAART, and returned for care, the estimated viral load was approximately 1.9 $\log_{10}$ copies/ml (80-fold) lower, whereas the estimated CD4 cell count was $> 100 \times 10^6$ cells/l higher than baseline values after 1 year of therapy, using an intent-to-treat analysis (Fig. 2 and Fig. 3). For the adults who were antiretroviral drug naive, received 2NRTI, and returned for care, the estimated viral load was more than 1.4 $\log_{10}$ copies/ml (25-fold) lower, while the estimated CD4 cell count was $> 70 \times 10^6$ cells/l higher, than baseline values, after 1 year of therapy. Moreover, approximately 25% of adults

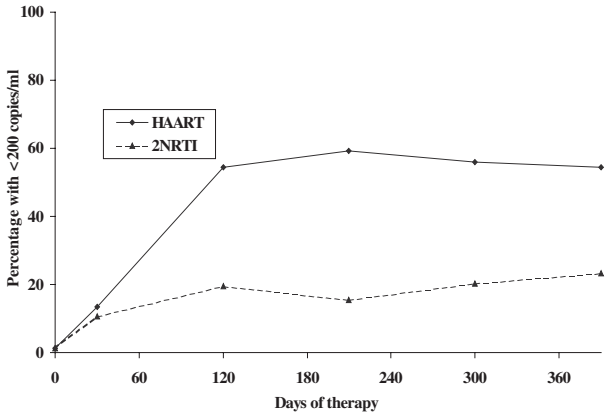

Fig. 4. Percentage of adults who were antiretroviral drug naive, received highly active antiretroviral therapy (HAART) or two nucleoside reverse transcriptase inhibitor (2NRTI), and returned for care, with viral loads < 200 copies/ml after initiation of therapy, using an intent-to-treat analysis[a].

No. of assay measurements at each time interval by type of therapy

Days	0	1–30	31–120	121–210	211–300	> 300
HAART	236	79	222	115	67	35
2NRTI	166	73	153	133	135	229

[a]The analysis includes 256 (93%) of 276 adults on HAART and 192 (94%) of 204 adults on 2NRTI, respectively, who had at least one viral load after initiation of therapy. Regression estimates were calculated at 30 days after initiation of therapy, then at 90-day intervals. The results were similar, using an as-treated analysis.

on 2NRTI and 50% of those on HAART had undetectable viral load (< 200 copies/ml) after 1 year of therapy (Fig. 4). Findings in as-treated analyses, with respect to virologic and immunologic responses to HAART and 2NRTI in our adult population, were consistent with those in intent-to-treat analyses.

Of the 480 HIV-1-infected adults who were antiretroviral drug naive, received 2NRTI or HAART, and returned for follow-up, 359 had hematology and serum chemistry results that were normal or abnormal and of grade 1 or 2 severity at baseline (Table 2). A total of 69 (19%) of 359 patients, as indicated in Table 2 had grade 3 or 4 adverse events during follow-up. The probability of an adverse event occurring within 6 months of starting therapy was 0.20. In addition, 16 patients had more than one type of adverse event.

For the 480 adults who were antiretroviral drug naive, received HAART or 2NRTI, and returned for care, the probability of survival for at least 1 year was 0.84 (95% CI, 0.80–0.89). In a multivariate analysis, factors associated with shortened survival time were baseline CD4 count $< 50 \times 10^6$ cells/l (hazard ratio 5.9; 95% CI, 2.0–17.5) and history of an AIDS-defining condition prior to starting antiretroviral therapy (hazard ratio 3.2; 95% CI, 1.5–7.0). Insufficient data were available to determine survival differences between adults who initiated HAART and those who initiated 2NRTI.

Discussion

The HIV-infected patients enrolled in the DAI pilot project in Abidjan, Côte d'Ivoire had successful virologic and immunologic outcomes, a low probability of adverse events, and a high rate of survival on antiretroviral therapy. After 1 year of HAART, the estimated viral load of adults was approximately 1.9 $\log_{10}$ copies/ml (80-fold) lower, while the estimated CD4 cell count was $> 100 \times 10^6$ cells/l higher than baseline values, and approximately 50% of adults had viral loads < 200 copies/ml. The probability of an adverse event occurring within 6 months after starting therapy was 0.20. The probability of survival for at least 1 year was 0.84 (95% CI, 0.80–0.89).

After 1 year of therapy, the virologic and immunologic changes in adults enrolled in the DAI were remarkably similar to those in patients enrolled in clinical trials in the USA and Europe. For example, in a multicenter, phase 3, randomized, double-blind trial to evaluate antiretroviral equivalence and safety of an abacavir–lamivudine–zidovudine regimen compared with an indinavir–lamivudine–zidovudine regimen, the median decrease in viral load from baseline was 1.96 $\log_{10}$ copies/ml in the abacavir–lamivudine–zidovudine group and 1.84 $\log_{10}$ copies/ml in the indinavir–lamivudine–zidovudine group, while the estimated increase in CD4 cell count from baseline were approximately 145 and 135×10^6 cells/l, respectively, after 48 weeks of therapy [19]. Moreover, the proportion of patients who had a viral load of 400 copies/ml or less at 48 weeks was 51% in both groups. Furthermore, in an analysis of 23 clinical trials conducted in North America, Europe, and Australia during 1994–2000 and involving more than 3200 patients, the mean percentage of patients who had a viral load of 400 copies/ml or less at 48 weeks was 55% (95% CI, 51–58%), whereas the mean increase in CD4 cell count from baseline was 160×10^6 cells/l (95% CI, 146–175) [20].

The proportion of patients enrolled in the DAI who developed laboratory abnormalities on therapy was also similar to that in clinical trials. For example, in the trial of abacavir–lamivudine–zidovudine versus indinavir–lamivudine–zidovudine described above, 16% of the abacavir group and 19% of the indinavir group had laboratory abnormalities of grade 3 or 4 severity, when the results of 11 laboratory tests, including eight of 10 labo-

Table 2. Adverse events after initiation of antiretroviral therapy for 359 HIV-1-infected adults who were antiretroviral drug naive, returned for therapy, and had hematology and serum chemistry results that were normal or were abnormal and of grade 1 or 2 severity at baseline[a].

Abnormal laboratory measurement of grade 3 or 4 severity	No. of patients[b]	No. of patients who had adverse event (%)[c]	Probability of adverse event occurring within 6 months
Hematology			
Hemoglobin < 7 g/dl	356	10 (2.8)	0.029
Neutrophils < 750/μl	356	3 (0.8)	0.005
Platelets < 50 000/μl	355	1 (0.3)	0.003
Renal serum chemistry			
Creatinine > 3.0 × ULN[d]	355	2 (0.6)	0.006
Hepatic serum chemistry			
AST[e] > 5.0 × ULN[d]	356	8 (2.2)	0.023
ALT[f] > 5.0 × ULN[d]	356	4 (1.1)	0.011
Alkaline phosphatase > 5.0 × ULN[d]	354	6 (1.7)	0.017
Bilirubin > 2.5 × ULN[d]	355	24 (6.8)	0.067
Pancreatic serum chemistry			
Amylase > 2.0 × ULN[d]	351	32 (9.1)	0.088
Any adverse event[g]	359	69 (19.2)[h]	0.198

[a]Abnormal hematology and serum chemistry results of grade 1 or 2 severity included those which were less than grade 3 or 4 severity at the initial screening, as defined in the first column of the table.
[b]This category includes the number of patients who had the laboratory measurement prior to initiation of therapy and at one or more times after therapy was prescribed.
[c]This percentage is calculated by dividing the number of patients who had the adverse event by the number of patients who had the laboratory measurement prior to initiation of therapy and at one or more times after therapy was prescribed.
[d]The manufacturer's ULN (upper limit of normal) values are the following: creatinine, 1.25 mg/dl (males), 1.1 mg/dl (females); AST, 34 IU/l (males), 31 IU/l (females); ALT, 43 IU/l (males), 36 IU/l (females); alkaline phosphatase, 110 IU/l; bilirubin, 1.0 mg/dl; amylase, 108 IU/l.
[e]AST indicates aspartate transaminase.
[f]ALT indicates alanine transaminase.
[g]Patients, who had any grade 3 or 4 adverse event during follow-up, were included in this category.
[h]Since 16 patients had more than one type of abnormal laboratory measurement of grade 3 or 4 severity, the number of adverse events is greater than the number of patients who had any adverse event during follow-up.

ratory tests monitored in the DAI, were examined. Moreover, in the INCAS Trial, which was a double-blind, controlled, randomized trial comparing zidovudine–nevirapine plus didanosine placebo, zidovudine–didanosine plus nevirapine placebo, and zidovudine–didanosine–nevirapine, 27% of patients treated with nevirapine developed at least one laboratory abnormality of grade 3 or 4 severity, compared with 11% of patients treated with zidovudine–didanosine [21].

To implement this project, UNAIDS, the Côte d'Ivoire Ministry of Health, and the project staff had to overcome several important programmatic challenges. The most difficult challenge was the need to develop a clear political and financial commitment by the national government, the local medical community, donor organizations, and the pharmaceutical industry [6]. After the government decided that the DAI should be implemented in Côte d'Ivoire, funds were identified, and agreements were negotiated with pharmaceutical companies. To address the second major challenge, Projet RETRO-CI agreed to fund and perform the laboratory tests for the project, which required substantial commitment of laboratory capacity, especially equipment and trained personnel. The third challenge was the enrollment and follow-up of HIV-infected patients in the programme. The large number of persons lost to follow-up at each stage of the programme demonstrates how difficult this challenge was. Of 2878 persons who were screened, only 605 (21%) initiated therapy and returned for care.

The results of this evaluation identified successes as well as opportunities for improving programmes in the future. Increasing community awareness, accessing patients earlier in the course of their disease, and improving patient education and adherence would greatly improve programme efficiency and the likelihood of success. Furthermore, increased attention to prevention as a component of comprehensive HIV care needs more attention to prevent secondary transmission, loss of efficacy of prevention messages, and transmission of drug resistance in the community.

Each of the analyses had limitations. For example, in the analysis of the proportion of patients remaining in care, we enumerated how many persons were lost to follow-up, but could not determine why patients did not

return. The analysis of the response to therapy may have been limited by differences in frequency and timing of testing and by the number of laboratory measurements available at 1 or more years. In the intent-to-treat analysis, the crossover of the majority of 2NRTI patients to HAART regimens may have contributed to a greater clinical benefit than was observed among those who started with a 2NRTI regimen but who did cross over to a HAART regimen. In the evaluation of adverse events, we may have underestimated the probability of occurrence because of the lack of information about clinical symptoms of drug toxicity, as well as the exclusion of patients with severe laboratory abnormalities at baseline from the analysis. Finally, in the survival analysis, we may have underestimated mortality because data about vital status were incomplete and patients lost to follow-up may have died. In addition, differences in length of follow-up may have biased our assessment of factors associated with survival time.

The principal lesson of this project is that antiretroviral treatment programmes in Africa can successfully treat large numbers of HIV-infected patients. Several African countries are exploring the possibility of establishing large-scale HIV treatment and care programmes [22,23]. The cost of antiretroviral drugs has dropped sharply, and the production of generic drugs will probably reduce the cost even further [24]. In April 2002, the Global Fund to Fight AIDS, Tuberculosis, and Malaria awarded US$378 million for 40 programmes in 31 countries and plans to approve an additional US$238, raising the expected total of grants to $616 million over the next 2 years [25]. Furthermore, in April 2002, the World Health Organization published guidelines for scaling up antiretroviral therapy in resource-limited settings, which recommends standardization and simplification of antiretroviral regimens, as well as minimal laboratory tests to reduce the costs and complexity of antiretroviral therapy [26]. The success of the DAI pilot programme in Côte d'Ivoire and these recent developments to make antiretroviral therapy more readily available in resource-limited settings suggest that it might be possible to implement and expand national programmes in the near future.

Acknowledgements

The authors would like to thank staff of the UNAIDS/WHO country team: Programme National de Lutte Contre le SIDA; Services des Maladies Infectieuses et Tropicales, CHU de Treichville; Unite de Soins Ambulatoires et de Conseils; Montagnier Foundation CIRBA Clinic; Service de Pediatrie, CHU de Yopougon; Hôpital Militaire d'Abobo; Centres Antituberculeux d'Adjamé; Pharmacie de la Santé Publique; Lumière Action Association of Persons Living with AIDS; Institut de Recherche et Dévelopment (IRD); Agence Nationale de Recherche sur le SIDA (ANRS); the US Embassy of Côte d'Ivoire; and Projet RETRO-CI. They would also like to thank the study participants and their families.

Author contributions: G.D., T.R., T.E., D.H., F.D., J.N., A.K., S.W., E.L., J.S. and T.C. contributed to the concept for the study. G.D., T.R., D.H., F.D., B.M., C.M., J.N., R.K.-K., A.K., S.W., E.L., J.S. and T.C. helped to design the study. G.D., T.R., T.E., D.H., F.D., B.M., C.M., J.N., E.L. and T.C. contributed to the analysis. All of the authors participated in writing and revising the manuscript.

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Changes in levels of immune activation and reconstitution markers among HIV-1-infected Africans receiving antiretroviral therapy

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Objective: To describe changes in immune activation and reconstitution markers among HIV-1-infected patients receiving antiretroviral therapy (ART) in Abidjan, Côte d'Ivoire.

Methods: Between November 1998 and February 2001, we analyzed changes in immune activation and reconstitution markers among 52 patients. Good virologic responders (n = 26) were defined as those who had suppressed and maintained plasma viral load (VL) below the detection limit of the assay for at least 12 months. Poor virologic responders (n = 26) were defined as those with a detectable VL at 6 and 12 months after beginning ART.

Results: Of the 26 good virologic responders, 20 (77%) were on highly active antiretroviral therapy (HAART) compared with one (4%) of the poor responders. Among the 26 good responders, baseline median levels of CD38+CD8+ T cells were elevated, but had decreased significantly at 6 months ($P < 0.001$) and at 12 months of therapy ($P < 0.001$). Median levels of HLA-DR+CD8+ T cells also decreased from baseline at 6 months ($P < 0.001$) and at 12 months of therapy ($P < 0.001$). Levels of CD62L+CD4+ T cells increased steadily during the 6 and 12 months of therapy and reached levels observed among HIV-negative blood donors ($P = 0.07$). Among the 26 poor responders, median levels of CD38+CD8+ T cells decreased significantly at 12 months of therapy ($P = 0.006$), but were higher than levels in blood donors ($P = 0.005$). Levels of HLA-DR+CD8+ T cells decreased significantly at 12 months of therapy ($P < 0.001$). Levels of CD62L+CD4+ decreased over time.

Conclusion: Our results suggest that HAART can be successfully used in African populations with elevated baseline immune activation markers.

AIDS 2003, **17** (suppl 3):S17–S22

Keywords: Antiretroviral therapy, CD62L, CD38, HIV-1, HLA-DR, immune activation, immune reconstitution, viral load

Introduction

Highly active antiretroviral therapy (HAART) suppresses viral replication, increases CD4+ T cells, and reduces mortality rates [1–3]. Because of the cost of HAART and complexity of administration and laboratory monitoring, it has not been widely used in Africa. However, due to international efforts and the drastic reduction of

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prices of antiretroviral therapy (ART) drugs, several countries in Africa have started pilot programmes to treat HIV-infected persons. Effective use of antiretroviral (ARV) drugs requires patient monitoring for CD4+ T-cell count, plasma RNA viral load, and ARV resistance testing. In Africa, it will be necessary to evaluate various biologic factors that may influence virologic response to therapy. A study conducted in Italy has shown that high pre-treatment levels of markers of immune activation, such as levels of expression of CD38+ on CD8+ T cells, predict maintenance of high viremia in HIV-1-infected patients receiving HAART [4]. Immune activation markers are prognostic markers of HIV disease progression prior to HAART or effective antiretroviral treatment [5–7] suggesting that activation may strongly correlate with HIV-RNA viral load. For instance, one study found that a 10% increase in levels of CD38+CD8+ T cells resulted in an 88% increase in the risk for AIDS [6]. Compared with persons in Europe and United States, HIV-infected and uninfected Africans have three- to four-fold higher levels of immune activation markers such as expression of CD38+ and HLA-DR on CD8+ T cells [8–11]. To date, no studies have been published examining immune activation and reconstitution markers among patients receiving ARV drugs in Africa. Understanding the interaction of these markers and antiretroviral therapy may provide valuable information for successful use of ART in Africa. In this study, we compared changes in markers of immune activation and reconstitution among HIV-1-infected patients receiving HAART or dual nucleoside therapy which resulted in good and poor virologic responses, respectively in Abidjan, Côte d'Ivoire.

Methods

Study population

Patients were recruited from the UNAIDS–Drug Access Initiative (UNAIDS-DAI) that started in Côte d'Ivoire in 1998 to expand patient's access to comprehensive HIV care including subsidized HAART. Details of how the DAI was set up, and the clinical, virologic and immunologic outcomes of patients enrolled and followed up have been described in detailed elsewhere [12]. In brief, our experience with the DAI shows that, after starting antiretroviral therapy, HIV-1-infected patients in Côte d'Ivoire had similar virologic and immunologic outcomes, probability of an adverse event, and estimated survival, as patients enrolled in clinical trials in the USA and Europe. At the start of the initiative, we systematically measured the levels of immune activation and reconstitution markers among 159 patients; however, the data presented in this study is a sub-analysis, which includes only a proportion of the 159 patients who had information on markers of immune activation and reconstitution at three time points. Thus, of the 159 patients, 52 had data on markers of immune activation and reconstitution at baseline, at 6 months and at 12 months of follow-up. We selected two groups of these patients: good virologic responders were defined as patients who had maintained viral load below the detection limit of the assay (< 200 copies/ml) at 6 months of therapy and had maintained viral load below detection limit at 12 months of follow-up. Poor virologic responders were defined as patients who had detectable viral loads (> 200 copies/ml) at 6 and 12 months. For patients in each group, we analyzed changes in CD4+ T cells, immune activation, and reconstitution markers at baseline, 6 months, and 12 months.

As a comparison group for normal levels of immune activation and reconstitution markers among HIV-uninfected Africans, we included 19 HIV-negative blood donors who were recruited at the National Blood Transfusion Center in Abidjan, Côte d'Ivoire. This study received approval from the CDC Institutional Review Board and the ethical committee of the Ministry of Health of Côte d'Ivoire.

Laboratory testing

Whole blood was collected from participants into ethylenediamine tetraacetic acid tubes (Becton Dickinson, San Jose, California, USA). Within 4 h, plasma was separated from cells by centrifugation at 200 ***g***, and then aliquoted and stored at –70°C.

We determined HIV-1 antibody status using an enzyme-linked immunosorbent assay (ELISA)-based testing parallel algorithm [13]. For HIV type-specific serodiagnosis, we used a combination of monospecific ELISAs [14]. HIV-1-RNA viral load in plasma was quantified by Amplicor HIV-1 Monitor Assay, version 1.5 (Roche Diagnostics Systems, Branchburg, New Jersey, USA). The assay's limit of detection was 200 copies/ml.

The CD4+ and CD8+ cell counts were determined by three-color flow cytometry using FACScan (Becton Dickinson). The Tritest kit and Multiset software (Becton Dickinson) were used for labeling and analysis. The following markers were analyzed on the surface of T cells: CD38 and HLA-DR on CD8+ T cells (CD38+CD8+ and HLA-DR+CD8+), as markers of immune activation [15], and CD62L+ CD4+ T cells were used as proxy markers of immune reconstitution. These cells correlate with CD45RA+CD62L+ T cells, which are direct markers of immune reconstitution [16–18]. CD38 is re-expressed on primed cells upon activation in HIV-infected persons, and its expression on CD8+ T cells increases significantly with disease progression. HLA-DR is a MHC class II antigen that is expressed on activated T cells. In HIV-positive persons, the expression of HLA-DR is significantly increased both on CD4+ and on CD8+ T cells [5].

Analysis of data

Immune activation markers were analyzed as a percentage of the major lymphocyte subsets (CD4+ and CD8+

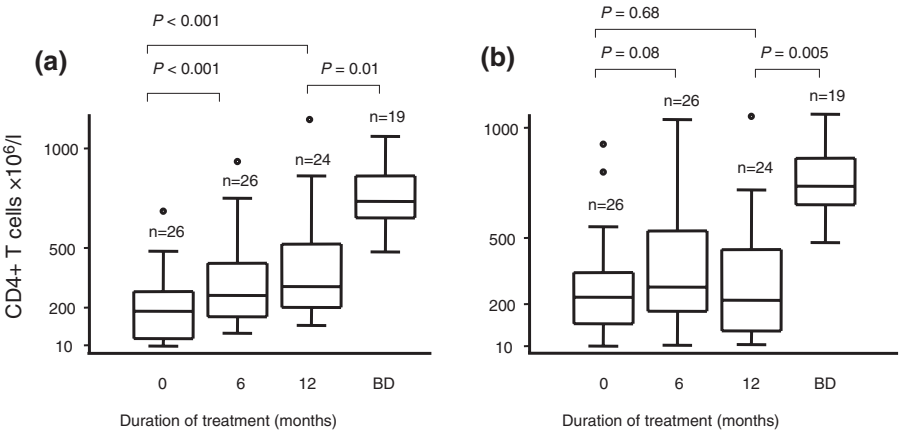

Fig. 1. Comparison of changes in median CD4+ T cell counts among good (a) and poor (b) virologic responders. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles). Dark dots represent outlier values; n, number of patients tested; BD; HIV-negative blood donors used as controls.

T cells) stained with a combination of monoclonal antibodies. Data were summarized with medians and interquartile ranges (IQR). Using the non-parametric Wilcoxon signed-rank test for paired data, median of the differences between 6, 12 months and baseline in levels of expression of immune activation and reconstitution markers were compared between good and poor virologic responders. Statistical analysis was carried out with the software STATA, version 7 (Stata Corporation, College Station, Texas, USA). All statistical tests were two-sided tests with a significance level of 0.05 and adjusted *P*-values were determined using the Horn procedure.

Results

Characteristics of the study participants

Of the 52 patients receiving ART, for whom we had information on immune activation and reconstitution markers, 26 were categorized as good responders and 26 as poor responders. Median (IQR) age for the different groups were 36 years (32–41) for good responders, 34 years (30–42) for poor responders, and 24 years (21–26) for blood donors. Median CD4+ T-cell counts at baseline were 182×10^6 cells/l (44–280) among the good responders, 231×10^6 cells/l (111–343) for poor responders ($P = 0.2$), and 734×10^6 cells/l (651–862) for HIV-negative blood donors. Plasma RNA viral load at baseline was similar for good and poor responders [5.1 $\log_{10}$ copies/ml (4.2–5.4) versus 5.2 $\log_{10}$ copies/ml (4.7–5.6) ($P = 0.56$)]. Good responders maintained viral load below the detection limit for at least 12 months. In contrast, among poor responders, median viral load was 4.2 $\log_{10}$ copies/ml after 6 months and 4.9 $\log_{10}$ copies/ml after12 months of therapy. Twenty (77%) of the 26 good responders were receiving HAART and six patients were receiving dual therapy (five patients received zidovudine + didanosine and one received didanosine + stavudine). Of the 26 poor responders one (4%) was prescribed HAART and 25 (96%) were receiving non-suppressive dual therapy: 12 received zidovudine + didanosine, five patients received zidovudine + zalcitabine, five received didanosine + stavudine, and three received zidovudine + lamivudine. At baseline, no opportunistic infections were observed for good and poor responders. After 12 months of therapy, opportunistic infections were reported for 8.3% of good responders and 8.7% of poor responders.

Changes among good virologic responders

In the good-responder group, median levels of CD4+ T-cell counts increased steadily from 182 cells $\times 10^6$ cells/l at baseline to 261×10^6 cells/l at 6 months ($P < 0.001$) and to 306×10^6 cells/l after 12 months of therapy ($P < 0.001$) (Fig. 1a). At baseline, median levels of expression of CD38+CD8+ T cells were elevated [93%; IQR (90–98%)], but they then decreased to 78% (69–88%) after 6 months of therapy ($P < 0.001$), and to 76% (70–84%) after 12 months of therapy ($P < 0.001$) (Fig. 2a). Levels of expression of CD38+CD8+ T cells at 12 months were comparable with those observed among HIV-negative blood donors ($P = 0.9$) (Fig. 2a). Median levels of expression of HLA-DR+ CD8+ T cells were also elevated at baseline [53% (37–66%)], then decreased to 33% (22–44%) after 6 months of therapy ($P < 0.001$), and to 28% (15–42%) after 12 months of therapy ($P < 0.001$)(Fig. 2b). Median levels of expression of HLA-DR on CD8+ T cells after 12 months of therapy were comparable to those observed among blood donors ($P = 0.5$) (Fig. 2b).

Median levels of expression of the immune reconstitution marker, CD62L on CD4+ T cells, rose steadily from baseline [57% (46–76%)] to 12 months [68% (56–78%)] of therapy ($P = 0.03$) (Fig. 3a).

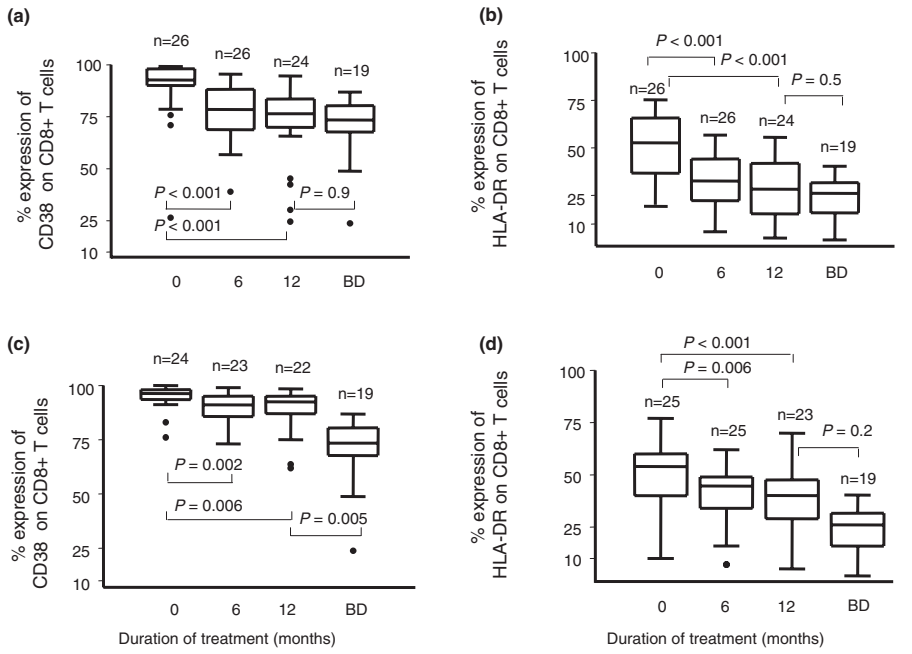

Fig. 2. Comparison of changes in median percentage of immune activation markers CD38 and HLA-DR on CD8+ T-cells among good (a, b) and poor (c, d) virologic responders. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles). Dark dots represent outlier values; n, number of patients tested; BD; HIV-negative blood donors used as controls.

Changes among poor virologic responders

In the poor-responder group, CD4+T-cell levels after 12 months of therapy [218 × 10^6 cells/l (79–448)] were similar to baseline values [231 × 10^6 cells/l (111–343)] ($P = 0.68$) (Fig. 1b). Levels of expression of CD38+CD8+ T cells decreased significantly from baseline after 6 ($P = 0.002$) and 12 months of ART ($P = 0.006$) (Fig. 2c). However, although median levels of CD38+CD8+ T cells had decreased to 92% (87–95%) after 12 months of ART, they remained significantly higher than levels observed among HIV-negative blood donors ($P = 0.005$) (Fig. 2c). Median levels of expression of HLA-DR+ CD8+ T cells also declined significantly at 6 and 12 months with respect to baseline values ($P = 0.006$ and $P < 0.001$, respectively) (Fig. 2d) but remained higher than levels observed among the blood donors.

Although median levels of expression of CD38 and HLA-DR on CD8+ T cells decreased significantly at 12 months compared with baseline values, the decrease was less profound among poor responders than among good responders. For instance, CD38+CD8+ T cells decreased by –17.4% among good responders compared with –4.0% among poor responders ($P = 0.01$), and levels of HLA-DR+CD8+ T cells decreased by –22% among good responders compared to –11% for poor responders ($P = 0.02$). Among poor responders, median levels of CD62L on CD4+ T cells decreased rather than increased over time, although this decrease was not significant ($P = 0.21$) (Fig. 3b).

Discussion

Our results indicate that with successful viral suppression in HIV-infected West Africans receiving HAART, elevated baseline levels of markers of immune activation (CD38 and HLA-DR on CD8+ T cells) decreased steadily. During a 12-month period, these values decreased to those observed among HIV-negative individuals. Immune reconstitution increased steadily among only those patients with good virologic responses. Our data confirm earlier reports from developed countries where levels of immune activation are several-fold lower than those observed in African populations [10,11]. In a French study, Bouscarat *et al.* [19] observed significant decreases for levels of CD8+CD38+ and CD8+HLA-DR+ T cells only among 14 patients who had sustained suppression in plasma RNA viral load. Autran and colleagues [20] also observed a decrease both in CD38+ and in HLA-DR+CD8+ T cells after triple-drug ART. To our knowledge, this is the first report on changes in immune activation and immune reconstitution markers among patients receiving ART in Africa.

Several aspects of our results should be highlighted: Firstly, in developed countries, high levels of immune activation, such as increased CD8+CD38+ T cells, are known to predict a decrease in CD4+ T cells and the concomitant progression of disease in HIV-1-infected persons [19,21]. Furthermore, immune activation is usually directly correlated with viral replication [6,22].

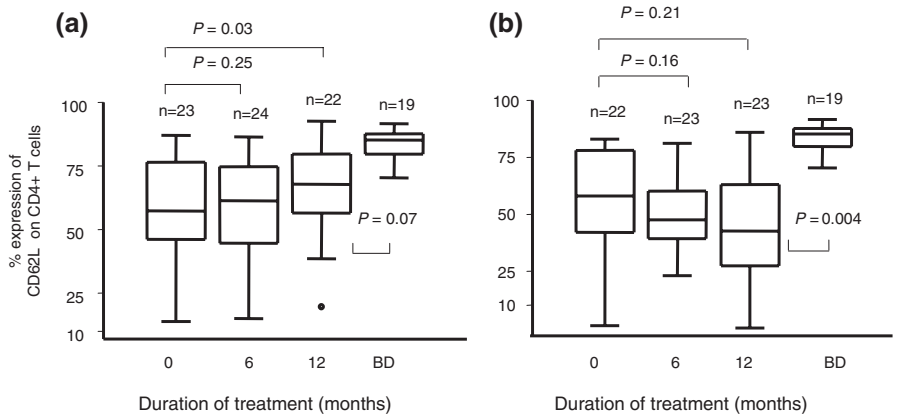

Fig. 3. Comparison of changes in median percentage of reconstitution markers CD62L on CD4+ T cells among good (a) and poor (b) virologic responders. Horizontal lines are medians and interquartile ranges (25th and 75th percentiles). Dark dots represent outlier values; n, are number of patients tested; BD; HIV-negative blood donors used as controls.

Thus, ascertaining the extent to which levels of immune activation decrease during ART in Africa may provide some insight into the risk for therapeutic failure and disease progression among HIV-infected Africans receiving ART. Indeed, Viganò and colleagues [4] have shown that persistence of viremia in HAART-treated individuals is associated with higher pretreatment levels of CD38+ CD8+ T cells; they have suggested that CD38 on CD8+ T cells should be analyzed in all HIV-infected patients receiving HAART and particular attention given to those in whom high levels are detected. However, our findings suggest that a reduction in the levels of immune activation depends on the successful suppression of viremia and may represent an affordable surrogate marker for the expensive viral load testing during treatment in resource-poor settings.

Secondly, profound changes in immune activation occurred and normalized at 12 months of therapy, predominantly among patients who were receiving HAART and had suppressed and sustained viral replication. This finding supports the concept that immune activation is antigen-driven and may represent an ongoing immune response to continuous HIV production [21,22]. Moreover, the low prevalence of opportunistic infections among our patients suggests that HIV replication was the principal cause of immune activation. Thirdly, the fact that viral load was not suppressed among patients who were prescribed regimens other than HAART underscores the limitations of using other regimens in African populations.

Finally, among good responders, levels of immune reconstitution markers increased steadily, through 12 months of therapy. This observation suggests that the high immune activation background that is characteristic of HIV-infected Africans may not affect immune reconstitution if appropriate ARV drugs are used.

In summary, despite having initially elevated levels of markers of immune activation (CD38 and HLA-DR on CD8+ T cells), Africans receiving HAART for 12 months and having sustained viral suppression appear to have steady decreases in levels of these markers. Levels declined to values observed among HIV-negative persons. However, immune reconstitution appears to occur steadily only among those patients who have good virologic responses. Our results do not support the possibility that HIV-infected Africans receiving ART may still be at greater risk for therapeutic failure and disease progression due to elevated levels of immune activation [4], but rather they do support the possibility that levels of immune activation simply reflect the suppression of HIV viremia with successful ART.

Acknowledgements

The authors thank Tossou Odette, Kadjo Jean Claude, Moïse Kouassi, and Pascal Ebby for data support and analysis. They also thank Dr Konaté Seydou and his staff at the National Transfusion Center for providing the blood donor samples used as control group.

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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

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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

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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 socio-demographic 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 ($\times 10^6$ 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.

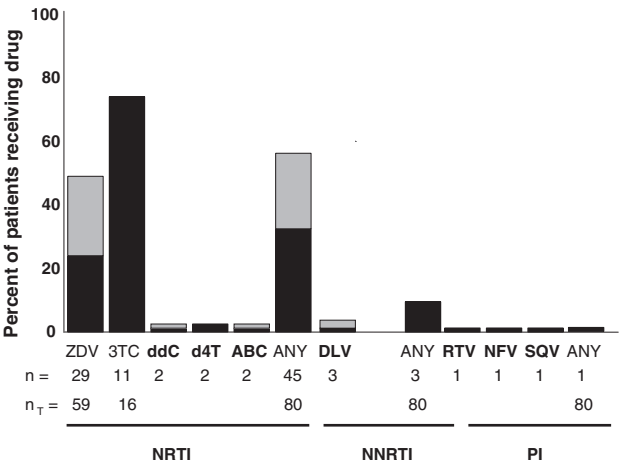

Fig. 1. Prevalence of phenotypic antiretroviral drug resistance among the 80 patients evaluated. White bars indicate the percentage of patients with viruses exhibiting an intermediate 4- to 10-fold reduced susceptibility, and black bars indicate patients whose viruses had high level (> 10-fold) reduced 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.

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Low rate of genotypic HIV-1 drug-resistant strains in the Senegalese government initiative of access to antiretroviral therapy

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Objective: To monitor the prevalence of antiretroviral (ARV)-resistant HIV-1 viruses, and the genotypic mutations in patients enrolled in the Senegalese initiative for access to antiretroviral treatment (ART).

Methods: A total of 80 patients with a virological follow-up of at least 6 months were selected, 68 were ART-naive and 12 ART-experienced. Genotypic resistance to ARV was studied at baseline for a random subset of patients and at each rebound in plasma viral load during ART, by sequencing the protease and reverse transcriptase genes.

Results: At baseline, 66 patients received highly active antiretroviral therapy (HAART) [2 nucleoside reverse transcriptase inhibitors (NRTIs) +1 protease inhibitor (PI) (n = 64) or 2 NRTIs + 1 non-nucleoside reverse transcriptase inhibitor (NNRTI) (n = 2)] and 14 patients (17.5%) started with a dual therapy because of ongoing anti-tubercular therapy or efficient previous bitherapy for the ART-experienced patients. The emergence of drug-resistant viruses (n = 13) during follow-up was more frequent in ART-experienced patients than in ART-naive patients, 41.7 versus 11.8%, resistant viruses emerged at comparable follow-up periods, a median of 17.8 and 18.3 months, respectively. In patients receiving zidovudine and lamivudine in their drug regimen, resistance to lamivudine was more frequent than to zidovudine. Two of the three patients, with viruses resistant to PIs, acquired mutations associated with cross-resistance. Strikingly, five (39%) of the 13 patients developed resistances to drugs that they had never received (n = 3) or that they received 18 or 36 months ago (n = 2). Didanosine/stavudine pressure had selected zidovudine-resistant viruses in four patients, and indinavir had selected a nelfinavir-resistant virus in one patient.

Conclusion: In contrast to other reports from developing countries where patients had received ARVs in an uncontrolled manner, our study showed that implementation of HAART together with good clinical, biological and logistical monitoring can reduce the emergence of resistant strains in Africa.

AIDS 2003, **17** (suppl 3):S31–S38

Keywords: Africa, antiretroviral therapy, drug resistance mutations, HIV-1 subtypes

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Introduction

Highly active antiretroviral therapy (HAART) has greatly reduced HIV/AIDS-related morbidity and mortality in the industrialized countries. However, in sub-Saharan Africa, where more than 70% of all HIV-infected patients live, access to antiretroviral therapy is still restricted despite the strenuous efforts of governments, international institutions and pharmaceutical companies to reduce therapy costs. The need for relatively sophisticated laboratory facilities for treatment monitoring, and the infrastructure required to provide an uninterrupted supply of drugs are additional limitations on widespread use of HAART in poor countries.

Antiretroviral drugs (ARV) have been designed, tested and validated against the European and North American subtype B strains, but non-B subtypes predominate worldwide, notably in Africa. The efficiency of antiretroviral treatment (ART) can be influenced by the viral diversity. Like HIV-2, HIV-1 group O viruses are naturally resistant to non-nucleoside reverse transcriptase inhibitors (NNRTIs) [1]. Within group M, some subtype F samples are less susceptible to the tetrahydroimidazo (4,5,1-jk) (1,4)-benzodiazepin-2-(1H)-one and -thione (TIBO) derivate, a NNRTI [2], and subtype G strains have decreased *in vitro* susceptibility to protease inhibitors (PIs) [3]. Among women receiving single-dose nevirapine prophylaxis to prevent mother-to-child transmission, subtype D viruses may develop resistance to nevirapine more rapidly than subtype A [4]. Many amino acid mutations associated with minor resistance to PIs have been reported as natural variants in treatment-naive patients infected with non-subtype B HIV-1 strains [5–7], but their biological consequences remain to be studied.

One of the first antiretroviral therapy initiatives to be sponsored by an African government was launched in Senegal, in August 1998. This initiative provided the opportunity to examine certain key operational questions concerning the use of HAART in the African context. In this cohort, clinical and biological results in ART-naive patients were comparable with those seen in western cohorts, despite differences in the HIV-1 subtype distribution and an advanced disease stage when the treatment was initiated [8]. Replication of HIV-1 with drug-resistant viruses during combination therapy is considered to be a major cause of treatment failure. Actually, no data are available on development of resistance to ARVs in Africa in a well-documented group of patients. In this study, we describe the prevalence and the genotypic mutations of ARV-resistant viruses in patients enrolled in the Senegalese initiative of access to ARVs.

Patients and methods

Patients

A total of 80 patients, enrolled in the Senegalese initiative of access to ARVs (ISAARV) between August 1998 and February 2001, with a virological follow-up of at least 6 months, were selected for this study. Among these 80 patients, 68 were ART-naive and 12 were ART-experienced at inclusion.

The consenting patients were eligible if they bore certain medical and social criteria as previously described [8]. Briefly, ART-naive patients were eligible if they were asymptomatic with CD4 cell counts below 350×10^6 cells/l and plasma HIV-1-RNA levels above 100 000 copies/ml, or mildly symptomatic with CD4 cell counts below 350×10^6 cells/l, or at clinical AIDS-stage. No such criteria were mandatory for patients with previous ART history. The patients were clinically monitored on a monthly basis in one of the three major hospitals in Dakar. Initially, the first line antiretroviral regimen was based on two nucleoside reverse transcriptase inhibitors (NRTIs) and one PI, except for mildly symptomatic patients with plasma HIV-1 RNA below 10 000 copies/ml who received only two NRTIs. Late in 2000, following the updated international recommendations from the International AIDS Society [9], HAART based on a combination of two NRTIs plus one PI or one NNRTI became the first line regimen for all patients. Four NRTIs [stavudine (d4T); didanosine (ddI); zidovudine (ZDV); and lamivudine (3TC)], one PI [indinavir (IDV)] and one NNRTI [nevirapine (NVP)] were available. Adverse effects were assessed using the WHO toxicity scale. Adherence was assessed on the basis of the patients' statements to the physicians at each monthly visit. It was calculated as the ratio between the number of respected doses and the number of prescribed doses. The national ethics committee on AIDS approved this study.

Plasma HIV-1-RNA assay and CD4 cell counts

Plasma HIV-1-RNA levels were initially determined using the Bayer branched DNA HIV-1 Quantiplex assay (Bayer Diagnostics, Emeryville, California, USA) version 2.0 (bDNA 2.0, measurement range 500 to 800 000 copies/ml), and subsequently with the ultrasensitive version 3.0 (bDNA 3.0, measurement range 50 to 500 000 copies/ml). Plasma samples were stored at –80°C until assay. CD4 cell counts were determined with a FACSCount apparatus (Becton Dickinson, Mountain View, California, USA) in freshly collected whole blood. Plasma HIV-1 RNA and CD4 cell values were done at baseline (J0), after one month of treatment (M1, plasma HIV-1 RNA only), at 6 months of treatment (M6) and subsequently every 6 months.

Table 1. Demographic and clinical baseline characteristics of the 80 patients by antiretroviral-experience groups (Dakar, Senegal, 1998–2001).

Characteristics	ART-naive patients (n = 68)		ART-experienced patients (n = 12)		*P*
Demography					
Sex – no. (%)					
Male	38	(55.9)	4	(33.3)	
Female	30	(44.1)	8	(66.7)	0.1
Median age (IQR[a]) (years)	42	(32–47)	38	(33–43)	0.4
Clinical data					
CDC class – no. (%)					
Class A	1	(1.5)	3	(25.0)	
Class B	20	(29.4)	4	(33.3)	
Class C	47	(69.1)	5	(41.7)	0.01
Median CD4 cell count × 10^6/l (IQR[a])	112	(34–217)	237	(148–354)	0.02
Median plasma HIV-1 RNA (IQR[a]), (copies/ml)	95740	(22170–225200)	1032	(662–53360)	< 0.001
Median body mass index (IQR[a])	20.6	(18.5–22.6)	23.5	(20.1–26.4)	0.01
Antiretroviral treatment – no. (%)					
2 NRTI	9	(13.2)	5	(41.7)	
2 NRTI + 1 PI	57	(83.8)	7	(58.3)	
2 NRTI + 1 NNRTI	2	(2.9)	0	–	0.07
Median length of follow-up (IQR[a]) (months)	18.4	(11.9–30.0)	30.0	(24.3–32.7)	0.04

[a] IQR, interquartile range. NRTI, nucleoside reverse transcriptase inhibitor; NNRTI non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Genotypic resistance testing

Genotypic resistance to ARVs was studied by sequencing the protease and reverse transcriptase (RT) genes as previously described [5]. Briefly, the viral RNA was extracted from plasma with QIAamp Viral RNA mini kit (QIAgen, Courtaboeuf, France) and retrotranscribed to complementary DNA by using Expand RT (Boehringer Mannheim, Mannheim, Germany) with a reverse primer. A 1800-bp fragment encompassing the protease and RT genes was amplified by nested-polymerase chain reaction and was directly sequenced (ABIPRISM Big Dye Terminator cycle sequencing ready reaction kit, Applied Biosystem, Roissy, France). Genetic subtypes were determined with phylogenetic tree analysis, using the Clustal W program as previously described [5,10]. The deduced amino acid sequences were compared to a reference sequence to detect mutations associated with resistance. These mutations were classified into minor mutations and major mutations, according to the consensus statements on ARV resistance of the Stanford HIV RT and Protease Sequence database [11].

Genotypic resistance testing was done at baseline for a random subset of patients and at each rebound in plasma viral load during ART. Viral rebound was defined as detectable viral load above 1000 copies/ml, which is also the detection limit of the genotypic resistance test, after having been undetectable. Genotypic resistance testing was also carried out for patients with non-optimal virological response after at least 6 months of treatment, defined as viral load above 1000 copies/ml without having ever been undetectable

Statistical analysis

Data were analysed using EPI-INFO 6.04 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and STATA Release 7.0 (STATA Corporation, College Station, Texas, USA) software. The chi-square test and Fisher's exact test, for small sample sizes, were used to compare the distribution of qualitative variables between ART-naive and ART-experienced patients. For continuous variables, comparisons were based on the non-parametric Mann–Whitney test. The same tests were used for analysis of factors associated with development of resistant viruses. For this analysis, the data for the patients concerned were censored at the moment that resistant viruses emerged. All statistical tests were interpreted at the 5% significance level and 95% confidence intervals (CI) calculated by the binomial exact method were computed for proportions.

Results

Baseline characteristics of the patients

Eighty patients, with a virological follow-up of at least 6 months, were selected for this study. Table 1 summarizes the patient characteristics at inclusion. Twelve

patients used ART before inclusion [median, 7.5 months; interquartile range (IQR), 2–18 months]. Overall, patients were predominantly of middle age (median, 40.5 years; IQR, 32.0–45.5 years), 52.5% were male and the majority were at an advanced stage of HIV disease (81.3% had AIDS). In comparison with ART-naive patients, those that had experienced ARV use had less advanced disease, had lower viral load and had higher CD4 cell counts.

Globally, antiretroviral therapy combined NRTIs with one PI or NNRTI. At baseline, in addition to indinavir, 46 patients (57.5%) were prescribed d4T and ddI; 11 (13.8%) ZDV and 3TC; three (3.8%) ZDV and ddI; three (3.8%) d4T and 3TC; and one (1.3%) ddI and 3TC. Only two patients (2.5%) received nevirapine (NVP), one with d4T and ddI, and one with ZDV and 3TC. Fourteen patients (17.5%) started with a dual therapy because of ongoing antitubercular therapy including rifampicin, efficient previous bitherapy in ART-experienced patients, or for mildly symptomatic patients with plasma HIV-1-RNA load below 10 000 copies/ml. Of them, 13 patients received d4T and ddI and one ZDV and 3TC. The median length of follow-up was 18.4 months for ART-naive patients and 30 months for ART-experienced patients. The longer follow-up of ART-experienced patients can be explained by the fact that the first patients included in the Senegalese initiative of access to ARV were those already receiving ART but having difficulties in continuing to pay for their treatment.

Genotyping at baseline in ART-naive and ART-experienced patients

At inclusion, only one of the 65 treatment-naive patients tested had an undetectable viral load (< 500 copies/ml) versus four of the 12 ART-experienced patients. As two of the treatment-naive patients were infected with an HIV-1 group O virus, undetectable by commercial viral load assays, we used an in-house semi-quantitative assay [12] to measure viral load in these patients; the plasma HIV-1-RNA levels ranged between 2×10^3–2×10^4 copies/ml and 2×10^4–2×10^5 copies/ml, respectively.

To determine whether drug resistance was present at baseline, we sequenced the protease and RT genes of specimens from 41 (60.3%) of the 68 ART-naive patients. Similarly, six of eight ART-experienced patients with detectable viral load were also genetically characterized to optimize their treatment, two patients could not be analysed because their viral load was below the detection limit of the genotypic resistance assay (< 1000 copies/ml).

Phylogenetic tree analysis of the 47 *pol* sequences revealed a high genetic diversity; CRF02 was predominant but multiple subtypes and other circulating recombinant forms (CRF) co-circulated: CRF02-AG (n = 25, 53.2%), A (n = 5, 10.6%), C (n = 5, 10.6%), B (n = 3, 6.4%), CRF06 (n = 3, 6.4%), G (n = 2, 4.2%), D (n = 1, 2.2%), a unique recombinant U/K (n = 1, 2.2%) and HIV-1 group O (n = 2, 4.2%).

Among the treatment-naive patients, no major mutations conferring resistance to NRTIs, NNRTIs and PIs were observed, except for the two HIV-1 group O strains which were, like all previously described group O viruses, also naturally resistant to NNRTIs (Y181C). Many minor mutations were observed in the protease gene: M36I (n = 37), K20M/R/I/V/C (n = 30), L63P/A/S/T/N (n = 19), L10I/V (n = 10), I93L (n = 4, all subtype C), V82I [n = 3, specific to subtype G (n = 2), and also for 1 subtype C], I93V (n = 2, all subtype G), D60K/N (n = 2, group O only), A71V (n = 2, group O only), V77I (n = 2), and K45R (n = 1). The RT gene was less polymorphic: R211K (n = 20), V179I/D/E (n = 7 including the two group O viruses), A98S (n = 2, subtype G), A98G (n = 2, group O), V118I (n = 1), K219N (n = 1) and G333E (n = 1).

Among the six ART-experienced patients, only one patient was resistant to ARV, more precisely to ZDV and possibly also to d4T and abacavir, related to the combination of the following mutations: M41L, D67N, L210W, and T215Y. This patient had been treated with ZDV and ddI, before baseline. Another patient, who had previously been treated with ddI, had selected a minor mutation, K65R, associated with a possible resistance to zalcitabine (ddc) and ddI. Similarly as in the treatment-naive population, many minor mutations were also observed in the protease gene (L10I, K20I, M36I, K45R, and L63P) and only a few in the RT gene (V179I, R211K, and G333E).

Viral load rebound and genotypic resistance during patient follow-up

The plasma HIV-1 RNA level fell markedly after treatment initiation and became undetectable (< 500 copies/ml) after 1 month in the majority (77.9%) of the patients. Genotypic resistance testing was carried out for each viral rebound (> 1000 copies/ml) observed during follow-up. The two group O patients were responding well to their therapy (ddI/d4T/IDV), viral load was below 1000 copies/ml at 30-month follow-up.

ART-naive patients

For 30 of the 68 patients, viral rebounds were observed after between 6 and 36 months of follow-up. Certain viral rebounds (n = 22) were associated with partial or total treatment interruption due to adverse effects or incompatibilities with treatment regimens for opportunistic infections, or to poor adherence. No resistance mutations were observed and viral load became again undetectable in these patients after reinstatement of HAART. The viral rebounds were associated with the

Table 2. Emergence of resistant viruses in antiretroviral therapy-naive and ART-experienced populations (Dakar, Senegal, 1998–2001).

Samples	Baseline (JO)/ viral rebound	Treatments	Mutations in the reverse transcriptase gene												Mutations in the protease gene									Selected resistances to following drugs	*pol* subtypes
			M41	K65	D67	K70	**V75[d]**	A98	118	**M184**	L210	R211	**T215**	K219	L10	K20	M36	L63	A71	G73	**V82**	**I84**	**N88**		
101HALD	JO	ZDV, 3TC, IDV																A							B
	M12									**V**								A						3TC	
34HALD	JO	ZDV, 3TC, IDV														I	I								CRF02-AG
	M24									**V**						I	I							3TC	
65HALD	JO	ddI, d4T[a]										K			V	I	I								CRF02-AG
	M2			R								K			V	I	I								
	M18	ZDV, 3TC, IDV								**V**		K			V	I	I	P						3TC	
69HPD	JO	ddI, d4T, IDV[b]															I								A
	M24	ZDV, 3TC, IDV	L						I	**V**			**Y**				I	P	V	S		**V**		3TC, PIs, (NRTIs)	
42HPD	JO	ddI, d4T[c]										K				I	I	N							CRF02-AG
	M18	ddI, d4T, IDV					**T**					K				I	I							d4T	
46HALD	JO	ddI, d4T, IDV										K				I	I								CRF02-AG
	M18					R						K		E		I	I							ZDV (intermediate)	
160HALD	JO	ddI, d4T, IDV										K					I	P							D
	M12											K					I	P	T				**D**	NFV	
63HPD	JO	ddI, d4T, IDV						S							I	I	I	I	P		I				G
	M30							S							I	I	I	I	P		**T**			IDV, RTV, NFV (AMP)	
20HALD	<JO-JO	ZDV, 3TC, IDV																						nt	
	M6									**V**					V									3TC	B
21HALD	<JO-JO	ZDV, 3TC, IDV																						nt	
	M18									**V**														3TC	B
74HALD	<JO-JO	ddI, d4T										K				I	I								
	M12											K	**Y**			I	I							ZDV	CRF02-AG
53HPD	<JO	ZDV, ddI																						nt	
	M18	ddI, d4T	L		N				I		W	K	**Y**			I	I							ZDV (d4T, ABC)	CRF02-AG
55HPD	<JO	ZDV, ddC																						nt	
	M36	ddI, d4T			N							K	**Y**	R			I							ZDV (d4T, ABC)	D

For each patient, previous (<JO= before ART initiation, for ART-experienced population) and actual treatment molecules and duration (in months) are indicated as well as genotyping results. nt (not treated) indicates no genotypic results because of undetectable viral load. [a]This patient interrupted his treatment between M5 and M7. [b]This patient interrupted PI between M4 and M6 (bi-therapy). [c]This patient received this treatment during 5 months. [d]The major mutations are noted in bold. Drugs used: IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; AMP, amprenavir; PI, protease inhibitors; ZDV, zidovudine; 3TC, lamivudine; ddI, didanosine; ddC, zalcitabine; d4T, stavudine; ABC, abacavir.

emergence of resistant strains in only eight patients. The prevalence of resistant viruses in the 68 naive patients was thus 11.8% (CI, 5.2–21.9%). Table 2 summarizes the treatment regimens for the eight patients who developed resistant viruses and shows the selected mutations compared to baseline profiles if applicable. Resistant viruses appeared after between 12 and 30 months (median, 18.3 months) of therapy, with mutation profiles conferring resistance to: 3TC (n = 3), nelfinavir (n = 1), ZDV (n = 1), d4T (n = 1), NRTIs/3TC/PIs (n = 1), indinavir/ritonavir/nelfinavir (n = 1). Resistance to 3TC (n = 4) was frequently and rapidly selected after 10 to 24 months of exposure to 3TC. Cross-resistance to several PIs was observed in one of two subtype G samples, the genotypic profile in the protease gene is almost similar between baseline and 30 months of follow-up, except the substitution at position 82. The natural mutation V82I, a characteristic feature of subtype G, could be a mutation allowing a faster switch to the resistance mutation V82T.

Strikingly, two of the eight patients developed mutations associated with resistance to molecules that they had never received, after 12 and 18 months of tritherapy (IDV/ddI/d4T). One patient was resistant to nelfinavir (A71T, N88D) and one had an intermediary resistance to ZDV (K70R, K219E). For both patients, these mutations were absent at baseline.

ART-experienced patients

For six patients, viral rebounds were observed after between 6 and 36 months of ARV treatment. For one patient, this rebound was related to a treatment interruption and no resistant mutations were present. However, the other five patients developed resistances to 3TC (n = 2) and ZDV (n = 3) (Table 2). The prevalence of resistant viruses in the 12 ART-experienced patients was thus 41.7% (CI, 15.2–72.3%) and resistant viruses emerged after a median follow-up of 17.8 months. The M184V mutation conferring resistance to 3TC appeared rapidly, after between 6 and 18 months of treatment, but these patients had already received 3TC before baseline. Similar to the ART-naive patients, three patients developed mutations (T215Y) associated with ZDV resistance while they were not receiving ZDV. This mutation was selected after 12 months (74HALD), 18 months (53HPD) and 36 months (55HPD) of ddI/d4T treatment, only the two latter patients took ZDV before baseline. Overall, among the 12 ART-experienced patients, three of five (60%) patients receiving bitherapy (ddI/d4T) developed resistant strains, versus two of the seven (28.6%) patients receiving triple therapy.

Factors associated with emergence of ARV-resistant viruses

During follow-up, the resistant viruses appeared more frequently in ART-experienced patients than in ART-naive patients (41.7 versus 11.8%, $P = 0.02$). The length of follow-up in patients who developed resistance was similar or lower than for those who did not develop resistance, thus allowing comparison between groups. Although not significant, except for CD4 cell counts, ART-naive patients in whom resistant viruses were observed, seemed to be at a more advanced stage of HIV disease at baseline than those without resistance (Table 3). This trend was not found in ART-experienced patients but this group was too small. As expected, a temporary or permanent intake of bitherapy was more frequent in patients who developed resistance. The average monthly adherence was very similar in both groups as well as the adverse effects that can favour lower plasma concentration of drugs and adherence difficulties.

Discussion

Our results showed that among 80 patients, receiving ARV in the Senegalese initiative for access to ARV treatment, 13 (16.3%) harboured resistant viruses after a median follow-up of 24 months. The selection of resistant viruses was lower in the ART-naive population than in the ART-experienced population, 11.8 versus 41.7%. In both populations, resistant viruses emerged after comparable treatment duration, with a median of 18.3 and 17.8 months, respectively. The overall prevalence of resistant viruses was thus lower than in previous preliminary studies in Gabon and Ivory Coast, where more than 50% of patients receiving ARV, mainly as mono- or bi-therapy with limited or no biological monitoring for treatment efficiency, were resistant in less than 18 months of ARV use [13–15]. However it is important to note that the data on populations with uncontrolled ARV use are similar to those observed in our ART-experienced group, and more precisely to the group of patients receiving bitherapy only [13–15].

Many other viral rebounds (n = 23) were observed, but they were associated with treatment interruption for social or medical reasons or poor adherence.

In patients receiving ZDV and 3TC in their drug regimen, resistance to 3TC related to the M184V mutation was more frequent than resistance to ZDV conferred by the T215Y mutation, in accordance with another study in Uganda [16]. This could be in concordance with previous studies suggesting that the M184V mutation could have a protective effect on the emergence of ZDV-associated mutations [17]. Two of the three patients with viruses that were resistant to PIs acquired mutations associated with cross-resistance to PIs, which compromised the further use of PIs in these patients.

One of the most striking observations was that five (39%) of the 13 patients developed resistances to drugs that they never received (n = 3) or for which treatment was interrupted for 18 or 36 months (n = 2). The ddI/d4T pressure had selected ZDV-resistant viruses in

Table 3. Analysis of factors associated with occurrence of antiretroviral drug resistance in the antiretroviral therapy-naive and ART experienced patients (Dakar, Senegal, 1998–2001).

	ART-naive patients			ART-experienced patients		
Characteristics	ARV resistance (n = 8)	No ARV resistance (n = 60)	*P*	ARV resistance (n = 5)	No ARV resistance (n = 7)	*P*
Demography						
Sex (%)						
Male	62.5	55.0		60.0	14.3	
Female	37.5	45.0	1.0	40.0	85.7	0.2
Median age (IQR[a]) (years)	32 (29–49)	42 (32–46)	0.4	39 (33–44)	37 (32–43)	0.6
Baseline clinical data						
CDC class, (%)						
Class A	0.0	1.7		20.0	28.6	
Class B	12.5	31.7		60.0	14.3	
Class C	87.5	66.7	0.5	20.0	57.1	0.3
Median CD4 cell count × 10^6/l (IQR[a])	28 (7–92)	124 (55–228)	0.049	308 (223–344)	203 (62–472)	0.3
Median plasma HIV-1 RNA (IQR[a]),	108900	89515		947	1987	
(copies/ml)	(19560–155300)	(22845–235950)	0.9	(824–1000)	(500–89720)	0.4
Median body mass index (IQR[a])	19.8 (17.9–22.5)	20.6 (18.6v22.8)	0.8	23.6 (19.9–26.6)	23.4 (20.3–26.2)	0.8
Follow-up						
Median length of follow-up (IQR[a]), (months)	18.3 (16.4–23.1)	18.0 (11.7–30.0)	0.9	17.8 (12.4–19.7)	30.0 (6.0–30.1)	0.5
Lifetime bitherapy, (%)	37.5	26.7	0.7	60.0	28.6	0.6
Median average monthly adherence, (%)	96.5	96.0		99.7	99.7	
	(91.5–99.0)	(91.0–99.0)	0.7	(81.9–99.8)	(98.2–99.8)	0.7
Median number of adverse effects (IQR[a])	0 (0–1)	1 (0–1.5)	0.3	0 (0–1)	1(0–2)	0.2

[a] IQR, interquartile range.

four patients, and indinavir had selected nelfinavir-resistant virus in one patient. *In vivo* both ddI and d4T have the potential to select thymidine analog mutations (TAM: M41L, D67N, K70R, L210W, T215Y/F and K219Q/E) associated with ZDV resistance but this pressure is not as great as that exerted by ZDV. Viral isolates possessing such mutations have previously been described but with modest or no phenotypic resistance to ddI or d4T *in vitro* [18,19]. However, in our study, the appearance of these viruses was associated with an increase in viral load, thus suggesting phenotypic resistance. Phenotypic and clinical studies are needed to explain the mechanisms involved in the selection of resistant viruses to drugs that were not administered. It will also be important to find out whether this is more frequently observed in non-B HIV-1 strains.

In addition to the lack of ART efficacy related to resistant strains for treated patients, a real public health problem concerns the possible transmission of these resistant strains. In industrialized countries, 11% of new HIV-1 infections already have variants that are resistant to one or more ARVs and recent studies have shown that this continues to increase [20]. In Senegal, no ART-naive patients harboured resistant viruses at baseline, but ARVs were only recently introduced.

Non-B viruses have a higher prevalence of naturally occurring minor mutations, especially in the protease gene, which could lead to faster development of drug resistance than in B viruses. Frater *et al.* [21] studied the impact of baseline polymorphisms in RT and protease genes on the outcome of HAART in HIV-1-infected African patients, with a 1-year follow-up. The patients who were infected with non-B subtypes, notably subtypes A, C and D, responded efficiently to HAART. Similarly, in our study with a longer follow-up, numerous minor mutations in the protease gene did not seem to influence therapy outcome, except maybe for subtype G, which could develop resistance to PIs more rapidly, due to the pre-existing V82I mutation and many minor (n = 4) mutations in the protease. However, more long-term studies are necessary to determine the clinical significance of these mutations.

Our studies in Senegal show that implementation of HAART is possible in developing countries, and that tritherapy and good clinical, biological and logistical monitoring can reduce the emergence of resistant strains. With the recent efforts to lower the price of ARV, these drugs will now be massively introduced in many developing countries. In order to avoid the rapid emergence of resistant viruses on a large scale in the develop-

ing world, it is important that the infrastructures necessary to monitor ART are also rapidly implemented in these countries and that clinicians are trained in the appropriate use of ARV. There is a need for alternative, less sophisticated and cheaper tools to monitor CD4 cell counts and/or viral load closely. A continuous surveillance of the circulation of ARVs and ARV drug-resistant viruses has to be organized to guide ARV treatment strategies and policies.

Acknowledgements

This work was partially supported by a grant of the French National Agency for Research on AIDS (ANRS) and by European Union (contract B7-6211/99/005). C.L. was the recipient of a doctoral fellowship from ANRS.

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Development of phenotypic and genotypic resistance to antiretroviral therapy in the UNAIDS HIV drug access initiative – Uganda

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Objective: We describe phenotypic drug resistance, response to therapy, and genotypic mutations among HIV-infected patients in Uganda taking antiretroviral medications for ≥ 90 days who had a viral load ≥ 1000 copies/ml.

Methods: HIV-1 group and subtype, virologic and immunologic responses to antiretroviral therapy, phenotypic resistance to antiretroviral drugs, and associated genotypic mutations among patients at three treatment centers in Uganda between June 1999 and August 2000 were assessed. Therapy was two nucleoside reverse transcriptase inhibitors (NRTIs) or highly active antiretroviral therapy (HAART).

Results: All HIV identified was HIV-1, group M, subtypes A, C, and D. Sixty-one (65%) of 94 patients with a phenotypic resistance result had evidence of phenotypic resistance including resistance to a NRTI for 51 of 92 (55%) taking NRTIs, to a non-nucleoside reverse transcriptase inhibitor (NNRTI) for nine of 16 (56%) taking NNRTIs, and to a protease inhibitor (PI) for eight of 37 (22%) taking PIs. At the time of the first specimen with resistance, the median change from baseline viral load was –0.56 log copies/ml [interquartile range (IQR), –1.47 to +0.29] and CD4+ cell count was $+35 \times 10^6$ cells/l (IQR, –18 to +87). Genotypic resistance mutations, matched with phenotypic resistance assay results and drug history, were generally consistent with those seen for HIV-1, group M, subtype B infections in industrialized countries.

Conclusion: Initial phenotypic resistance and corresponding genotypic mutations among patients treated in Uganda were similar to those with subtype B infections in North America and Europe. These data support policies that promote the use of HAART regimens against HIV-1, group M, non-B subtypes in a manner consistent with that used for subtype B infections.

AIDS 2003, **17** (suppl 3):S39–S48

Keywords: Africa, antiretroviral, HIV, resistance, subtypes, Uganda

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Sponsorship: Funding sources for this project were the US Centers for Disease Control and Prevention, Atlanta, Georgia, USA, and the United Nations AIDS Program, Geneva, Switzerland. Virco NV Belgium performed phenotypic and genotypic resistance testing.

Use of trade names is for identification purposes only and does not imply endorsement by the US Centers for Disease Control and Prevention, the US Department of Health and Human Services or the UNAIDS. This research was approved by the Uganda National Council for Science and Technology and the Institutional Review Board of US Centers for Disease Control and Prevention, Atlanta, Georgia, USA. Presented in part at the XIIIth International AIDS Conference, Durban, South Africa, July 2000 and the Eighth Conference on Retroviruses and Opportunistic Infections, Chicago, Illinois, USA, February 2001.

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Introduction

Increasing access to antiretroviral drugs for patients living with HIV/AIDS in developing countries has attained global focus [1]. However, concerns have been raised that emerging drug resistance may hinder treatment efforts in countries with weak infrastructure, and these concerns are sometimes cited as a caution to extending antiretroviral therapy in Africa. Resistance to antiretroviral drugs has mostly been studied in North America and Europe where HIV-1, group M, subtype B viruses predominate; the available guidance on interpretation of associated genotypic mutations is based primarily on such information [2,3]. Limited data from African patients infected with HIV-1, non-B subtypes, who are treated with antiretroviral drugs in Uganda, Côte d'Ivoire, and Europe indicate similar patterns of development of resistance to antiretroviral drugs [4–6]. However, knowledge of antiretroviral resistance in relation to virologic and immunologic response among non-B subtypes is incomplete. This is complicated by the fact that several polymorphisms that are associated with resistance are found naturally in non-B subtypes at sites considered to be minor mutations [7–9]. Some evidence indicates that these will not adversely affect the response to antiretroviral drugs [6]. Additionally, existing evidence suggests that despite these mutations, HIV-1, group M, non-B subtype isolates collected from antiretroviral-naive patients in Africa, South America, and Europe are generally as sensitive to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors as are subtype B viruses [4,10–17], with the possible exception of decreased sensitivity among HIV-1, group M, subtype G [14]. HIV-2 and HIV-1, group O viruses are resistant to the currently available NNRTIs [18,19].

The UNAIDS HIV Drug Access Initiative (DAI) was a pilot programme developed in 1997 to increase access to AIDS care and drugs in four countries: Uganda, Côte d'Ivoire, Chile, and Vietnam [20]. The pilot UNAIDS/Uganda Ministry of Health HIV DAI was evaluated from 1 August 1998 to 31 July 2000. The US Centers for Disease Control and Prevention (CDC), in collaboration with the Uganda Ministry of Health and UNAIDS, evaluated the virologic and immunologic response to treatment, sustainability of treatment, survival, and emergence of drug resistance among patients accessing the DAI. We have previously reported good virologic and immunologic responses from this initiative similar to those reported from more developed settings, as well as the aggregate findings of the phenotypic resistance testing [21]. We now describe patterns of phenotypic drug resistance and related genotypic mutations in relation to specific antiretroviral therapy taken by these Ugandan patients infected with HIV-1, group M, non-B subtypes. We also further describe their virologic and immunologic response to antiretroviral medications in relation to the development of resistance.

Methods

Sites and study participants

Five health care facilities near Kampala, the capital of Uganda, were accredited to provide antiretroviral drug treatment through the initiative as previously described [21]; four of these facilities became fully operational. For this evaluation, we used clinical and laboratory information from three accredited centers that had completed standardized medical record forms and follow-up data (Nsambya Hospital, Mildmay Palliative Care Center, and Mulago Hospital) to assess patterns of resistance development.

Choice of drugs, frequency of follow-up visits, and laboratory monitoring were based on the patient's clinical status, availability and cost of drugs, patient's finances, and laboratory test results. The patients paid for all drugs and medical care. Drug therapy was characterized as highly active antiretroviral therapy (HAART) if regimens were consistent with those recommended by international standards at the time [22–24]. This included two NRTIs plus abacavir, an NNRTI, and/or a protease inhibitor. Therapy was characterized as two NRTIs if the regimen included two NRTIs with or without hydroxyurea.

Virologic and immunologic assays

Plasma levels of HIV-1 RNA (viral load) (Amplicor HIV-1 Monitor Version 1.5 Assay; Roche Diagnostics, Branchburg, New Jersey, USA) and CD4+ cell counts (FACSCount; Becton Dickinson, San Jose, California, USA) were provided free of charge by the Uganda Virus Research Institute/CDC-Uganda, beginning on 7 June 1999. Blood samples were collected in ethylenediamine tetraacetic acid Vacutainer tubes (Becton Dickinson, Le Pont-de-Claix, France) and processed within 6 h; results were returned to the treating physician within 7 to 14 days. Patients gave informed consent for simultaneous collection of an additional specimen in a CPT Vacutainer (Becton Dickinson, Le Pont-de-Claix, France) and plasma was stored at –80°C.

Phenotypic and genotypic resistance testing and subtype determination

Plasma specimens stored between 7 June 1999 and 31 July 2000 were identified for resistance testing if they were obtained ≥ 90 days after therapy was begun, if they had a viral load ≥ 1000 copies/ml, and if sufficient information was recorded to determine the patient's drug therapy. Physicians did not receive resistance testing results in time for patient management decisions, but summary reports were provided as the testing was completed. Phenotypic testing (Antivirogram; VIRCO, Mechelen, Belgium) and full-sequence ABI-based genotypic (VircoGen;VIRCO, Cambridge, UK) testing were performed [25]. The Antivirogram utilizes recombinant viruses derived by incorporation of patient-derived viral

sequences into a wild-type HIV-1 laboratory strain (HXB2, subtype B). Phenotypic resistance was determined by comparing the concentration at which virus replication is inhibited by 50% (IC_{50}) of the patient-derived recombinant virus to the IC_{50} of the HXB2 reference wild type [10]. This technique has been demonstrated to be a robust method for measurement of phenotypic resistance for a wide variety of group M, HIV-1 isolates and has similar viral load input performance characteristics between subtypes [26]. Genotypic mutations associated with resistance [2,3] were identified by comparing the amino acid sequence of the reverse transcriptase and protease genes of the patient's isolate to a consensus subtype B reference. HIV subtype was determined using the Stanford HIV Reverse Transcriptase and Protease Sequence Database Analysis programme of nucleic acid sequences available from genotypic resistance testing, and results are reported as protease/reverse transcriptase (RT) [27].

Statistical analyses

To test for statistical significance, we used the Wilcoxon two-sample rank sum test for continuous variables and the chi-square test for dichotomous variables. We used Stata, Version 6.0, (College Station, Texas, USA) for all statistical analyses.

Results

Patients, treatment regimens, and virologic response

A total of 912 patients were enrolled at the five DAI centers. A total of 476 patients were enrolled at three centers available for this analysis: Nsambya Hospital (286 patients), Mildmay HIV Care and Rehabilitation Center (157 patients), and Mulago Hospital (33 patients). Median baseline viral load was 193 817 copies/ml [n = 360; interquartile range (IQR), 37 013–651 716], and CD4+ cell count was 73×10^6 cells/l (n = 342; IQR, 15–187). Antiretroviral therapy was prescribed at the initial visit for 399 (84%) patients, and follow-up for ≥ 90 days and at least one viral load test was performed between 7 June 1999 and 31 July 2000 for 167 (42%) patients (Fig. 1). For these 167 patients, there were 365 viral load tests performed (median 2 per patient, range 1–6) and this did not differ between those prescribed HAART and two NRTIs ($P = 0.84$). For 51 (31%) of 167 patients, all viral load values were < 1000 copies/ml; more so for those prescribed HAART [32 (41%) of 78] than those prescribed two NRTIs [19 (21%) of 89; $P = 0.006$]. The remaining 116 (69%) first had their viral load ≥ 1000 copies/ml at a median of 182 days (IQR, 116–288) after starting in the DAI. At least one phenotypic resistance result was available for 94 patients (median 1, range 1–5), the first of which was obtained at a median of 203 days (IQR, 133–308) after starting therapy in the DAI.

HIV-1 subtype analysis

As determined from available nucleic acid sequences for 93 patients, all were HIV-1, group M; none were subtype B. Subtype in the protease and RT genes were, respectively, D/D for 42 (45%) patients, A/A for 41 (44%), A/D for four (4%), C/C for three (3%), D/A for two (2%), and D/C for one (1%).

Phenotypic resistance

Of the 94 patients with at least one phenotypic resistance result, 61 (65%) had HIV with phenotypic resistance to at least one drug (Table 1). Resistance to more than one class of drugs was found in 17 (18%) patients. Cross-resistance among lamivudine, abacavir, zalcitabine, and/or didanosine was evident for 27 (29%) patients; all 27 were taking one of those drugs.

Virologic and immunologic response at time of phenotypic resistance

For the 61 patients with phenotypic resistance, the first specimen with resistance was obtained after a median of 202 days (IQR, 121–274). At that time, median viral load was 29 708 copies/ml (IQR, 11 811–185 337) and < 10 000 copies/ml for 11 (18%). The median change from baseline was –0.56 log copies/ml (n = 42; IQR, –1.47 to +0.29). The median change from baseline for CD4+ cell count was $+35 \times 10^6$ cells/l (n = 45; IQR –18 to +87), and CD4+ cell count was still above baseline for 32 of 45 (71%). Similar results were noted for patients prescribed two NRTIs and HAART (Table 2).

Estimated prevalence of resistance

To estimate the prevalence of resistance among all patients, we considered those who always had a viral load < 1000 copies/ml to have sensitive viruses. We considered those who had a viral load ≥ 1000 copies/ml, but no phenotype completed to have the same prevalence of resistance as those tested. Any phenotypic resistance was estimated to have been present for 78 of 167 (47%) patients – for those initially prescribed HAART for 28 of 78 (36%) and prescribed two NRTIs for 50 of 89 (56%) [odds ratio 0.44, 95% confidence interval (CI) 0.22–0.85, $P = 0.009$].

Correlation of phenotypic resistance to genotypic mutations

Because sometimes patients received different antiretroviral drugs at different times, we examined the mutations in the individual specimens to analyze the relationship between drug history and phenotypic resistance to genotypic mutations. Both a phenotypic resistance result and genotypic sequence were completed for 150 specimens and are the basis for the remainder of the analysis.

Nucleoside reverse transcriptase inhibitors

For 24 specimens with phenotypic resistance to zidovudine and/or stavudine, 23 (96%) had at least one commonly recognized mutation for zidovudine resistance. A

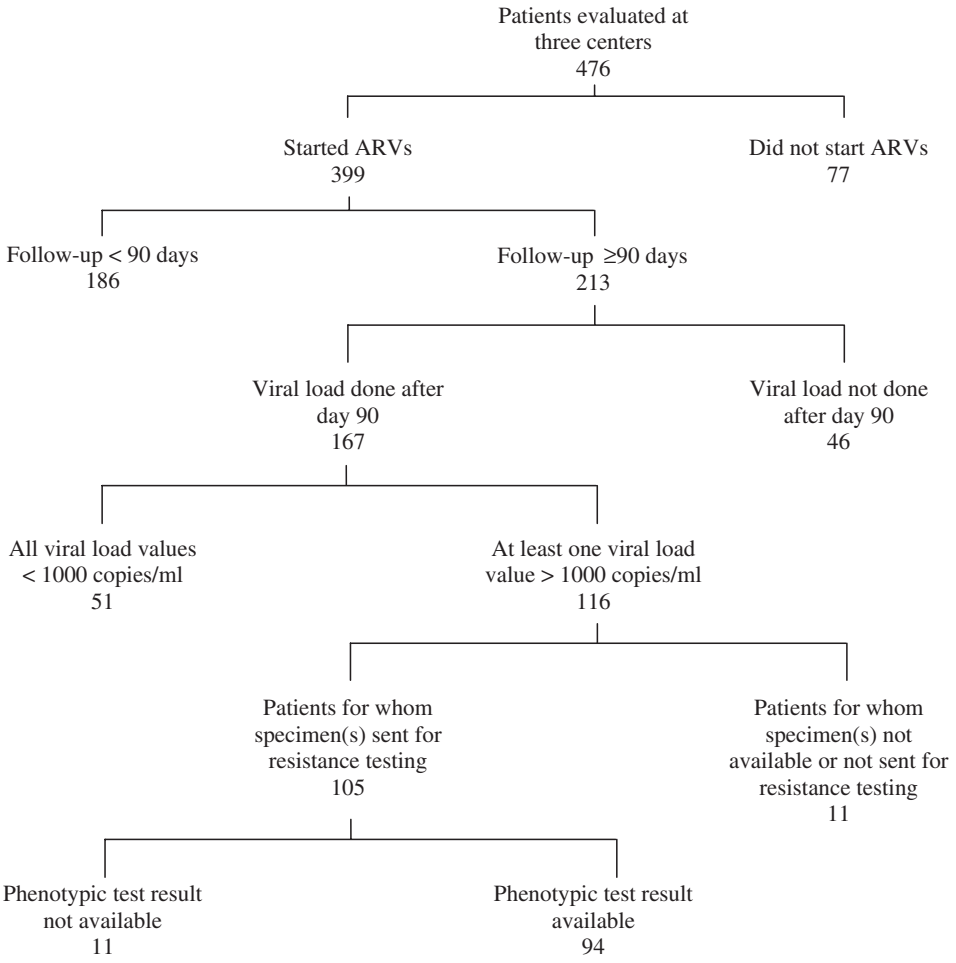

Fig. 1. Patients, treatment regimens, and virologic response. ARVs, antiretroviral drugs.

mutation was present in the RT gene at codon 215 for 22 of 24 (92%) as follows: T215Y for 18 and T215F for four. The mutation M41L or M/L mixture was present for 19 (79%), K70R or K/R for 10 (42%), D67N or D/N for 16 (67%), and L210W or L/W for nine (38%). An absence of phenotypic resistance to zidovudine and stavudine but at least one mutation associated with zidovudine resistance was found in 22 specimens, all of which were from patients with a current or past history of taking zidovudine or stavudine.

Phenotypic resistance to lamivudine was found in 77 (51%) specimens; a mutation was present for 74 of 77 (96%) at codon 184 of the RT gene as follows: M184V for 64, M184M/V for eight, and M184I for two. Of 73 specimens without phenotypic resistance to lamivudine, only three (4%) had the mixed mutations M184M/V (n = 2) or M184V/I (n = 1). These three were from patients with a current or past history of taking lamivudine.

Phenotypic resistance to both zidovudine and lamivudine was found in 16 specimens. Mutations at positions 215 and 184 were found in 13; three of these (from the same patient) also had a G333E mutation. Two specimens from patients taking zidovudine and zalcitabine had mutations at positions 41, 67, and 215; one other specimen from a patient taking zidovudine and lamivudine had mutations at positions 41 and 184.

Non-nucleoside reverse transcriptase inhibitors

Phenotypic resistance was present to at least one NNRTI for 21 specimens (Table 3). However, only 10 of these were from patients prescribed an NNRTI at the time the specimen was obtained (n = 9) or in the past (n = 1), although further review of the medical records indicated that four additional specimens were obtained at a time when the patient may have been receiving an NNRTI from another source. An NNRTI mutation was found for only two specimens without phenotypic resistance to an NNRTI. One specimen was from a

Table 1. Phenotypic resistance patterns in specimens from 94 patients for whom at least one phenotypic resistance result was available[a].

	All patients (n = 94)	Patients taking the drug[b]	
Antiretroviral drug	No. with resistance[c,d] (%)	No. taking drug	No. with resistance[c] (%)
Nucleoside RTIs			
Lamivudine	49 (52)	67	43 (64)
Zalcitabine	17 (18)	7	0 (0)
Zidovudine	16 (17)	63	12 (19)
Abacavir	15 (16)	2	1 (50)
Didanosine	7 (7)	29	0 (0)
Stavudine	6 (6)	36	3 (8)
Any nucleoside RTI	52 (55)	92	51 (55)
Non-nucleoside RTIs			
Nevirapine	14 (15)	5	2 (40)
Delavirdine	11 (12)	3	1 (33)
Efavirenz	9 (10)	8	4 (50)
Any non-nucleoside RTI	17 (18)	16	9[e] (56)
Protease inhibitors			
Nelfinavir	8 (9)	12	5 (42)
Indinavir	4 (4)	19	2 (11)
Ritonavir	4 (4)	6	1 (16)
Saquinavir	3 (3)	7	1 (14)
Amprenavir	3 (3)	0	–
Lopinavir (data from 52 patients)	2 (4)	0	–
Any protease inhibitor	9 (10)	37	8 (22)

[a]Specimens were selected from patients with a viral load ≥ 1000 copies/ml and that were obtained ≥ 90 days after start of therapy. For 105 patients at least one specimen was sent for resistance testing and at least one phenotypic resistance test result was completed for 94 patients. The percentage listed with resistance is of patients from whom specimens were tested and should not be interpreted to indicate the percentage of resistance among all patients in the DAI. [b]Indicates patients for whom the drug was prescribed and believed to be taking at the time the specimen was obtained, although adherence to therapy at the time of specimen collection was not confirmed. [c]Indicates the number of patients who ever had resistance documented. Fold-resistance above which phenotypic resistance was considered present: lamivudine, 4.5; zalcitabine, 3.5; zidovudine, 4.0; abacavir, 3.0; didanosine, 3.5; stavudine, 3.0; nevirapine, 8.0; delavirdine, 10.0; efavirenz, 6.0; nelfinavir, 4.0; indinavir, 3.0; ritonavir, 3.5; saquinavir, 2.5; amprenavir, 2.5; lopinavir, 2.5. [d]Seventeen (18%) patients had resistance to at least one drug in more than one class of drugs. [e]One patient taking delavirdine had resistance to efavirenz, but not delavirdine. Another patient taking efavirenz had resistance to delavirdine and nevirapine, but not efavirenz. RTI, reverse transcriptase inhibitor.

Table 2. Virologic and immunologic response at time the first specimen with documented phenotypic resistance to at least one drug was obtained.

	Plasma HIV-RNA				CD4+ cell count		
	HIV RNA (copies/ml) [Median (IQR)]	No. of specimens at HIV RNA concentrations (copies/ml)			Change from baseline[a] [Median (IQR)]	Change from baseline[b] [Median (IQR)]	No. of patients with CD4 cell count above baseline[b]
		1000–9999	10 000–99 999	≥ 100 000			
Two NRTIs	21 338 (13 027, 161 842)	6 (16)	19 (50)	13 (34)	–0.62 (+0.14, –1.43)	+38 (+2, +76)	23 (77)
HAART	65 000 (10 904, 253 040)	5 (22)	7 (30)	11 (48)	–0.11 (+0.75, –1.51)	+35 (–116, +141)	9 (60)
P-value[c]	0.45	0.55[d]			0.47	0.93	0.25

The median time between enrollment and collection of the first specimen for 38 patients receiving two nucleoside reverse transcriptase inhibitors (NRTIs) was 153 days (IQR, 105–239), and for 23 patients receiving highly active antiretroviral therapy (HAART; see below) was 250 days (IQR, 155–408; $P < 0.0001$). [a]There were 28 patients receiving two NRTIs and 14 receiving HAART who had a viral load at baseline from which to calculate a change. [b]There were 30 patients receiving two NRTIs and 15 receiving HAART who had a CD4 cell count at baseline from which to calculate a change. [c]*P*-values calculated by Wilcoxon rank sum or chi-square tests. [d]P-value for probability of viral load 1000–9999 versus ≥ 10 000 copies/ml. HAART, highly active antiretroviral therapy that included two NRTIs plus either abacavir, a non-nucleoside RTI, reverse transcriptase inhibitor or a protease inhibitor; IQR, interquartile range.

Table 3. Specimens with non-nucleoside reverse transcriptase inhibitor resistance.

Specimen no.[b]	Days since started in DAI	Subtype in RT gene	NNRTI prescribed at the time specimen obtained	Fold-resistance			Mutations in reverse transcriptase gene[a]										
				Nevirapine (8.0)[c]	Delavirdine (10.0)	Efavirenz (6.0)	A98	L100	K103	V106	V108	Y181	Y188	Y190	P225	M230	P236
1a	511	D	[d]	71.8	122.2	1.6						Y/C					
1b	546	D	[d]	72.3	134.4	2.2			K/N			Y/C					
1c	656	D	Efavirenz	88.7	595.2	67.4			N								
2a	312	D	Delavirdine	3.9	90.5	0.8			K/N								L
2b	447	D	[e]	8.2	15.2	3.3			K/N								P/L
3	574	A	Efavirenz	39	183.5	300.3	G		N								
4	486	D	Nevirapine	37.6	7.6	108											
5	175	D	Delavirdine	7	9.4	6.2											
6	414	A	Nevirapine	40.5	154.3	520.8			N		V/I						
7	148	A	Efavirenz	88.7	484.2	85.4	G		N								
8	204	D	Efavirenz	56.3	106.2	14			N								
9	196	D	Efavirenz	26.7	52.6	3.5											
10a	239	A	[d]	72.3	63.8	28.8			N								
10b	362	A	[d]	88.7	595.2	52			N						P/H		
11	358	D	[f]		7	9.2											
12	408	A	[f]	25	27.5	2.9											
13	230	A	[f]	9.3	9.1	3.3											
14	202	D	[f]	2.3	10	2.4											
15	217	D	[f]	9.9	4.6	1.3											
16	206	D	[f]	12.3	1.7	2.4											
17	308	A	[f]	10.3	15.7	1.3											

[a]Mutations in reverse transcriptase gene. The letter preceding the position number corresponds to the expected amino acid at the position number of the reverse transcriptase gene. The letters in the data cells correspond to the observed mutant amino acid [2]. [b]The number indicates different patients, and the letter indicates specimens, in chronological order, for the same patient. Patients 1 and 2 have results reported on Table 4 as well. [c]Fold-resistance value above which resistance was considered present. [d]Patient may have been receiving an NNRTI from another source. [e]Patient had taken delavirdine in the past. [f]No evidence in records that patient had ever taken an NNRTI. DAI, drug access initiative; RT, reverse-transcriptase; NNRTI, non-nucleoside reverse transcriptase inhibitor.

patient who was prescribed efavirenz and had K103N/K and M230L mutations. The other was from a patient who had not taken an NNRTI but had an Y188Y/H mutation.

Protease inhibitors

Phenotypic resistance to at least one protease inhibitor was found in 17 specimens. Two were from patients who had never received a protease inhibitor, and one each was subtype A and subtype D in the protease gene, had moderate fold-resistance (3.3 to 6.8-fold) with no major mutations noted. Table 4 contains data from specimens from patients prescribed protease inhibitors. Of note, nine specimens from patients prescribed nelfinavir had resistance to that drug. Six of these were from patients who were infected with a virus that was subtype D in the protease gene, had a D30N or D30D/N mutation present, and did not demonstrate cross-resistance to other protease inhibitors. The other three specimens were from patients whose HIV was subtype A in the protease gene and did not have a mutation at position 30; two had an M46I mutation, and the other had an N88S mutation. Minor protease mutations were present for all three specimens, and cross-resistance to other protease inhibitors was evident for one.

Only one specimen without resistance to a protease inhibitor had a major mutation noted. This was from a patient taking ritonavir plus saquinavir who had an L90M mutation on day 201 and later developed phenotypic resistance to multiple protease inhibitors on day 334 (Table 4, patient 19). Other minor mutations (positions 10, 20, 36, 71, and 77) were found in specimens with and without phenotypic resistance to protease inhibitors.

Discussion

Resistance to antiretroviral drugs among patients in Uganda who are infected with HIV-1, group M, subtypes A, C, or D and receiving antiretroviral therapy appears to develop in a similar manner to that seen in subtype B infections in patients in industrialized countries [2]. Virologic and immunologic parameters indicated that many patients improved, even when taking antiretroviral drugs to which phenotypic resistance could be demonstrated. The amount of phenotypic resistance we observed is comparable to that found in a recent large survey in the United States [28] and to that found in clinic patients receiving HAART in Italy [29].

The amount of resistance that will occur among patients receiving antiretroviral therapy is difficult to quantify. In the United States in 1999, an estimated 87% of patients with detectable viremia receiving treatment with antiretroviral drugs had evidence of genotypic mutations associated with HIV resistance to at least one drug - 70% for NRTI, 31% for NNRTI, and 42% for protease inhibitors [28]. In one Italian clinic between 1996 and 1998, the estimated proportion of patients treated with protease-inhibitor-based HAART for at least 18 months who had genotypic resistance mutations to protease inhibitors was 18.3% at 1 year and 33.9% at 2 years [29]. Although not directly comparable, our results showing that about one-third of those prescribed HAART and just over one-half of those prescribed two NRTIs had resistance to at least one drug appear to be similar to results from more developed settings. The resistance we observed reflects the practice within the DAI wherein drug selection and modification of therapy were constrained by the patient's ability to afford drugs, the evolving availability of individual drugs, and resistance testing results not being available to the clinicians in time for patient management decisions. Throughout most of the evaluation, the least expensive two NRTIs combination was Combivir®, GlaxoSmithKline, UK (zidovudine plus lamivudine), making it a preferred regimen with or without a third drug. Most of the documented resistance was to lamivudine and was associated with a major genotypic mutation that would be predicted from what is found in subtype B. Although this occurred most frequently in patients receiving two NRTIs combinations, it also occurred in patients receiving HAART when virologic control was lost, consistent with the findings of others [30]. This is similar to the findings, reported in an earlier publication, of resistance among clinical specimens stored at one center in Uganda from 1996 to 1998, before the DAI, in which the major genotypic mutation for resistance to lamivudine was found among all nine specimens from patients taking lamivudine who developed phenotypic resistance to that drug [4].

The prices of antiretroviral drugs declined in 2001 in Uganda, and there has been a shift to increasing use of HAART among patients entering care and a switch to HAART among those already receiving care [31]. This would be expected to lead to more durable virologic responses and lowered or delayed resistance in this population. Inevitably, an increase in the absolute numbers of patients with resistance to NNRTIs and protease inhibitors would be expected as more patients access these drugs and remain on treatment longer, as has been documented in more developed settings [28,29]. Regardless, there have been substantial reductions in morbidity and mortality in North America and Europe during that same time [32,33] consistent with the common clinical perception that resistance, associated with loss of optimal virologic control, will not necessarily lead to widespread near-term failure of antiretroviral drugs [34].

Nearly all instances of phenotypic resistance to specific drugs were associated with use of that drug (either current or in the past), or cross-resistance to another drug in a manner that would be predicted from subtype B infections [2,3,35]. Genotypic mutations associated with

Table 4. Specimens with protease inhibitor resistance.

Specimen no.[b]	Days since started in DAI	Subtype in protease gene	Protease inhibitors prescribed at the time the specimen was obtained	Fold-resistance						Mutations in protease gene[a]																				
				IND (3.0)[c]	RTV (3.5)	NFV (4.0)	SQV (2.5)	AMP (2.5)	LPV (2.5)	L10	K20	L24	D30	V32	L33	M36	M46	I47	G48	I50	I54	A71	G73	V77	V82	I84	N88	L90
1a	511	D	Indinavir	5.5	35.3	12.2	2.8	1.2	nd	V/I	V/M					I					V				A			
1b	546	D	Indinavir	18.1	48.7	27.6	4.3	2.9	nd	V/I						I					V				A			
1c	656	D	Indinavir	2.9	25.5	14.2	1.4	1	20.4	V/I	V/M					I					V				A			L/M
18a[d]	398	D	Indinavir	5.9	2.6	10.9	3.3	1.3	nd	L/I	M					I								I				
18b[d]	610	D	Indinavir	1.4	1.6	1.2	0.4	2.9	1.1	I	M					I							T	I				
19	334	C	Saquinavir, ritonavir	8.6	15.8	9.2	11.9	1.7	3.7							I						A/V				V		M
2b	447	D	Nelfinavir, ritonavir	0.7	0.5	22	0.7	0.8	nd				D/N											I				
2c	545	D	Nelfinavir	0.6	0.3	51	0.3	0.3	nd				N				M/L							I				
2d	640	D	Nelfinavir	1.2	0.5	27.4	0.6	2.2	0.6	F			N											I		N/D		
2e	690	D	Nelfinavir	0.7	0.2	59	0.5	1	1.4	L/F			D/N											I				L/M
20	448	D	Nelfinavir	0.7	0.3	16.9	0.9	1.3	nd				N											I		N/D		
21	274	D	Nelfinavir	1.8	0.9	46.6	0.9	1.3	1.1	I	T		N			I										D		
22	427	A	Nelfinavir	2.4	1.9	4.3	0.4	1.1	nd	F	R			I		I	I											
23	626	A	Nelfinavir	8.9	3.5	17.6	1.8	2.5	1.9	F	R			I		I	I											
24	328	A	Nelfinavir	1.7	0.4	4.7	0.7	0.3	0.5		R					I											S	

[a]The letter preceding the position number corresponds to the expected amino acid of the protease gene. The letters in the data cells correspond to the observed mutant amino acid [2]. [b]The number indicates different patients, and the letter indicates specimens, in chronological order, for the same patient. Patients 1 and 2 have results reported on Table 3 as well. [c]Fold-resistance value above which resistance was considered present. [d]Low-level phenotypic resistance to one or more protease inhibitors found on two occasions without evidence of a major mutation. The patient's viral load on these two occasions was 3862 and 2365 copies/ml, respectively, suggesting adherence to medication. IND, indinavir; RTV, ritonavir; NFV, nelfinavir; SQV, Saquinavir; AMP, amprenavir; LPV, lopinavir; DAI, drug access initiative; nd, not done.

resistance were generally consistent with those found in subtype B infections [2,3]. The few instances of phenotypic resistance or corresponding genotypic mutations not explained in this manner probably reflect variability in the testing process or incomplete data on drug prescribing rather than naturally occurring resistance.

Phenotypic resistance to NNRTIs, but without major mutations, was found in some patients who had no evidence of taking these drugs; others have described this finding among NNRTI-naive patients who have received extensive prior treatment with NRTIs and protease inhibitors [36]. Similarly, others have found genotypic mutations associated with resistance to NNRTIs in some NNRTI-naive patients [36,37], although we found this in only one instance. The absence of a mutation at position 30 of the protease gene among all three subtype-A specimens with documented resistance to nelfinavir in patients prescribed that drug raises the possibility that this subtype may preferentially use a mechanism for resistance that is different from that of subtype B. The few subtype-A specimens tested and the relatively low level of phenotypic resistance among two of the specimens precluded our drawing any firm conclusions regarding their potential importance.

The strength of our study was the ability to correlate antiretroviral drug history and virologic response to phenotypic resistance and corresponding genotypic mutations. Antiretroviral drug histories were obtained as part of actual patient care, which was constrained by drug cost and availability, and blood specimens were not collected at regular intervals, potentially biasing the availability of specimens for resistance testing. Our sampling strategy would be expected to detect many patients harboring HIV with resistance to these drugs as we only tested those who were receiving therapy and had an elevated viral load. We attempted to account for this by estimating the likely resistance among all patients who had viral load measured after 90 days of therapy, but our estimates could be biased since viral loads were not measured with the same frequency in all patients. Thus, our estimate of prevalence should be interpreted cautiously since it may not reflect actual resistance in this population. We chose a viral load value of < 1000 copies/ml as evidence of virologic control and lack of resistance to correlate with the sampling strategy for selection of specimens for resistance testing. Although specimens with a viral load < 1000 copies/ml may harbor resistant viruses [38], we would have been unable to detect them with our methods and resistance had not yet manifested itself clinically as a loss of virologic control.

Phenotypic resistance and corresponding genotypic mutations for these HIV-1, group M, subtypes A, C, and D from this evaluation of patients in Uganda receiving antiretroviral drugs occurred in a similar manner to those seen with subtype B infections in patients in North America and Europe. Beneficial virologic responses were consistent with those found in more developed settings. One consequence of expanding access to HAART in Africa and elsewhere will be the development of drug resistance; a challenge to be incorporated into the design of rational programmes that provide antiretroviral therapy. Standardized approaches to introducing antiretroviral drugs based on the knowledge of likely toxicity and resistance are being advocated for resource-poor settings [39]. Our data support policies that promote the use of HAART regimens against HIV-1, group M, non-B subtypes in a manner consistent with those used for subtype-B infections within the context of programmes that provide sustainable access to and administration of antiretroviral therapy.

Acknowledgements

The authors thank all those involved in the Drug Access Initiative, including the Uganda Ministry of Health, the National Advisory Board of the Drug Access Initiative, the Uganda AIDS Commission, Nsambya Hospital, Joint Clinical Research Center, Mulago Hospital, Mildmay HIV Care and Rehabilitation Center, Mengo Hospital, Medical Access Uganda, Ltd., the Uganda Virus Research Institute, and the Joint United Nations Programme on HIV/AIDS. They also thank Scott Holmberg, MD, MPH, of the CDC for review and editing.

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Antiretroviral therapy in HIV-2-infected patients: changes in plasma viral load, CD4+ cell counts, and drug resistance profiles of patients treated in Abidjan, Côte d'Ivoire

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Objective: To describe changes in plasma viral load, CD4+ cell counts, and drug resistance profiles of HIV-2-infected patients receiving antiretroviral (ARV) therapy in Abidjan, Côte d'Ivoire.

Methods: Consecutive blood samples were collected from 18 HIV-2-infected ARV-naive patients who had received ARV therapy in the UNAIDS drug access initiative (UNAIDS–DAI) in Abidjan between August 1998 and July 2000. Changes in HIV-2 plasma viral load, CD4+ cell counts, and genotypic and phenotypic drug resistance testing were determined.

Results: At baseline, 11 (61%) of the 18 patients initiated highly active antiretroviral therapy (HAART) and seven (39%) received dual therapy. No significant change in median viral load was observed at 2 months ($P = 0.09$), at 6 months ($P = 0.06$), and at 12 months of therapy ($P = 0.26$). No significant increase in CD4+ cell counts was observed at 12 months ($P = 0.10$). All four patients on indinavir-containing HAART had undetectable viral loads at 2–4 months of therapy. However, none of seven patients on nelfinavir-containing HAART had a substantial decrease in viral load. Viruses from 14 patients were analyzed, 12 of which (86%) had at least one primary resistance mutation that is known to confer resistance to HIV-1 virus. Three patients had the multi-drug-resistant mutation, Q151M, two of whom showed reduced susceptibility to zidovudine, didanosine, stavudine and zalcitabine.

Conclusion: Our limited findings show that nelfinavir-containing regimens may have limited virologic benefit to HIV-2-infected patients.

AIDS 2003, **17** (suppl 3):S49–S54

Keywords: Genotypic mutations, HIV-2, indivavir-based highly active antiretroviral therapy, nelfinavir-based highly active antiretroviral therapy, phenotypic resistance, viral load response

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Introduction

Human immunodeficiency virus type 2 (HIV-2) is endemic in West Africa and has spread in the last decade to India and Europe [1–3]. In comparison with persons infected with HIV-1, those infected with HIV-2 have slower disease progression, lower rates of vertical transmission, and lower viral loads while asymptomatic [4–6]. Much information has been published about virologic and immunologic response and antiretroviral (ARV) drug resistance profiles of patients infected with HIV-1 who are receiving therapy [7–9], but less for patients infected with HIV-2 [10–12]. Most cases of HIV-2 infection occur in developing countries, where supplies of ARV drugs are limited. HIV-2 isolates are intrinsically resistant to at least some non-nucleoside reverse transcriptase inhibitors (NNRTIs) [13]. This study describes changes in viral load, CD4+ cell counts, and drug resistance profiles of a case series of 18 HIV-2-infected patients receiving ARV therapy in Abidjan, Côte d'Ivoire.

Materials and methods

Study population

Details of the UNAIDS–Drug Access Initiatives has been published elsewhere in this issue of the journal [14]. For the purpose of this study, we included all HIV-2-infected patients enrolled in the UNAIDS–DAI between August 1998 and October 2001 who gave consent to use information from their medical charts. Patients were eligible in this study if they had received ARV therapy for at least 5 months. Patients were considered to be on highly active antiretroviral therapy (HAART) if they received one protease inhibitor (PI) and two nucleoside reverse transcriptase inhibitors (NRTIs) or were on three NRTIs, and on dual therapy if they received two NRTIs.

Laboratory testing

Blood samples were collected into Vacutainer CPT tubes (Becton Dickinson, San Jose, California, USA). Within 4 h of blood collection, plasma was separated from cells by centrifugation at 200 ***g***, then aliquoted and stored at –70°C. To confirm HIV-2 infection, DNA polymerase chain reaction (PCR) testing was performed on uncultured peripheral blood mononuclear cells (PBMCs) as described previously [15,16]. CD4+ cell counts (FACScan flow cytometer; Becton Dickinson) and RNA viral load (prototype assay; Roche Molecular Systems, Alameda, California, USA) testing for HIV-2 was measured as described previously [17].

Drug resistance testing HIV-2

HIV-2 RNA was extracted by the NucliSens method (NucliSens Isolation kit, Organon Teknika, Boxtel, the Netherlands). The RNA was then used in a reverse transcriptase (RT)–PCR to amplify 1.4 kilobase pairs (kb) (from nt 241 to nt 1687) of the *pol* gene by using specific primers [10,16] and sequencing done by fluorescent dye-labeled sequencing terminators run on an automated DNA sequencer (Applied Biosystems 373, 3100; Applied Biosystems, Foster City, California, USA). Analysis of phenotypic resistance was based on the susceptibility of the RT activity of HIV from plasma to inhibition by the triphosphate form of nucleoside analogs. RT activity in plasma was detected by using the Amp-RT assay [9,18].

Adherence

Adherence at last visit was defined on the basis of several parameters: number of pills skipped, failure to take drugs with or without meals, and numbers of times therapy was interrupted. Each parameter was given a score and the total expressed as percentage adherence.

Statistical analysis

Median levels of viral load and CD4+ cell counts at baseline were compared with levels measured at 2, 6 and 12 months of therapy by a non-parametric sign test for paired data. Statistical analysis was carried out using the software STATA version 7 (Stata Corporation, Texas, USA). All statistical tests were two-sided tests with a significance level arbitrarily set at 0.05.

Results

Patients and HIV-2 characterization

Of the 25 HIV-2-infected patients receiving ARV therapy, 18 (78% male) had no history of prior ARV therapy (ARV-naive). At time of initiation of therapy, median age was 41 years [interquartile range (IQR), 36–47], median CD4+ cell count was 82×10^6 cells/l (IQR, 52–188), median viral load was 4.5 $\log_{10}$ RNA copies/ml (IQR, 4.1–5.2), and median duration of therapy was 11 months (IQR, 7–12). At baseline, the 18 patients received different combinations of therapies with adherence varying from 60 to 90% (Table 1). Mean adherence values were 85.7% for patients who initiated dual therapy, 82.5% for patients on indinavir (IDV)-based-HAART and 82.8% for those on nelfinavir (NFV)-based-HAART.

Changes in plasma viral load and CD4+ cell counts

Overall, compared with baseline levels (4.5 $\log_{10}$ copies/ml; IQR, 4.1–5.2), no statistically significant changes in viral load were observed at 2 months (3.9 $\log_{10}$ copies/ml; IQR, 3.1–4.5; $P = 0.09$), at 6 months (4.0 $\log_{10}$ copies/ml; IQR, 2.8–4.7; $P = 0.06$), or at 12 months of therapy (4.1 $\log_{10}$ copies/ml; IQR, 3.2–4.9; $P = 0.26$). CD4+ cell counts increased gradually, albeit insignificantly, from a median 82×10^6 cells/l (IQR, 52–188) at baseline to 162×10^6 cells/l (IQR, 115–208) at 2 months ($P = 0.08$), 154×10^6 cells/l (IQR, 68–275) at 6 months ($P = 0.12$), and 163×10^6 cells/l (IQR, 132–244) at 12 months of therapy ($P = 0.1$).

Table 1. Types of antiretroviral regimens, adherence, and genotypic mutations among 18 HIV-2-infected patients treated with antiretroviral therapy.

Patient number	ARV treatment[a]		Percentage adherence at time of sequencing	Duration of treatment at time of sequencing	Genotypic resistance mutations[b]
	Initiated at baseline	At time of sequencing			
	Non-HAART				
P0071	ZDV + ddI	ZDV + ddI	70	14 months	**G48V, Q151M**
P0025	ZDV + ddI	D4T + 3TC + **NFV**	80	14 months	**Q151M**
P0145	ddI + D4T	ZDV + 3TC + **NFV**	90	11 months	K70N
P0146	ZDV + ddI	ZDV + 3TC + **NFV**	90	13 months	**S215Y**, F221Y
P0548	ZDV + ddI	ddI + D4T	90	10 months	none
P0574	ZDV + ddI	ZDV + ddI	90	10 months	Undetectable
P1809	ddI + D4T + 3TC	ZDV + 3TC + **NFV**	90	12 months	**M184V**
	IDV-based HAART				
P1077	D4T + 3TC + **IDV**	ZDV + 3TC + **IDV**	90	11 months	Undetectable
P2760	ZDV + 3TC + **IDV**	ZDV + 3TC + **IDV**	90	10 months	Undetectable
P1028	ZDV + 3TC + **IDV**	ZDV + 3TC + **NFV**	60	6 months	Undetectable
P2491	ZDV + 3TC + **IDV**	ddI + D4T + **NFV**	90	8 months	None
	NFV-based HAART				
P1458	ddI + D4T + **NFV**	ddI + D4T + **NFV**	70	12 months	**L90M, Q151M**
P3336	ddI + D4T + **NFV**	ZDV + 3TC + **IDV**	80	2 months	**M184V**
P1584	ZDV + 3TC + **NFV**	D4T + 3TC + **NFV**	90	13 months	**M184V**
P1797	ZDV + 3TC + **NFV**	ZDV + 3TC + **NFV**	70	14 months	**M184V**
P1891	ZDV + 3TC + **NFV**	ZDV + 3TC + **NFV**	90	7 months	**M184V**
P2187	ZDV + 3TC + **NFV**	ZDV + 3TC + **NFV**	90	11 months	**M184V**
P3935	ZDV + 3TC + **NFV**	ZDV + 3TC + **NFV**	90	7 months	**M184I**

[a]Protease inhibitors are in bold type. [b]Primary genotypic mutations that have been shown to confer phenotypic resistance to the antiretroviral drugs (validated for HIV-1 viruses) are shown in bold. HAART, highly active antiretroviral therapy; ZDV, zidovudine; 3TC, lamivudine; D4T, stavudine; ddI, didanosine; NFV, nelfinavir; IDV, indinavir; SQV, saquinavir; ND, not done.

Response to IDV-containing HAART

All five patients who initiated treatment with, or switched to, IDV-containing HAART had sustained suppression of viral load (Fig. 1, first four patients: 1077, 1028, 2491, 3336). At baseline, viral load for patient 1077 was 4.3 $\log_{10}$ RNA copies/ml and the CD4+ cell count was 19×10^6 cells/l. At 1, 7 and 11 months his viral load became undetectable with a rebound at 4 months. His CD4+ cell count increased to 291×10^6 cells/l at 11 months (Fig. 1). The viral load for patient 1028 declined from 4.1 $\log_{10}$ copies/ml to undetectable levels and CD4+ cell count increased from 43 to 81×10^6 cells/l. A switch to NFV-based HAART resulted in rising viral load and stable CD4+ cell counts (Fig. 1). Patient 2491 started with an IDV-based HAART regimen [zidovudine (ZDV) + lamivudine (3TC) + IDV]; at baseline his viral load was 4.5 $\log_{10}$ copies/ml and CD4+ cell count was 232×10^6 cells/l. At 3 months, viral load decreased to undetectable level and CD4+ cell count decreased to 160×10^6 cells/l. However, when the patient was switched to an NFV-based HAART regimen, CD4+ cell count increased to 270×10^6 cells/l. For patient 3336, his viral load declined from 5.2 to 4.9 $\log_{10}$ copies/ml at 2 months of therapy (Fig. 1). When the patient was switched to an IDV-based regimen at 4 months, viral load decreased drastically by 1.5 $\log_{10}$ copies/ml (from 4.9 $\log_{10}$ copies/ml at 2 months to 3.4 $\log_{10}$ copies/ml at 5 months) and CD4+ cell count also increased from 52 to 153×10^6 cells/l at 5 months. Viral load was undetectable at baseline, and at follow-up visits for patient 2760 (figure not shown). None of these patients had genotypic-resistant mutations.

Response to NFV-containing HAART

By contrast, none of the six patients who initiated and continued with NFV-containing HAART had substantive virologic response. Change in viral load ranged from –0.52 to +1.72 $\log_{10}$ copies/ml during the follow-up period. However, in some patients (P1458, 1797, 1891, 3935, and 2187) CD4+ cell counts did not change remarkably (Fig. 1). All seven patients who initiated NFV-based HAART at baseline had viruses with genotypic drug resistance: six had the M184V/I mutation and the seventh had both the L90M and Q151M mutations (Table 1).

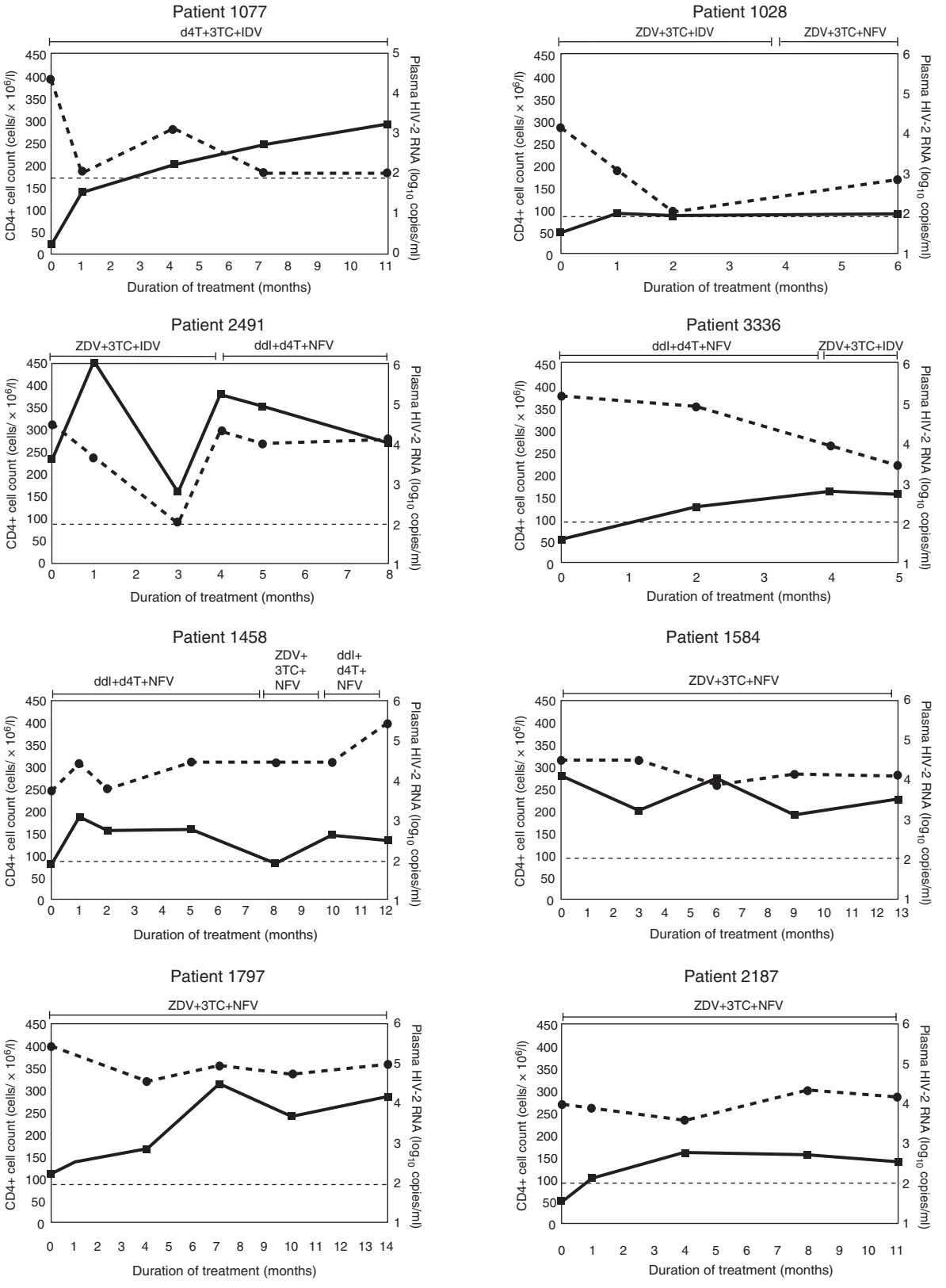

Fig. 1. Continued opposite

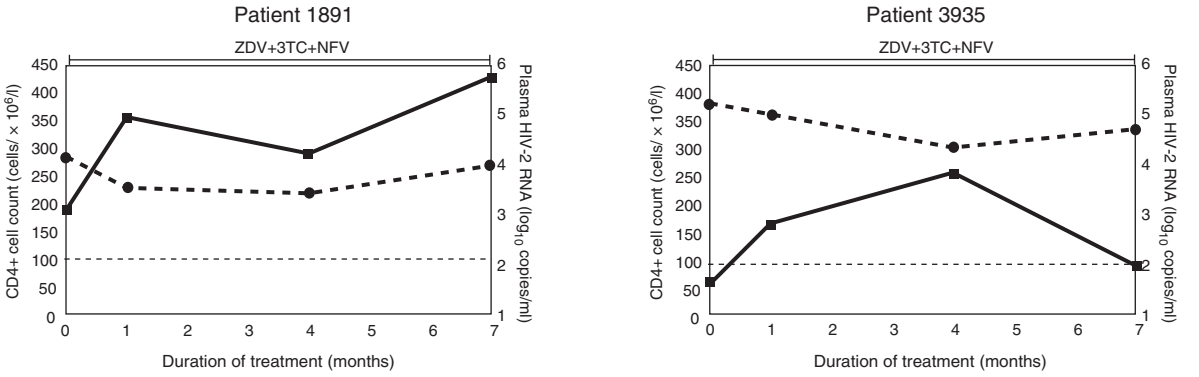

Fig. 1. Changes in plasma viral load and CD4+ cell counts among HIV-2-infected patients on indinavir-based highly active antiretroviral therapy with good responses versus those on nelfinavir-based HAART with poor responses. Viral loads are indicated in thick broken lines and CD4+ cell counts in thick continuous lines. The thin hatched line below represents the lower limit of detection of the HIV-2 viral load assay. The horizontal line above indicates the duration and changes in antiretroviral (ARV) therapy. ZDV, zidovudine; 3TC, lamivudine; D4T, stavudine; ddI, didanosine; SQV, saquinavir.

Genotypic drug resistance

Of the 18 HIV-2 patients, RT-PCR testing was negative for both RT and protease genes for four plasma samples. For the viruses obtained at 2 to 14 months from the remaining 14 patients, 11 (78.6 %) had genotypic resistance mutations to at least one reverse transcriptase inhibitor (RTI) (Table 1). Of the 13 patients who had received ZDV-containing regimens, one (7.7%) had a virus that contained the S215Y mutation. Seven (70%) out of 10 patients who had ever received 3TC-containing therapy had viruses with the M184V/I mutation that confers primary resistance to 3TC in HIV-1. Of the nine patients who had received a regimen with didanosine (ddI)-containing NRTI together with either stavudine (D4T) or ZDV, three (33.3%) harbored viruses with the Q151M mutation.

Of the 14 patients, DNA PCR testing was successful in the protease region of the viruses from eight patients. Only one (12.5%) of these patients who had ever received PI-containing regimens had a known primary PI mutation, the L90M mutation (Table 1). The Q151M mutation was only found among patients who had used ddI in combination with D4T or ZDV. Similarly, the M184V/I mutation was detected only among patients who were on 3TC-containing regimens (Table 1).

Phenotypic resistance to nucleoside reverse transcriptase inhibitors

We analyzed in detail the phenotypic profiles of three of the four patients who had the Q151M or S215Y mutations. At baseline, no genotypic and phenotypic resistance was detected for patient 0146. At 9 months of therapy, we detected the S215Y mutation, without changes in ZDV and 3TC susceptibility. At 15 months of therapy we found the M184V mutation in addition to the S215Y mutation, and phenotypic resistance to 3TC was observed.

For patient 0025, the Q151M mutation was detected at 12 and 14 months of therapy with corresponding low-level phenotypic drug resistance [3.9- to 5-fold increase in 50% inhibitory concentration (IC_{50})] to ZDV, ddI, and zalcitabine (ddC).

Similarly the Q151M mutation was detected in patient 0071 at 14 months, with a corresponding low-level phenotypic resistance to ZDV, ddI, and ddC (1.5- to 17.3-fold increase in IC_{50}).

Discussion

Our results show no significant reduction in viral load among 18 HIV-2-infected patients after a median of 11 months of ARV therapy. Indeed, our findings contrast with what is known for HIV-1 infection, where even ARV mono- or dual-therapy results in at least a temporary short-term reduction in viral load [19,20]. The observed lack of significant decrease in viral load, albeit based on a limited number of patients, is unlikely to be due to poor adherence to ARV because 12 out of the 18 patients reported at least 80% adherence (Table 1).

Our results on patients receiving IDV- or NFV-based HAART are consistent with the few studies that have been published: Van der Ende *et al.* [21] reported that 10 out of 11 HIV-2-infected patients treated with ZDV + 3TC + IDV had sustained viral suppression, whereas six patients receiving other ARV combinations had poor virologic responses; Schutten *et al.* [22] reported good viral load responses among six HIV-2-infected naive patients treated with ZDV + 3TC + IDV but not for two treated with ZDV + 3TC + NFV. Poor virologic responses were also observed in a case series of seven HIV-2-infected patients treated with NFV-containing regimens [23]. If virologic response depends on

the type of PI used, therapeutic options for HIV-2-infected patients and those co-infected with HIV-1 and HIV-2 may be more limited, since HIV-2 isolates are also known to be naturally resistant to NNRTIs [13].

In HIV-1 infection, the multi-drug-resistant mutation (Q151M) usually develops in 3 to 17% of patients after more than 2 years of therapy with ddI in combination with ZDV or D4T [24,25]. In our study, the Q151M mutation developed in three (33.3%) of the nine patients receiving ddI and ZDV or D4T within 12 months of therapy. These preliminary data indicate that the Q151M mutation may represent a major pathway for nucleoside resistance for HIV-2 viruses and also leads to NRTI cross-resistance.

In summary, our limited study shows that among HIV-2-infected naive patients, virologic response to HAART may depend on the choice of PI, and that the Q151M mutation may represent a major pathway for nucleoside resistance in HIV-2 viruses. Studies of a larger number of HIV-2-infected patients receiving different PI-based HAART are needed to develop appropriate guidelines for treating HIV-2-infected persons.

Acknowledgements

The authors thank Dr Danuta Pienazek for HIV-2 sequence analysis, Debra R. Adams, Richard Respess, Gaston Djomand, Thierry Roels, Maran Mathieu, Bile Celestin, Nguessan Kabran and Pascal Ebi for technical assistance, Shirley Kwok and Cindy Christopherson, formerly of Roche Molecular Systems for support with HIV-2 load assay. C.A.-T. received a travel grant from the American Society for Microbiology Minority and International Activities (UNESCO-ASM Travel Award 2000) to do this work at the Centers for Disease Control, Atlanta, Georgia, USA.

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Clinical, immunological and virological response to different antiretroviral regimens in a cohort of HIV-2-infected patients

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Objective: To assess the clinical, immunological and virological response and the emergence of resistance towards antiretroviral therapy (ART) in a cohort of HIV-2-infected patients.

Design: Observational study.

Patients: HIV-2-infected patients residing in the Netherlands.

Results: From 1995 to 2001 seven patients failed various ART regimens. The resistance mutations were analysed retrospectively. Development of mutations proved to be similar to that observed in HIV-1-infected patients, with the exception of a higher occurrence of the Q151M mutation within the reverse transcriptase gene. In a prospective study, comprising 13 consecutive naive HIV-2-infected patients, all patients achieved plasma HIV-2-RNA suppression below the detection limit (500 copies/ml). The antiretroviral regimen consisted of two nucleoside reverse transcriptase inhibitors (NRTIs) and indinavir, with a boosting dose of ritonavir; the median follow-up was 91 weeks. Two patients experienced a temporary virological rebound, while at the same time therapeutic drug monitoring showed sub-therapeutic plasma levels of indinavir.

Conclusion: Sustained viral suppression in HIV-2-infected patients can be achieved using an antiretroviral regimen of two NRTIs and boosted indinavir or lopinavir.

AIDS 2003, **17** (suppl 3):S55–S61

Keywords: Antiretroviral drug resistance, antiretroviral therapy, HIV-2, HIV-2 RNA, nucleoside analogues, protease inhibitors

Introduction

In HIV-1 infection various combinations of three or more antiretroviral agents can achieve durable suppression of viral replication [1–4]. This has resulted in declined morbidity and prolonged survival for HIV-1-infected patients [5]. At present there are few reported data on the treatment of HIV-2 infection [6–8]. Most HIV-2 infections occur in the developing world, where antiretroviral drugs are largely unavailable. In Europe and

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the United States in-vivo monitoring of HIV-2 infection has been hampered by the absence of validated assays to measure plasma HIV-2 RNA. Efficacy *in vitro* of nucleoside analogue reverse transcriptase inhibitors (NRTIs) [9] and protease inhibitors (PIs) [10,11] is comparable for HIV-1 and HIV-2, with the exception of amprenavir (APV) [12]. The third available class of drugs, the non-nucleoside reverse transcriptase inhibitors (NNRTIs) has shown minimal in-vitro inhibiting effect on HIV-2 replication [13].

Genotypic resistance testing of HIV-1, in combination with a detailed antiretroviral therapy (ART) history, can be used to predict a salvage regimen with an optimal virological response [14]. This is due to the large amount of data available on mutations in the HIV-1 genome associated with antiretroviral drug resistance. The currently available data on HIV-2 genome mutations that emerge during ART failure suggest minor differences between HIV-1 and HIV-2. In-vitro and in-vivo data on drug sensitivity of HIV-2 variants are scarce [15–17].

In this study we first documented the emergence of resistance-associated mutations and the results of salvage therapy in HIV-2-infected patients who failed ART. Furthermore, we prospectively observed the clinical, immunological and virological response of therapy with zidovudine (ZDV), lamivudine (3TC) and indinavir (IDV)/ritonavir (RTV) in 13 naive HIV-2-infected patients in the Netherlands.

Materials and methods

Patients

Approximately 45 HIV-2-seropositive individuals visit HIV outpatient clinics in one of the HIV hospitals in the Netherlands. Twenty of these individuals currently receive or have received ART consisting of a combination of one protease inhibitor (PI) and two NRTIs, two PIs or three NRTIs.

Group I

The first seven HIV-2-infected patients were all analysed retrospectively. None of the patients received ART before the start of combination therapy, except for patient RH2-5, who was treated with ZDV monotherapy for 15 months and ZDV + 3TC for 22 months [15]. Patients RH2-19 and RH2-20 were dually infected with HIV-1 and HIV-2 and have in part been described elsewhere [7]. From these seven patients stored ethylenediamine tetraacetic acid samples were available for retrospective assessment of plasma HIV-2-RNA load and sequence analysis.

Group II

The next 13 HIV-2-infected patients were enrolled in an open-label, non-randomized study from May 1997 to January 2002. Eligible patients were at least 18 years old, had a CD4+ T-cell count of 300×10^6 cells/l or less and had never received ART. The only exclusion criterion was the presence of an active opportunistic infection. All patients started with ZDV 300 mg twice daily (bid), or stavudine (d4T) 40 mg bid, combined with 3TC 150 mg bid and IDV 800 mg/RTV 100 mg bid. In all patients clinical assessments, plasma HIV-2 RNA, CD4 cell count and plasma IDV levels were monitored before and during therapy at 3-monthly intervals.

HIV viral load assay and HIV-2 protease and reverse transcriptase genotyping

The plasma HIV-2 RNA load was measured with a previously described in-house-developed real-time reverse transcriptase–polymerase chain reaction (RT-PCR) detection assay [18]. For genotyping antiviral drug resistance HIV-2 RNA was reverse transcribed with AMV RT (Promega, Leiden, the Netherlands) and primer HIV-2 pol rpr5 (5′-TGTATAGCTTCCCCT-TTCTGTTGA-3′) for 2 h at 55°C in 25 µl final volume. HIV-2 protease (prot) and RT amplicons were generated in a nested PCR format using for the first round PCR: 5 µl complementary DNA, 1 µl 20 µmol/l forward primer HIV-2 pol fpr6 (5′-ACCTGCCCC-TATCCCATTCGC-3′), 1 µl 20 µmol/l reverse primer HIV-2 pol rpr5, 20 µl 1 mmol/l dNTPs (Amersham Life Science, Roosendaal, the Netherlands), 10 µl 25 mmol/l $MgCl_2$, 52 µl H_2O, 10 µl 10 × PCR buffer II and 1 µl AmpliTaq Gold (Roche Diagnostics, Almere, the Netherlands). In the nested PCR 2.5 µl of the first round PCR product was used with 1 mmol/l dNTPs (Amersham Life Science), 10 µl 25 mmol/l $MgCl_2$, 54.5 µl H_2O, 10 µl 10 × PCR buffer II and 1 µl AmpliTaq Gold. For HIV-2 Prot 1 µl 20 µmol/l forward primer HIV-2 prot fpr1 (5′- AGGGCTGCTGGAAGT-GTGGTAA-3′) and 1 µl 20 µmol/l reverse primer HIV2 prot rpr1 (5′- TCAGTTAGGAATTCCACA-CCCAGCA-3′) and for HIV-2 RT 1 µl 20 µmol/l forward primer HIV-2 RT fpr1 (5′-GACAGGCGA-CACCCCAATC-3′) and 1 µl 20 µmol/l reverse primer HIV-2 RT rpr1 (5′-AAGAAGCACTAGTCATTT-GGGGA-3′), were used. Amplicons were sequenced with internal sequence primers using the DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech, Roosendaal, the Netherlands) and detected on an ABI Prism 373 DNA sequencer (Applied Biosystems, Niewerkerk a/d IJssel, the Netherlands). Sequences were analysed using the Lasergene Software Package (DNASTAR, Madison, Wisconsin, USA).

Results

Study population

Baseline characteristics of patients in group I and group II are shown in Table 1. The median observation follow-up since start of ART in group I was 195 weeks (range, 91–234). Six out of the seven patients received more than one ART regimen (Table 2). The table shows

Table 1. Baseline characteristics of HIV-2-infected patients.

		Group I (n = 7)	Patients	Group II (n = 13)	Patients
Gender	Male	3		8	
	Female	4		5	
Nationality	Cape Verdian	3	RH2-5, 19 and AH2-5	8	RH2-16, 18, 21, 23, 24, 25, 27 and RH2-28
	Portuguese	–		1	RH2-17
	Dutch	1	AH2-1	4	RH2-26 and AH2-4, 6 and 7
	Ghanese	2	AH2-2, 3		
	Ivory Coast	1	RH2-20		
Transmission	Heterosexual	7		13	
Age (years)	mean (SD)	39 (± 6.1)		50 (± 16.9)	
HIV-2 RNA (copies/ml)					
	> 100 000	2		5	
	> 500 and < 100 000	5		3	
	< 500	0		2	
CD4 cell count (cell × 10^6/l)	< 200	7		10	
	> 200 and < 500			3	
CDC status	A	3			
	C	4	RH2-5 and AH2-2,3 RH2-19, 20 and AH2-1,5	6	RH2-16, 17, 18, 23, 24, 28 and AH2-7,8 RH2-21, 25, 26, 27

the time on each regimen. The median follow-up in group II was 91 weeks (range, 52–234). One patient in each group repatriated to Portugal after 91 and 104 weeks of follow-up (RH2-19 and RH2-17). In group I two patients developed a CDC-defined AIDS diagnosis (RH2-2 and AH2-2), as opposed to none of the patients in group II.

Virological and immunological outcome

In group I two of seven patients retrospectively responded to first-line therapy with HIV-2-RNA suppression below the detection limit (500 copies/ml). Subsequently, all patients experienced virological failure. The regimens were changed because of virological failure (RH2-2, RH2-20, AH2-3 and AH2-5), side effects requiring treatment interruption (AH2-1) or inadequate drug levels due to malabsorption (AH2-2). The median CD4+ T-cell count, 0.06×10^6 cells/l (range, $0.01–0.18 \times 10^6$) at $t = 0$ and 0.06×10^6 cells/l (range, $0.02–0.36 \times 10^6$) at the end of follow-up, did not increase.

In a salvage regimen containing lopinavir (LPV)/RTV, with or without tenofovir (TDF), viral suppression was achieved in five patients.

All patients in group II achieved initial HIV-2-RNA suppression to below the detection limit of 500 copies/ml (Table 2). Eleven out of 13 patients (85%) in group II remained below the detection limit (500 copies/ml) during the entire period of follow-up. The ART regimens and plasma HIV-2-RNA results during therapy are shown in Table 2. One patient failed at 12 weeks of follow-up and had low IDV plasma levels, due to a drug interaction with omeprazol (AH2-7). Another patient (RH2-24) showed a temporary failure at weeks 78 and 91 (HIV-2 RNA 27 000 and 12 000 copies/ml, respectively) due to non-adherence. Both patients have been counselled, are still on the same regimen, with renewed HIV-2-RNA suppression < 500 copies/ml. The median CD4 cell count at baseline was 90×10^6 cells/l (range, $10–360 \times 10^6$). This number increased to 140×10^6 cells/l (range, $70–310 \times 10^6$), 220×10^6 cells/l (range $60–340 \times 10^6$), 230×10^6 cells/l (range $40–380 \times 10^6$), and 270×10^6 cells/l (range $60–410 \times 10^6$) after 4, 12, 24, and 52 weeks of therapy, respectively.

Side effects were comparable with those observed in HIV-1 infection, including nausea, dry skin, paronychia and lipodystrophy. In two patients the IDV dosage was adjusted because of IDV plasma levels. None of the patients developed urological symptoms. Two patients switched to LPV/RTV after 52 and 65 weeks respectively, because of nausea due to IDV.

Genotyping *Prot* and *RT* genes

HIV-2 *Prot* and *RT* genes were sequenced and compared with the HIV-1 and HIV-2 predicted amino acid consensus sequence from the Los Alamos Database (http://hiv-web.lanl.gov/) at positions known to be associated with resistance to antiretroviral drugs in HIV-1 infection (Table 3 and Table 4). Within the

Table 2. Results of antiretroviral treatment in HIV-2-infected patients

Patient	ART	HIV-2 RNA (copies/ml)					
		$t = 0$ weeks	$t = 4$ weeks	$t = 12$ weeks	$t = 26$ weeks	Last follow-up	Total time on regimen
RH2-5	RTV+SQV	3.2×10^5	NT	2.0×10^3	8.1×10^5	5.7×10^5	69
	ddI+D4T+ABC	5.7×10^5	1.1×10^5	6.5×10^5	7.3×10^5	7.3×10^5	24
	TFV+LPV+3TC	1.4×10^4	5.3×10^2	< 500			12
RH2-19	RTV+SQV	9.3×10^3	< 500	N.T.	4.3×10^4	1.9×10^4	91
RH2-20	ZDV+3TC+NFV	2.3×10^3	< 500	8.0×10^3	2.1×10^4	1.3×10^4	60
	ZDV+3TC+IDV	1.3×10^4	3.9×10^3	1.9×10^4	1.6×10^5	1.6×10^5	24
	ZDV+3TC+LPV/RTV	1.6×10^5	1.7×10^5	2.9×10^4	1.4×10^4	2.8×10^3	52
	ZDV+3TC+LPV/RTV+TFV	2.8×10^3	< 500				4
AH2-1	D4T+3TC+RTV+SQV	1.5×10^4	< 500	< 500	< 500	2.7×10^4	78
	D4T, 3TC	1.3×10^4	NT	2.5×10^5	1.7×10^5	3.4×10^4	42
	ZDV, 3TC, LPV/RTV	3.4×10^4	NT	< 500	< 500		26
AH2-2	ZDV+3TC+IDV	3.2×10^5	2.1×10^3	3.0×10^4	1.0×10^5	1.7×10^5	52
	D4T+3TC+RTV+SQV	4.0×10^5	5.6×10^5	NT	4.0×10^5	1.6×10^3	117
AH2-3	ZDV+3TC+ddI+ABC	1.0×10^4	NT	NT	NT	2.5×10^3	72
	ABC+3TC+IDV+RTV	2.5×10^3	< 500	< 500	4.3×10^3	< 500	83
	3TC+LPV/RTV	< 500	< 500	< 500		< 500	12
AH2-5	D4T+3TC+ddI	5.0×10^3	NT	NT	NT	15	
	ZDV+3TC+LPV/RTV	6.4×10^4	7.2×10^2	< 500	2.3×10^3	12	
RH2-16	ZDV+3TC+IDV/RTV	< 500	< 500	< 500	< 500	< 500	260
RH2-17	ZDV+3TC+IDV/RTV	< 500	< 500	< 500	< 500	< 500	104
RH2-18	ZDV+3TC+IDV/RTV	6.0×10^3	< 500	< 500	< 500	< 500	182
RH2-21	ZDV+3TC+IDV/RTV	7.3×10^3	< 500	< 500	< 500	< 500	156
RH2-23	ZDV+3TC+IDV/RTV	6.0×10^3	< 500	< 500	< 500	< 500	117
RH2-24	ZDV+3TC+IDV/RTV	5.0×10^4	< 500	< 500	< 500	< 500	104
RH2-25	ZDV+3TC+IDV/RTV	4.7×10^3	< 500	< 500	< 500	< 500	52
	ZDV+3TC+LPV/RTV	< 500	< 500	< 500	< 500	< 500	39
RH2-26	ZDV+3TC+IDV/RTV	3.3×10^5	< 500	< 500	< 500	< 500	78
RH2-27	ZDV+3TC+IDV/RTV	6.2×10^5	< 500	< 500	< 500	< 500	65
RH2-28	ZDV+3TC+IDV/RTV	3.8×10^5	< 500	< 500	< 500		38
AH2-4	ZDV+3TC+IDV/RTV	1.0×10^4	< 500	< 500	< 500	< 500	65
	ZDV+3TC+LPV/RTV	< 500	< 500	< 500	< 500	< 500	26
AH2-6	ZDV+3TC+IDV/RTV	1.0×10^5	4.0×10^4	< 500	< 500	< 500	52
AH2-7	ZDV+3TC+IDV/RTV	1.3×10^5	< 500	2.2×10^4	1.4×10^4	< 500	52

ART, Antiretroviral therapy; ZDV zidovudine; RTV, ritonavir; SQV, saquinavir; ddI, didanosine; D4T, stavudine; ABC, abacavir; TFV, tenofovir; LPV, lopinavir; 3TC, lamuvidine; NFV, nelfinavir; IDV, indinavir; NT, not tested.

HIV-2 consensus several mutations, known to be associated with resistance to PIs, were present relative to the HIV-1 consensus. These included 10V, 32I, 36I, 46I, 47V, 71V and 82I (Table 2). In patients receiving a combination of RTV and saquinavir (SQV) the 90M mutation within the *Prot* gene, together with either the 82F (AH2-2) or the 84V (RH2-5 and RH2-19) mutation, was consistently observed. In the virus from patient RH2-20, who was treated with nelfinavir (NFV), no mutations with known PI resistance had developed after 14 months of therapy. After one additional month on ZDV, 3TC and NFV and 3 months after switching from NFV to IDV, however, the 90M mutation was observed, and subsequently disappeared after four additional months of IDV therapy. These data suggest that the 90M mutation developed during the 15 months of NFV treatment and that IDV did not facilitate its selection.

The most frequently observed mutations within the *RT* gene were the 184V and 151M mutations, which have been shown in HIV-1 to give 3TC/ddI/abacavir (ABC) and multi-NRTI, resistance, respectively [11]. No ZDV resistance-associated mutations were observed except for the 151M mutation. In addition to the already present 151M and 184V mutations, the 65R mutation developed after 7 months on d4T, ddI and ABC treatment in patient RH2-5.

Table 3. Amino acid substitutions within HIV-2 protease at positions associated with PI resistance of HIV-1.

	ART regimen	Months on therapy	10	20	24	30	32	36	46	47	48	50	54	63	71	77	82	84	88	90
HIV-1			L[a]	K	L	D	V	M	M	I	G	I	I	L	A	V	V	I	N	L
HIV-2			V	V	L	D	*I*	*I*	*I*	*V*	G	I	I	E	V	T	I	I	N	L
RH2-5	ZDV/3TC	6							V											
	RTV/SQV	13							V									V		M
	D4T/ddI/ABC	7							V											
RH2-19	RTV/SQV	22											L		*I*			**V**		M
RH2-20	ZDV/3TC/NFV	14													***I***					
	ZDV/3TC/IDV	3		*I*											***I***					M
		7		***I***									*M*		***I***					
AH2-1	D4T/3TC/SQV	18						V												
AH2-2	Pre-therapy																			
	ZDV/3TC/IDV	9															F			
	D4T/3TC/RTV/SQV	9		*I*													**F**	L		M
AH2-3	ZDV/3TC/ddI(ABC)**V	18(4)						V												
AH2-5	Pre-therapy																			
	ddI/3TC/d4T	3		A																

[a]The HIV-1 and HIV-2 consensus predicted amino acids (HIV-1 and HIV-2 respectively) are taken from the Los Alamos Database (http://hiv-web.lanl.gov/). The amino acids in the HIV-2 consensus associated with protease inhibitor (PI) resistance of HIV-1 are indicated in italic only. Reported are mutations in the patient viruses, relative to the HIV-2 consensus. Mutations that are associated with resistance of HIV-1 against the medication taken by the patient are shaded: bold italic denotes mutations to amino acids other than those arising in HIV-1, bold upright denotes mutations comparable with those arising in HIV-1. ART, antiretroviral therapy; ZDV zidovudine; RTV, ritonavir; SQV, saquinavir; ddI, didanosine; D4T, stavudine; ABC, abacavir; 3TC, lamuvidine; NFV, .elfinavir; IDV, indinavir.

Table 4. Amino acid substitutions within HIV-2 reverse transcriptase at positions associated with NRTI resistance of HIV-1.

	ART regimen	Months on therapy	41	62	65	67	69	70	74	75	77	115	116	151	184	210	215	219
HIV-1			M[a]	A	K	D	T	K	L	V	F	Y	F	Q	M	L	T	K
HIV-2			M	A	K	D	N	K	L	I	F	Y	F	Q	M	N	S	E
RH2-26	Pre-therapy																	
RH2-27	Pre-therapy																	
RH2-5	ZDV/3TC	6												**M**	**V**			
	RTV/SQV	13												M				
	D4T/ddI/ABC	7			R									**M**				
RH2-19	RTV/SQV	22								M								D
RH2-20	ZDV/3TC/NFV	14													V			
	ZDV/3TC/IDV	3													**V**			D
		7													**V**			
AH2-1	D4T/3TC/SQV	18													**V**			
AH2-2	Pre-therapy																	
	ZDV/3TC/IDV	10												**M**	**V**			
AH2-3	ZDV/3TC/ddI(ABC)	18(4)												**M**	**V**			
AH2-5	ddI/3TC/d4T	3																

[a]The HIV-1 and HIV-2 consensus predicted amino acids (HIV-1 cons and HIV-2 cons respectively) are taken from the Los Alamos Database (http://hiv-web.lanl.gov/). Mutations in the patient viruses, relative to the HIV-2 consensus, are given. Mutations that are associated with resistance of HIV-1 against the medication taken by the patient are denoted in bold. ART, antiretroviral therapy; ZDV zidovudine; RTV, ritonavir; SQV, saquinavir; ddI, didanosine; D4T, stavudine; ABC, abacavir; TFV, tenofovir; LPV, lopinavir; 3TC, lamuvidine; NFV, nelfinavir; IDV, indinavir; NRTI, nucleoside reverse transcriptase inhibitor.

No mutations had developed within the *Prot* and *RT* genes from patient AH2-5, probably because of the short interval between starting therapy and sequencing.

Discussion

In the present paper we present retrospectively collected data on a cohort of HIV-2-infected individuals receiving ART. We conclude that first-line regimens consisting of three NRTIs, two PIs, or two NRTIs and NFV are inadequate for the treatment of HIV-2-infected individuals. When the *RT* gene mutation Q151M emerged, TDF seemed a valuable addition to the anti-HIV-2 regimen.

In the prospectively studied cohort of 13 antiretroviral-naive HIV-2-infected patients the combination of two NRTIs and IDV/RTV showed 100% efficacy in achieving and sustaining viral suppression. As in HIV-1 infection, LPV/RTV also proved to be able to suppress HIV-2 replication. These results agree with the experience of treating HIV-2 infected patients with ART in Africa, as reported by Nkengasong in this supplement.

The relative success of ART in the treatment of HIV-1 is largely dependent on the availability of potent drugs from several classes. The limited availability of effective antiretroviral drugs is a major problem in the treatment of HIV-2-infected individuals. The choice of the first-line regimen is even more important for the virological and clinical outcome of therapy than in HIV-1 infection. Wild-type HIV-2 contains several mutations within the *Prot* gene that are associated with PI resistance in wild-type HIV-1. The combination of 32I, 46I, 47V and 82I in wild-type HIV-2 protease sequences proved in HIV-1 to be associated with high-level resistance to APV and low-level resistance to IDV, RTV and NFV [19]. As with the mutations at position 77 (V to G/R/K/E/A) in HIV-1, the threonine at position 77 in wild-type HIV-2 probably also results in reduced susceptibility of HIV-2 towards NFV [20]

In our study the two patients (RH2-5 and RH2-19) receiving RTV+SQV as first-line therapy had a treatment failure, despite a low baseline plasma HIV-2 RNA. This is not in agreement with studies performed in HIV-1-infected patients, in whom dual PI therapy with RTV/SQV was sufficient in patients with baseline HIV-1-RNA concentrations < 100 000 copies/ml [3]. Furthermore, in the dually HIV-1/HIV-2-infected patient RH2-19, HIV-1-RNA levels decreased to below 500 copies/ml, whereas the HIV-2-RNA concentration rebounded after initial suppression, suggesting reduced susceptibility of HIV-2 to RTV/SQV. Reduced susceptibility of HIV-2 to NFV was demonstrated in another dually HIV-1/HIV-2-infected patient (RH2-20). Two additional HIV-2-infected patients were also reported to fail a NFV-containing regimen (Dr J. Breuer, personal communication). In HIV-1-infected patients with a low viral load, ART with a triple NRTI regimen resulted in suppression of viral replication [21]. In our study triple/quadruple NRTI regimens, applied in two (AH2-3 and AH2-5) patients with a low baseline viral load, failed. Although the numbers are very small, this may suggest that triple NRTI therapy is inadequate for HIV-2 suppression.

Due to the lack of phenotypic antiretroviral drug resistance data the interpretation of HIV-2 *Prot* and *RT* genotyping data after ART failure is difficult. In our study four out of five patients treated with PIs developed primary resistance-associated mutations within the *Prot* gene (at positions 82, 84 and 90), which have in HIV-1 infections been shown to give significant resistance to IDV, RTV, NFV and SQV [20]. Our data suggest that the development of PI resistance in HIV-2 is similar to that in HIV-1, and that HIV-1 resistance data can be used to interpret HIV-2 *Prot* sequencing data. In line with our previously published studies and those of others, the 151M mutation in the RT protein was detected in a high percentage of individuals treated with NRTIs (50 versus 1–4% in HIV-1-infected individuals) [22, 23]. The mutation at position 184 of the RT protein associated with 3TC resistance developed in a high percentage of the individuals treated with 3TC (83%). Mutations in the RT protein at the ZDV resistance-associated positions 41, 210, 215, 219 were not observed [15,16].

TDF is a recently approved nucleotide analogue [24]. It was shown to have a significant efficacy in patients infected with HIV strains with primary nucleoside-associated resistant mutations. For this reason we applied TDF in two of our patients (RH2-1, RH2-20). One of them had a known Q151M mutation (RH2-1). In both patients this resulted in HIV-2 suppression to < 500 copies/ml

These findings seriously limit the choice of ART combinations available to HIV-2-infected individuals. However, the results of the prospectively studied group II show that, with a well-chosen regimen, the initial decline of plasma HIV-2 RNA during the first 4 weeks of therapy and long-term suppression are comparable to the response observed in HIV-1 infection [25]. In view of the low threshold to the development of resistant HIV-2 strains [15], only a limited number of successful ART combinations are available. This should be carried out with great caution, including intensive counselling, regular therapeutic drug monitoring and plasma HIV-2-RNA assessments.

Acknowledgements

The authors are grateful to Prof. J.H.P. Wilson for his critical comments.

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Socio-economic and health characteristics of HIV-infected patients seeking care in relation to access to the Drug Access Initiative and to antiretroviral treatment in Côte d'Ivoire

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Objective: To compare socio-economic and health characteristics of HIV-infected patients in Côte d'Ivoire whether or not they had access to the Drug Access Initiative (DAI) and to antiretroviral drug (ARV) treatment.

Design and methods: Cross-sectional survey using medical files, blood sampling for CD4 cell counts and face-to-face interviews among all patients, informed of their HIV status, who attended during a 6-week period in the five DAI referral centres and three additional centres in charge of HIV care in Abidjan and Bouaké (participation rate = 65.4%). Multiple logistic regression using generalized estimating equations (GEE) to identify factors related to non-access to DAI and to ARV treatment.

Results: Among the 711 respondents, 23.0% were ARV-treated, 14.2% had been included in the DAI but were still waiting for initiation of ARV, and 62.7% were neither part of the DAI nor ARV-treated. In this latter group, less than one-third (29.6%) declared that they knew about the existence of the DAI. Among the 164 ARV-treated patients, 59.1% had benefited from DAI public subsidies partially covering the costs of drugs. In the non-DAI–non-ARV-treated group, 86% could have qualified for ARV treatment according to the DAI medical criteria (CD4 cell counts $< 500 \times 10^6$ cells/l), and only 32.9% of those medically eligible were prescribed cotrimoxazole prophylaxis. In multivariate analysis, not being in the DAI and not being ARV-treated was related to: being a male, not having health care insurance, having a low level of education, living in poor housing conditions (absence of refrigerator in the household, absence of ventilation in patient's bedroom), and not being under cotrimoxazole prophylaxis.

Conclusion: The Ivoirian DAI has facilitated access to ARV treatment for a significant number of patients with limited ability to pay. The majority of HIV-infected patients seeking care however face persisting socio-economic and informational barriers to access to these treatments.

AIDS 2003, **17** (suppl 3):S63–S68

Keywords: Access to antiretroviral treatment, Africa, gender, HIV infection, socio-economic status

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Sponsorship: This work was funded by French Research Agency on AIDS (ANRS Agence Nationale de Recherches sur le SIDA).

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Introduction

The pilot phase of the UNAIDS HIV Drug Access Initiative (DAI), aimed at providing wider access to HIV-related drugs in developing countries, was launched in November 1997. Four countries, Chile, Côte d'Ivoire, Uganda and Vietnam, participated and the Ministry of Health established its own national schedule in close collaboration with UNAIDS [1].

Côte d'Ivoire had the highest HIV prevalence in West Africa, with an estimated one million individuals already HIV infected in 1998, namely approximately 6% of the general population [2]. In addition, the national Ivoirian programme against AIDS had been integrated with the pre-existing national programme against tuberculosis which had already excellent expertise for delivery of care [3]. Finally, the Ivoirian authorities expressed strong commitments for facilitating access to antiretroviral drugs (ARVs) in the HIV-infected population of their country.

The stated objectives, jointly adopted by UNAIDS and the Ministry of Health of Côte d'Ivoire, for the DAI were to improve HIV-infected persons' access 'to essential drugs in remote primary-care centers', 'to drugs for the treatment of opportunistic infections (related to HIV/AIDS) in follow-up centers', and 'to antiretroviral therapies in referral centers'. Specific goals of the DAI included the promotion of rational selection of drugs by health care providers and their clients and the availability of affordable HIV care [1].

In Côte d'Ivoire, the health authorities opted for a public mechanism to subsidize the costs of ARVs: patients who were identified as 'low income' could receive a 50 to 75% subsidy. In addition, patients identified as active members of people living with HIV/AIDS (PLWAs) non-governmental organizations (NGOs), as well as women who had participated in clinical trials for prevention of mother-to-child transmission of HIV, could receive the maximum subsidy, namely 95%. Patients' out-of-pocket monthly costs of drugs for highly active antiretroviral therapies (HAART) was about US$15 in 2000. Public subsidies of ARVs were partly supported by financial donations from the International Therapeutic Solidarity Fund (ISTF) sponsored by the French government.

In Côte d'Ivoire, the DAI effectively started in August 1998. Selected patients for the DAI accessed ARVs at six 'referral centers' in Abidjan, the economic capital of the country. These referral centers were the hospital departments or medical centers with the greatest experience in clinical care for HIV-infected patients. The procedure for accessing ARV treatment through the DAI started with a face-to-face interview with either a physician or a social worker. This first interview included a rapid assessment of patient's socio-economic status and ability to pay for the drugs. Following this first interview a CD4 cell count, viral load and other tests were performed. One month later, physicians could prescribe ARV treatment for patients who met the DAI medical eligibility criteria (CD4 cell count $< 500 \times 10^6$ cells/l and/or viral load $> 10\,000$ copies/ml, absence of contraindications for ARVs) and who had the ability to pay. For those who required a public subsidy to pay for the costs of ARVs, an authorization had to be obtained from the public committee in charge ('Comité de Gestion de l'Initiative') [4].

The evaluation of the socio-economic and behavioural impacts of the DAI, carried out by ANRS (the French Agency for AIDS Research) in close collaboration with social science researchers from Côte d'Ivoire [4], included a cross-sectional survey among HIV-infected patients, aware of their serostatus and seeking HIV care, in order to describe the socio-economic and behavioural characteristics of those who did, or did not get access to ARV treatment.

Material and methods

Data collection

A cross-sectional survey was carried out from December 1999 to February 2000 in the five referral centers of the initiative dealing with adult patients as well as three additional health structures in charge of HIV care in Abidjan and Bouaké, the second most populated town in the country (Infectious Diseases Department in Treichville University Hospital, USAC, Abidjan Military Hospital, Antituberculous Center in Adjamé, Abidjan, CIRBA, Hôpital de jour de Bouaké, CAT de Bouaké, CAT de Treichville). In each of these eight centers, during a period of a month and a half, a face-to-face questionnaire was proposed by physicians to all HIV-infected patients who had been informed of her/his HIV status. Once the patient's consent to participate in the study had been obtained, the survey questionnaire was administered face-to-face by specially trained interviewers.

The questionnaire included 11 questions dealing with patients' socio-economic status including variables about the patient's practical conditions of living (such as living in collective housing, an indicator of poverty). Fifty-one additional questions dealt with the following items: knowledge of HIV status, circumstances of HIV testing and date of HIV diagnosis, sexual behaviours, psychological and health status, quality of life, health care-seeking behaviour and history of access to HIV care, impact of disease on resources of the household, family and social support, disclosure of HIV serostatus to others, knowledge and beliefs about HIV treatments and awareness of the DAI.

Medical data at time of the interview were obtained from consulting physicians and medical files. For the sub-sample of patients who were already in the DAI at

time of the survey, we obtained from the files of the Retro-CI project, in charge of the epidemiological and biological evaluation of the DAI, the CD4 lymphocyte counts at time of inclusion in the DAI before initiation of ARV treatment. In parallel, all other patients who agreed to answer the questionnaire were offered a CD4 lymphocyte count.

Statistical analysis

Four groups of respondents were compared: those included in the DAI and already ARV-treated at time of the survey (DAI–ARV group); those included in the DAI but still waiting for ARV treatment at time of the survey (DAI–non-ARV group); those having access to ARV treatment through other channels (non-DAI–ARV group), and those who did not access either ARV treatment or the DAI (non-DAI–non-ARV group). At first, univariate comparisons were made using 4×2 chi-square test for qualitative variables and Kruskall–Wallis test for quantitative variables. When the comparison between these four groups was significant, multiple comparisons were calculated using a 2×2 chi-square or a Mann–Whitney test.

In order to identify factors associated with failure to access ARV treatment we focused on the comparison between the non-DAI–non-ARV group and the rest of the sample and performed a multiple logistic regression using the generalized estimating equation method (GEE) [5,6].

Results

During the period of data collection, a total of 1087 HIV-infected adult patients, informed of their HIV status, consulted, at least once, at one of the participating health centers. Among these patients, 342 (31.5%) were not offered the questionnaire by their consulting physician. Among the 745 patients who were offered participation in the study, 95.4% (n = 711) gave consent and CD4 cell counts were obtained for 650 (91.4%).

When comparing the basic socio-demographic characteristics (sex, age, occupational status) and access to ARV treatment of the 342 patients who were not included with the 745 who were, the former were significantly older (36.6 versus 35.1 years of age, $P = 0.02$), less likely to be ARV-treated (12.6 versus 23.5%, $P < 0.001$), and more likely to have a high occupational status (middle and top management, civil servants) (30.6 versus 17.8%, $P < 0.001$). No significant differences were found between the 711 respondents and the 34 patients who refused to participate.

Of 711 respondents, about one-third (n = 242, 34.0%) were included in the DAI. At time of the interview, 141 of the DAI patients were ARV-treated (19.8% of total sample) (DAI–ARV group) whereas 101 patients (14.2% of total sample) were awaiting ARV treatment. (DAI–non-ARV group). Twenty-three non-DAI patients (3.2%) had access to ARVs (non-DAI–ARV group). The majority of respondents (n = 446, 62.7%) were neither part of the DAI nor ARV-treated (non-DAI–non-ARV group). Of these, less than one-third (29.6%) knew about the existence of an initiative offering access to ARVs. Although women accounted for 48.9% of the total sample, this proportion was significantly higher among those included in the DAI than in the rest of the sample (55.0 versus 45.8%, $P = 0.026$).

Table 1 shows that patients in the non-ARV–non-DAI group significantly differed from the rest of the sample for most socio-economic characteristics. They were also more likely to have been more recently informed of their HIV diagnosis (in the 9 months period prior to the survey). However, even after adjustment for this factor in multivariate analysis, not being ARV treated and not having access to the DAI was significantly related to being a male, not having health care insurance, having a low level of education and living in poor housing conditions (absence of refrigerator in the household, absence of ventilation in patient's bedroom) (Table 1).

Among the 164 ARV-treated patients, no significant differences in socio-economic conditions were found when comparing those who had access to ARVs through the DAI to the minority of those who used other channels for drug procurement.

As also shown in Table 1, patients in the non-ARV–non-DAI group tended to declare a poorer subjective self-estimation of their own health status. This fact has to be related to the significantly higher proportion of patients in this group with symptomatic HIV infection (CDC AIDS stage > A).

Among the 650 patients with available CD4 cell counts at time of the interview, 566 (87.1%) had a CD4 cell count less than 500×10^6 cells/l and 324 (49.8%) were under 200×10^6 cells/l, but only 29.0% of those with less than 500×10^6 cells/l and 24.4% of those with less than 200×10^6 cells/l were ARV treated. The majority (67.9%; n = 57) of the 84 patients with CD4 cell counts $\geq 500 \times 10^6$ cells/l belonged to the non-ARV–non-DAI group. When excluding these 84 patients from analysis, no difference was found in levels of immunodepression between groups (mean CD4 cell count $193 \pm 131 \times 10^6$ cells/l in the non-ARV–non-DAI group versus $175.3 \pm 127.5 \times 10^6$ cells/l in the rest of the sample, $P = 0.11$). Moreover, the proportion of patients with CD4 cell count $\leq 200 \times 10^6$ cells/l was similar in the non-ARV–non-DAI group (48.6%) to the rest of the sample (51.4%, $P = 0.54$).

Overall, only 24.9% of patients in the non-ARV–non-DAI-group had, however, been prescribed cotrimoxazole

Table 1. Access to the Drug Access Initiative and to antiretroviral treatment (ARVT) and socio-economic and health characteristics of HIV-infected patients consulting for HIV care in Côte d'Ivoire (December 1999–February 2000, n = 711).

	In DAI		Out of DAI					
Patients' characteristics	(1) ARV-treated 141 (100%)	(2) Non-ARV-treated 101(100%)	(3) ARV-treated 23 (100%)	(4) Non-ARV-treated 446(100%)	[a]Level of sign (*P*)	*P* [4 vs (1+2+3)]	[b]Not being in DAI and not being ARV-treated Adjusted OR (95%CI)	Total sample 711 (100%)
Male gender	73 (52%)	36 (36%)	15 (65%)	239 (54%)	0.006	0.080	1.5 (1.1–2.0)	363 (51%)
Age < 35 years	63 (44%)	64 (63%)	8 (35%)	248 (56%)	0.006	0.2		383 (54%)
Education level ≤ primary school	38 (27%)	49 (49%)	7 (30%)	287 (64%)	< 0.001	< 0.001	1.4 (1.0–1.8)	381 (54%)
Head of household (or spouse of)	101 (72%)	60 (59%)	20 (87%)	275 (62%)	0.012	0.07		456 (64%)
Head of household = manager or civil servant	64 (45%)	44 (44%)	12 (52%)	101 (23%)	< 0.001	< 0.001		221 (31%)
Lives in collective housing	39 (27%)	34 (34%)	10 (44%)	274 (62%)	< 0.001	< 0.001		357 (50%)
Access to tap water in household	130 (8%)	88 (87%)	22 (96%)	259 (58%)	< 0.001	< 0.001		499 (70%)
No refrigerator in household	39 (27%)	41 (31%)	9 (39%)	316 (71%)	< 0.001	< 0.001	1.7 (1.2–2.3)	405 (57%)
Absence of ventilation in bedroom	20 (14%)	29 (29%)	5 (22%)	207 (46%)	< 0.001	< 0.001	1.5 (1.1–2.0)	261 (37%)
No health insurance	90 (64%)	71 (70%)	18 (78%)	399 (89%)	< 0.001	< 0.001	1.7 (1.1–-2.5)	133 (19%)
Has lost job since HIV diagnosis	25 (18%)	27 (27%)	6 (26%)	156 (35%)	< 0.001	< 0.001		214 (30%)
Has disclosed HIV+ status to others	134 (95%)	86 (85%)	22 (96%)	246 (55%)	< 0.001	< 0.001		488 (69%)
Knows HIV status for less than 9 months	39 (28%)	47 (47%)	4 (17%)	337 (56%)	< 0.001	< 0.001	1.6 (1.2–2.1)	337 (48%)
Personally feels in bad health	56 (40%)	60 (59%)	8 (35%)	284 (65%)	< 0.001	< 0.001		408 (58%)
Declares declining health when compared with previous year	25 (18%)	40 (40%)	6 (26%)	165 (37%)	< 0.001	0.005		236 (33%)
Not under cotrimoxazole prophylaxis	69 (49%)	45 (45%)	14 (61%)	335 (75%)	< 0.00001	< 0.00001	2.0 (1.5–2.7)	463 (65%)
CDC AIDS Stage > A	56 (40%)	46 (46%)	11 (52%)	250 (58%)	0.004	< 0.00001		363 (52%)

[a]The chi-square test was calculated on the 4 × 2 table. [b]Odds ratios (ORs) and their standard error estimates were calculated by logistic regression based on generalized estimating equations. ARV, antiretroviral treatment; CDC, centers for disease control; CI, confidence interval; DAI, Drug Access Initiative.

prophylaxis and this proportion remained significantly lower than in the rest of the sample, even after multivariate adjustment (Table 1). When restricting the analysis to patients with available data on CD4 cell counts and CD4 cell counts $< 500 \times 10^6$ cells/l, coverage by cotrimoxazole prophylaxis was only 32.9% in the non-DAI–non-ARV group and 58.1% in the group of DAI patients still waiting for ARV treatment (DAI–non-ARV group).

Among the 242 patients who were included in the DAI, the proportion of those who were already ARV treated was significantly lower among the 133 female patients (51.1%) than among the109 male patients (67.0%, $P = 0.01$). DAI ARV-treated patients had logically longer follow-up in the initiative (65.2% with 6 months follow-up or more) than those still waiting for initiation of ARV treatment at the time of this cross-sectional survey (33.7%, $P < 0.001$). Among the 141 DAI patients who were ARV treated, about one-third (31.2%) had to pay the full costs of ARV drugs out of their pocket; whereas another third (34.1%) did benefit from the maximum level of public subsidy (95%); 17.0 and 17.7%, respectively, had a 75 and 50% subsidy.

Discussion

To our knowledge, this study was the first to compare socio-economic characteristics and health status of HIV-

infected patients consulting for HIV care in African medical centers, whether or not they had access to ARV treatment. Of course, this cross-sectional survey is not representative of the whole HIV-infected population in Côte d'Ivoire since only a minority of HIV-infected individuals in this country, as elsewhere in Africa, are aware of their HIV serostatus. In Abidjan, 20 to 26% of pregnant women who received adequate counselling refused to be tested for HIV and more than one-third (38.6%) did not return for notification [7]. Moreover, fewer than one out of ten adults seen in primary health-care centers in the most populated neighbourhoods of Abidjan had been tested for HIV [4]. Among those who know they are infected, the proportion who effectively consult health structures for HIV counselling and care remains unknown. Our survey was focused on an HIV-infected population that should logically be the easiest to target for access to ARV treatment, namely HIV-infected patients aware of their serostatus and seeking health care in the main hospital departments and medical centers providing HIV care.

At the individual patient's level, affordability of drugs remains a major barrier between HIV-infected individuals and access to ARV treatment in Africa. At the time of our survey, monthly costs of HAART were between 250 000 and 300 000 CFA Francs (~ US$450–540), when the legal minimum salary in Côte d'Ivoire was only 40 000 CFA Francs (US$75), annual health care expenditures per capita was only 6200 CFA Francs (US$12), and only 20% of the general population had some form of health insurance [8]. This cross-sectional survey shows that the introduction of the DAI in Côte d'Ivoire has allowed access to ARVs for groups of patients who would not have had ability to pay for ARVs in the absence of public subsidies. This is especially true for women who had previously participated in mother-to-child transmission prevention programmes and received the 95% maximum subsidy, and thus, whose proportion was higher among patients included in the DAI than in the rest of our sample.

This survey however shows that the DAI had not yet been able to reach the majority of HIV-infected patients medically eligible for ARV treatments, aware of their serostatus and in regular contact with the health care system. Indeed, 86% of non-DAI–non-ARV-treated patients in our sample could have qualified for ARV treatment according to the medical criteria of the DAI (CD4 cell counts $< 500 \times 10^6$ cells/l), and nearly half of them were already under 200×10^6 cells/l which means that initiation of ARV treatment should have been considered a medical priority [9]. In spite of public subsidies for ARVs, patients from the poorest social and economic background did not achieve access to ARVs.

A first explanation for limited access to DAI among HIV-infected patients consulting for care is that information on the existence of this initiative has not been widespread. Even in the population attending the referral or peripheral centers of the DAI: more than 60% of patients out of the DAI in our sample had never heard about it. This illustrates how difficult it is to provide accurate information in limited-resource settings on the benefits and costs of ARVs. Barriers to appropriate dissemination of information on ARVs could not be fully overcome. There are various explanations which may have contributed to this limitation. A recent knowledge of HIV status (less than 9 months) was associated with non-access to DAI, reflecting the complexity of the process of access to HIV care in general. Qualitative research suggested that some of the HIV-infected patients who were aware of the availability of ARVs self-excluded because they expected insuperable financial, practical and social difficulties [4]. In addition, in order to access ARV treatment, HIV-infected patients may need to disclose their serostatus to family members, including their main partner, and this requirement may have presented a barrier. Here, ARV-treated and DAI patients were far more likely to have disclosed their serostatus to significant other people (Table 1). Finally, health care professionals in referral and peripheral centers of the DAI may not have provided information about the initiative to HIV-infected patients whose living conditions appeared inappropriate for ARVs. Although, in developed countries, it has been shown that non-adherence to HAART cannot be reliably predicted by easily identified patient characteristics [10], in the Ivoirian context, DAI physicians and social workers may have anticipated non-adherence to occur more often among their patients with the lowest socio-economic status.

Comparison between HIV-infected patients attending medical centers in and out of the DAI also highlights another major limitation of the initiative. Two clinical trials have shown the positive impact of cotrimoxazole prophylaxis on the survival of non-ARV-treated, HIV-infected patients [11,12]. Such low-cost prophylaxis (< US$ 2 per month) is now recommended for all HIV-infected adults with symptomatic HIV infection and/or with CD4 cell counts $\leq 500 \times 10^6$ cells/l [13]. However, of those medically eligible in our sample, only one-third in the non-DAI–non-ARV group and less than 60% in the group waiting for ARV treatment in the DAI had received prophylaxis. Training for health professionals is therefore vital to scaling up access to HIV care in African countries [14].

Wider dissemination of information about the availability of effective treatments, including ARVs, among patients and the general population may also be a prerequisite for scaling up access to HIV/AIDS care. The current reluctance of public health authorities to disseminate information about ARVs to the population at large in countries like Côte d'Ivoire is certainly related

to fears that excessive demand for expensive drugs may create unmanageable economic demands. Therefore, promoting further decreases in prices of ARVs and evaluation of cheaper laboratory methods for monitoring HIV infection and ARV treatment [15–17], and 'adapting' clinical guidelines to limited resource settings [9], are key for access to HIV care and treatment. Lack of information about treatment currently remains a major cause of inequity in access to health care among the HIV-infected populations.

There are legitimate concerns that the use of public funds to subsidize antiretroviral treatment in developing countries may be inequitable, and will shift health resources from the poor to those who are less poor [18]. In terms of equity, the picture that emerges from the experience of the Ivoirian DAI is quite mixed. A national consensus defining the priority population groups had been reached and the provision of ARVs at a 50 to 95% subsidized price has allowed access to ARV treatment for a number of patients with limited ability to pay. Gender differences in access to ARVs have consequently been reduced. However, constraints on government expenditures have prevented the Ivoirian DAI from establishing consistent access to ARVs, even in the limited subgroup of HIV-infected individuals aware of their serostatus and in contact with health care providers. Complementary funding mechanisms, including insurance funds in the public and private sectors, should be developed [19]. Indeed, significant progress has recently been made in Côte d'Ivoire with involvement of the private business sector in funding ARV treatment.

Acknowledgements

The authors thank M. Coulibaly (ONUSIDA, Abidjan), I. M. Coulibaly (PNLS/MST/TUB, Abidjan), R. Aka and A. Kadio (Service des Maladies infectieuses du CHU de Treichville, Abidjan), Miss F. Twiyubahe and all social workers who participated in the completion of this study.

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Appendix

The Côte d'Ivoire HIV Drug Access Initiative Socio-Behavioural Evaluation Group includes: H. Aka-Dago, A. Ayo, M. Chevallier-Schwartz, T. Chorba, D. Coulibaly-Traore, G. Dacoury, K. Delaunay, A. Desgrées du Loû, H. Digbeu, G. Djomand, J.P. Dozon, C. Esse, G. Kponhassia, A. Juillet, C. Malaval, J.P. Moatti, P. Msellati, T. Roels, Y. Souteyrand, M. Souville, G. Tape, L. Vidal.

Access to antiretroviral treatment and sexual behaviours of HIV-infected patients aware of their serostatus in Côte d'Ivoire

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Objective: To compare sexual behaviours of HIV-infected patients in Côte d'Ivoire whether or not they had access to antiretroviral treatment (ARV).

Design and methods: Cross-sectional survey using face-to-face interviews among all patients, informed of their HIV serostatus, attending the eight health centers in charge of HIV care in Abidjan and Bouaké. Univariate comparisons of declared sexual behaviours during the prior 6 months between the 164 ARV-treated and the 547 non-ARV-treated respondents. Multiple logistic regressions to identify factors related to sexual abstinence in the total sample and to unprotected sexual intercourse in the subsample of sexually active respondents during the same period were performed.

Results: More than half of the 711 respondents (53.0%) declared an absence of sexual activity during the previous 6 months, with this proportion being significantly higher among women (60.6%), and among both men (85.7%) and women (92.4%) who were not living in a stable relationship. Among the 334 sexually active patients, 49.7% declared a low frequency of sexual intercourse ('once a month or less'), and 43.7% declared at least one episode of unprotected sexual intercourse. In multivariate analysis, recent diagnosis of HIV infection (< 9 months), having only one sexual partner and not knowing her/his serostatus, high alcohol consumption, absence of episodes of acute morbidity, not participating in household's expenditures and not being ARV-treated were significantly related to a higher likelihood of HIV-related risky sexual behaviours.

Conclusion: Sexual abstinence is the preventive strategy of choice for a majority of HIV-infected patients aware of their serostatus and consulting for care in Côte d'Ivoire. In these patients, access to ARV is not associated with an increase in HIV-related risky sexual behaviours.

AIDS 2003, **17** (suppl 3):S69–S77

Keywords: Africa, antiretroviral treatment, condom use, HIV infection, sexual behaviour

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Sponsorship: This work was supported by the French Agency for AIDS Research (ANRS).

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Introduction

Concerns have been raised world-wide that improvements in health status and quality of life of HIV-infected patients due to access to effective treatment such as highly active antiretroviral therapies (HAART) may indeed increase the opportunities for continued or relapse to risk behaviours among HAART-treated individuals [1]. Furthermore, they may create a new threat for public health through transmission of HIV viral strains that have already acquired genetic resistance characteristics against actual therapies [2]. Such concerns about the potential negative impact of access to HAART on the transmission of the HIV virus may be even more pronounced in the context of developing countries. In Africa, reported condom use remains low at 20 to 25% among men with non-spousal partners and 11 to 24% among women [3,4]. Data about West African women who learned of their HIV serostatus during pregnancy show that only a minority disclose this status to their main partner. Moreover they find it difficult to avoid unprotected sexual relations, as suggested by self-reports as well as by the high rate of new pregnancies in this population [5,6].

As the access to antiretroviral treatment (ARV) has been very limited in developing countries up to now, little is known about the evolution of HIV-related sexual behaviours among HIV-positive patients in the developing world once they have started antiretroviral treatment. The cross-sectional survey among HIV-infected patients, informed of their serostatus, which was carried out to contribute to the socio-economic and behavioural evaluation of the Drug Access Initiative (DAI) in Côte d'Ivoire gave us the opportunity to collect data about sexual behaviours in this population and to compare them between ARV-treated patients and patients who have access to HIV care but not to ARVs.

Material and methods

The methodology of the cross-sectional survey, carried out from December 1999 to February 2000, among patients, informed of their HIV serostatus, who attended one of the eight health centers in charge of HIV care in Abidjan and Bouaké (the Infectious Diseases department in Treichville University Hospital, USAC, Abidjan Military Hospital, Antituberculous Center in Adjamé, Abidjan, CIRBA, Hôpital de jour de Bouaké, CAT de Bouaké, CAT de Treichville) has been described elsewhere in this special issue [7]. The questionnaire administered to patients in the face-to-face interview included three multi-item questions about sexual behaviours and condom use, with regular as well as occasional partners, during the 6-month period prior to the survey. An additional question dealt with the circumstances of the last sexual intercourse. The wording of these questions was similar to that validated in the large-scale French survey on sexual behaviours in the general population [8] as adapted to the Ivoirian context. Two additional questions asked ARV-treated patients if they have changed their behaviours since initiation of treatment.

In addition to biomedical data obtained from medical files and socio-economic and behavioural data collected during the face-to-face interview, the questionnaire also included the French version of the Centre for Epidemiological Studies depression (CES–D) scale [9] that had been adapted and validated to the Ivoirian situation [10]. The CES–D score ranges from 0 to 60, with higher values corresponding to the highest likelihood of depression. The questionnaire also included the seven-items of the 'Physical Ability' sub-scale of the Medical Outcome Study Short Form Health Survey (MOS–SF), that has previously been validated in French [11]: the higher the score on this scale (graduated from 0 to 100), the better is the individual capability in physical functioning.

Chi-square test, Fisher's exact test, or Mann–Whitney test were used to perform univariate comparisons of socio-demographic, clinical and behavioural characteristics in the whole sample of respondents whether or not they had declared sexual activity in the previous 6 months. According to recommendations in the literature on sexual behaviours, these analyses were carried out separately by gender [8].

Similar comparisons were also performed in the sub-sample of sexually active patients whether or not they declared at least one episode of unprotected sexual intercourse during the same 6-month period. In this sub-sample, we also compared the characteristics of the last sexual intercourse whether or not respondents were ARV-treated at time of the survey.

Logistic regressions were performed to analyze factors associated with abstinence of sexual activity during the previous 6 months in both sub-samples of male and female respondents, as well as unprotected sexual intercourse in the sub-sample of sexually active respondents. They were also used to analyze whether being ARV treated had an impact on these behaviours after adjustment for potential confounding variables. In the three cases, all explanatory variables associated with the outcome variable (abstinence of sexual activity, unprotected sex) with a P-value ≤ 0.1 in univariate analyses were included in the initial logistic model. Age, antiretroviral treatment, and sex in the case of the sub-sample of sexually active respondents, were also introduced in the initial models even if they did not fulfil this condition of significance (P-value ≤ 0.1) in univariate analysis. Final multivariate models were obtained by using a backward stepwise procedure based on log-likelihood ratio to eliminate non-significant ($P > 0.05$) variables from initial models, and by forcing the "ARV treatment" variable

in the model, even when it did not fulfil this condition. As data about CD4 cell counts at time of the survey were missing for some respondents, all three multivariate models were also computed with an initial introduction of this variable on the samples of respondents who had available data about their immunological status.

Results

A total of 711 (65.4%) of the 1087 HIV-infected adult patients, informed of their HIV serostatus, who attended, at least once, at one of the participating health centers during the study period, were included in the survey. Among these 711 respondents, 51.1% were men, 53.9% were younger than 35 years and 50.2% lived in collective housing, which is an indicator of low socio-economic status in Ivoirian society. A total of 456 respondents (64.1%) declared that they had a stable relationship with a regular partner, this proportion being significantly higher among male than among female respondents (73.0 versus 54.9%, $P < 0.001$). Overall, more than half of HIV-infected patients (n = 377; 53.0%) declared an absence of sexual activity during the previous 6 months, this proportion being significantly higher among women (60.6%) than among men (45.7%) ($P < 0.001$).

Factors associated with sexual abstinence

The great majority of both men and women who were not living in a stable relationship with a regular partner (respectively 85.7 and 92.4%) declared having been sexually abstinent in the previous 6 months. Among the 454 respondents who had a stable relationship with a regular partner, less than one-third (n = 130, 28.6%) knew the HIV serostatus of this partner. The majority (63.2%) of these 130 respondents belonged to seroconcordant HIV-positive couples but both acknowledged seroconcordant and serodiscordant couples maintained sexual activity in similar proportions (70.3 and 65.3%, respectively). Multivariate analysis confirmed the relationship between sexual abstinence and absence of a regular partner (Table 1).

Overall, about a quarter of respondents (24.5%) reached the maximum value (100) for the score of physical functioning. This proportion of patients without any impairment of their physical capability tended to be higher among ARV-treated than non-ARV-treated patients (29.9 versus 22.9%; $P = 0.06$). For both men and women, a lower score of physical functioning was related to a greater likelihood of having been sexually abstinent. in univariate as well as multivariate analyses (Table 1).

Table 1 details all additional factors which were found to be associated ($P < 0.1$) with sexual abstinence in univariate analysis. Older age and being illiterate were clearly associated with the absence of sexual activity in the case of women whereas these factors did not seem to play a role, after multivariate adjustment, for men. On the contrary, although various indicators of lower socio-economic status were found to be related to sexual abstinence for both sexes, such a relationship was only confirmed by multivariate analysis in the case of men. Male respondents who lived in poor housing conditions (with no access to tap water) in a highly populated household (with more than eight members), who were unemployed and who did not have income to contribute to expenditures of their household were more likely to have refrained from any sexual activity. We also found that not having disclosed HIV serostatus to others was related to sexual abstinence for both men and women, although this was again confirmed by multivariate analysis only in the case of male respondents.

Data on CD4 cell counts were only available for 321 male and 328 female respondents (91.3% of total sample). When analysis was restricted to these patients, advanced immunodepression (having CD4 cell count $\leq 200 \times 10^6$ cells/l) was associated with sexual abstinence for men but not for women.

Finally, Table 1 shows that being ARV treated did not have any influence on the fact of being or not sexually abstinent, even after adjustment for potential confounders through multivariate analysis.

Sexual activity of HIV-infected patients

Among the 334 respondents who declared that they had been sexually active in the previous 6 months, 14.7, 35.6, and 49.7%, respectively, declared that they had sexual intercourse 'more than once a week', 'two or three times per month' and 'once a month or less' during this period. Frequency of sexual intercourse was higher among men than among women (17.8, 38.0, and 44.2% versus 10.2, 32.1, and 57.7%, respectively; $P = 0.05$), but was similar among ARV-treated and non-ARV-treated patients. Among the 164 ARV-treated patients, 44.5% had renounced sexual intercourse, 39.0% had 'reduced frequency of sexual intercourse' after treatment had been initiated, whereas the remaining 16.5% maintained the same frequency of sexual activity. For the majority of ARV-treated patients, initiation of treatment had occurred quite recently (71.3% for one year or less) and no relationship was found between time since initiation of ARVs and sexual activity. Among these sexually active HIV-infected patients, 22.8% declared more than one sexual partner during the previous 6 months. The proportion of those declaring multiple sexual partners was higher among men than among women (29.4 versus 13.1%, $P < 0.001$).

HIV-related risky sexual behaviours

A total of 146 out of the 334 sexually active patients (43.7%) declared at least one episode of unprotected sexual intercourse in the previous 6 months. The proportion of sexually active patients who declared such

Table 1. Factors associated with sexual abstinence among male (n = 363) and female (n = 348) HIV-infected patients consulting for HIV care in Côte d'Ivoire (December 1999–February 2000).

	Male respondents Sexual activity in prior 6 months				Female respondents Sexual activity in prior 6 months			
	No n = 166 (45.7)	Yes n = 197 (54.3)	*P*[b]	OR (95% CI) Multivariate[a]	No n = 211 (60.6)	Yes n = 137 (39.4)	P[b]	OR (95% CI) Multivariate[a]
Being ARV-treated	33 (19.9)	55 (27.9)	0.08	0.60 [0.30–1.19][c]	40 (19.0)	36 (26.3)	0.15	1.53 [0.72–3.22]
Age > 35 years	99 (59.6)	108 (54.8)	0.36	–	82 (38.9)	39 (28.5)	0.05	2.65 [1.32–5.34]
Illiterate	42 (25.3)	34 (17.3)	0.06	–	75 (35.5)	28 (20.4)	0.003	2.79 [1.47–5.34]
Education level lower than secondary school	74 (44.6)	108 (54.8)	0.05	–	82 (38.9)	66 (48.2)	0.09	–
No access to tap water in household	66 (39.8)	53 (26.9)	0.009	2.18 [1.17–-4.09]	67 (31.8)	26 (19.0)	0.009	–
Being unemployed	71 (42.8)	31 (15.7)	0.000	2.21 [1.16–4.22]	121 (57.3)	70 (51.1)	0.25	–
Does not contribute to household's expenditures	44(26.5)	13 (6.6%)	0.000 [1.02–6.53]	2.58	126 (59.7)	96 (70.1)	0.05	–
Has lost job since HIV diagnosis	44 (26.5)	23 (11.7)	0.000	–	92 (43.6)	42 (30.7)	0.015	–
No stable relationship with a regular partner	84 (50.6)	14 (7.1)	0.000	14.36 [7.01–29.41]	145 (68.7)	12 (8.8)	0.000	21.4 [10.6–43.4]
No. of persons in household > 8	50 (30.1)	38 (19.3)	0.02	1.81 [1.01–3.37]	72 (34.1)	34 (24.8)	0.06	–
CD4 cell count ≤ 200 × 10^6/l[d]	91 (61.5)	87 (50.0)	0.02	1.92 [1.05–3.52][f]	94 (46.1)	55 (44.4)	0.76	–
Personally feels in bad state of health	109 (65.7))	106 (53.8)	0.02	–	125 (59.2)	73 (53.3)	0.27	–
Already had information about ARV treatments	97 (58.4)	87 (44.2)	0.007	–	131 (62.1)	66 (48.2)	0.01	–
Has not disclosed HIV+ serostatus to others	68 (41.0)	59 (29.9)	0.03	1.20 [1.02–1.56]	72 (34.1)	23 (16.8)	0.000	–
Score of physical abilities (median and IQR)	71.4 [57.1–92.9]	85.7 [71.4–100.0]	0.000	0.98 [0.97–0.99][e]	78.6 [57.1–92.9]	85.7 [64.3–100.0]	0.004	0.98 [0.97–0.99][e]

[a]Multiple logistic regression model. [b]Chi-square test or Mann–Whitney test. [c]Variable forced in the logistic regression model. [d] For the 321 male respondents and the 328 female respondents who had available data on CD4 cell counts. [d]Odds ratio (OR) per unit increase of the score used. [f]When logistic regression model is applied to the sample of 321 male respondents with available data on CD4 cell count. ARV, antiretroviral treatment; CI, confidence interval; IQR, inter-quartile range.

HIV-related sexual behaviour was similar among men (42.6%) and women (45.2%) ($P = 0.64$), and also among the 308 respondents who declared living in a stable relationship with a regular partner (43.2%) and among the 26 who had no regular partner (50.0%) ($P = 0.50$). Episodes of unprotected sex also occurred in similar proportions with regular partners (46.5%) and with occasional partners (47.0%). However, respondents who declared multiple sexual partners were more likely always to have used condoms (65.8%) than those who only had one single partner during the previous 6 months (53.5%), although this difference was only close to statistical significance in univariate analysis ($P = 0.06$) (Table 2).

No relationship was found between age of respondents, stage of HIV disease as measured by CDC classification for clinical stage or CD4 cell counts and HIV-related sexual risk behaviour. However, patients who did not have any episode of acute morbidity in the same period were more likely to declare HIV-related risky sexual behaviour. A relationship was also found between HIV-related risky sexual behaviour and a higher score on the CES–D scale, but this was not confirmed by multivariate analysis (Table 2)

As shown in Table 2, ARV-treated patients were significantly less likely to declare episodes of unprotected sex (29.7%) than patients who did not have access to ARVs

Table 2. Factors associated with HIV-related risky sexual behaviour among sexually active HIV-infected patients consulting for HIV care in Côte d'Ivoire (n = 334) (December 1999–February 2000).

	Unprotected sexual intercourse in the previous 6 months				
	Yes n = 146 (%)	No n = 188 (%)	Level of sign P^b	OR (95% CI) univariate	OR (95% CI) multivariate[a]
Age			0.9		
≤ 35 years	81 (55.5)	106 (56.4)		1	
> 35 years	65 (44.5)	82 (43.6)		1.04 [0.67–1.60]	–
Sex			0.6		
Male	84 (57.5)	113 (60.1)		1	
Female	62 (42.5)	75 (39.9)		1.11 [0.72–1.73]	–
ARV-treated			0.002		
No	119 (81.5)	124 (66.0)		1	1
Yes	27 (18.5)	64 (34.0)		0.44 [0.26–0.74]	0.52 [0.29–0.93]
Has known his/her HIV serostatus since			0.000		
> 9 months	60 (41.1)	116 (71.7)		1	1
≤ 9 months	86 (58.9)	72 (38.3)		1.60 [1.24–2.05]	1.90 [1.1–3.12]
Knowledge of HIV serostatus of main partner			0.000		
Knows	22(15.1)	68 (36.2)		1	1
Doesn't know (or no regular partner)	124 (84.9)	120 (63.8)		3.19 [1.86–5.49]	3.15 [1.71–5.80]
Already had information about ARV treatments			0.000		
Yes	60 (41.1)	121 (64.4)		1	
No	86 (58.9)	67 (35.6)		2.59 [1.66–4.04]	–
High alcohol consumption			0.06		
No	116 (79.5)	164 (87.2)		1	1
Yes	30 (20.5)	24 (12.8)		1.77 [0.98–3.18]	2.32 [1.18–4.53]
Number of sex partners in previous 6 months			0.06		
1	120 (82.2)	138 (73.4)		1	1
2 or more	26 (17.6)	50 (26.6)		0.74 [0.52–1.03]	0.42[0.23–0.78]
Education level			0.03		
≥ Secondary school	66 (45.2)	108 (57.4)		1	
< Secondary school	80 (54.8)	80(42.6)		1.32 [1.03–1.69]	–
Access to tap water in household			0.000		
Yes	98 (67.1)	157 (83.5)		1	
No	48 (32.9)	31 (16.5)		2.48 [1.48–4.16]	–
Actively contributes to household's expenditures			0.05		
Yes	90 (61.6)	135 (71.8)		1	1
No	56 (38.4)	53 (28.2)		1.59 [1.00–2.51]	2.11 [1.04–4.30]
Had an episode of acute morbidity in the previous 6 months			0.01		
Yes	82 (56.2)	131 (69.7)		1	1
No	64 (43.8)	57 (30.3)		1.79 [1.15–2.82]	1.79 [1.09–2.93]
Participates in activities of NGOs			0.000		
Yes	22 (15.1)	60 (31.9)		1	
No	124 (84.9)	128 (68.1)		1.83 [1.26–2.68]]	
CES–D score (median and interquartiles)	19.0 [12.0–27.0]	16.5 [10.0–25.0]	0.02	1.02 [1.00–1.05][c]	

[a]Odd ratios (ORs) were calculated using multiple logistic regression. [b]Chi-square test or Mann–Whitney test. [c]OR per unit increase of score used. ARV, antiretroviral treatment; CI, confidence interval; NGO, non-governmental organization; CES–D, Centre for Epidemiological Studies depression scale.

Table 3. Behaviour during last sexual intercourse among sexually active HIV-infected patients consulting for HIV care in Côte d'Ivoire (n = 334) (December 1999–February 2000).

	AVR-treated patients		Non-ARV-treated patients			
	Male (1) n =55	Female (2) n = 36	Male (3) n = 142	Female (4) n = 101	*P* (1+2) vs (3+4)[a]	*P* (1+3) vs (2+4)[a]
When?						
Less than one month	37 (67.3%)	16 (44.4%)	84(59.2%)	51(50.5%)	0.66	0.03
One month or more	18 (32.7%)	20 (55.6%)	58 (40.8%)	50(49.5%)		
With who?						
Main partner	53 (96.4%)	34 (94.4%)	117 (82.4%)	94 (93.1%)	0.02	0.04
Occasional partner	2 (3.6%)	2 (5.6%)	25 (17.6%)	7 (6.9%)		
Use of condoms						
Yes	43 (78.2%)	30 (83.3%)	91 (64.1%))	52 (53.0%)	< 0.001	0.15
No	12 (21.8%)	6 (16.7%)	51 (35.9%)	49 (47.0%)		

[a]Chi-square or Fisher test. ARV, antiretroviral treatment.

(49.0%). Results of multivariate analysis, also presented in Table 2, show that the following factors remain significantly related to a higher likelihood of risky sexual behaviour: having learnt his/her HIV serostatus only recently (less than 9 months); not knowing HIV serostatus of regular partner; high alcohol consumption; having only one partner; not having experienced a recent episode of acute morbidity; not participating in household's expenditures; and not being ARV-treated.

Table 3 describes the circumstances of the most recent sexual intercourse for both male and female respondents, whether or not they were ARV treated. It shows that male non-ARV-treated patients were more likely to have their most recent intercourse with an occasional partner. Overall, 35.3% of patients did not use condoms during their most recent intercourse but this proportion was significantly lower among ARV-treated than non-ARV-treated individuals.

Discussion

Since the advent of the AIDS epidemic, extensive research has been carried out, in both developed [12–14] and developing countries [15–17], about sexual behaviours and their relationships with the dynamic of spread of the HIV viruses in various populations. By contrast, the sexual behaviours of HIV-infected persons had not received similar attention until quite recently [18]. As HAART has become widely available in developed countries, there have been disturbing reports of a decreased awareness of HIV risks in the general population [19], and of an increased incidence of sexually transmitted diseases [20–22] and of high-risk sexual behaviours among homosexual and bisexual men [23–27]. Such reports have legitimately raised concerns that access to HAART may favour an increase and resumption of risky sexual behaviours among the treated HIV-infected population [1,28]. However, data from cohort studies in developed countries remain unclear about whether or not HIV-positive individuals receiving HAART effectively tend to adopt risky sexual behaviours more frequently than those who are not on treatment [29–31].

Data about sexual behaviours among HIV-positive persons living in developing countries are scarce. In a cross-sectional study from Brazil, 60% of female partners of HIV-infected men reported safe sexual behaviour after they had been informed of their partner's serostatus [32]. In Tanzania, access to care has been associated with increased adoption of preventive behaviours among recently diagnosed HIV-infected patients [33]. To our knowledge, the survey carried out for the evaluation of the DAI in medical centers of Côte d'Ivoire was the first to compare sexual behaviours of HIV-infected patients in a developing country, whether or not they had access to ARV.

In the developed world, studies among HIV-infected patients in the pre-HAART era have shown how the expression of their sexuality has been affected by their HIV status and how sex life tapered off sharply or simply stopped for a large minority of them [18]. More than half of Ivoirian HIV-infected patients who participated in our survey declared sexual abstinence in the previous 6 months, and one-half of those who remained sexually active had a low frequency of sexual intercourse (once a month or less) during this period.

As in developed countries [34–36], absence of sexual activity in Ivoirian patients was also found to be related to physical impairment associated with HIV disease and, in the case of male patients, with more advanced immunodepression. However, results of the multivariate analysis about factors related to sexual abstinence in these patients clearly show that this behaviour is not only related to physiological and psychological factors

that may directly impede sexual activity. Sexual abstinence also seems to be the *de facto* preventive strategy of choice for a large portion of HIV-infected patients aware of their serostatus in Côte d'Ivoire. This is particularly true for women, for both men and women who do not live in stable relationships, and for men with the lowest socio-economic status and/or who live in social environments where disclosure of HIV serostatus to family and friends is highly difficult. Previous studies in Côte d'Ivoire have already mentioned that women who are aware of their HIV serostatus tend to prolong the traditional postnatal sexual abstinence period [37]. A high prevalence of sexual abstinence is also consistent with the fact that in Côte d'Ivoire, such behaviour is systematically recommended in counseling to HIV-infected patients by most health professionals and social workers and has been publicly supported by religious and spiritual leaders [38,39]. In addition, the survey shows that at the current limited stage of diffusion of the DAI, access to ARV did not change the proportion of patients who remained sexually abstinent.

More than 40% of sexually active patients in our sample declared HIV-related risky sexual behaviours in the previous 6 months and more than one-third did not use condoms during their last intercourse. Heterosexual intercourse is the main mode of transmission of HIV in developing countries such as Ivory Coast and sexual behaviours may be quite different than those observed among men having sex with men or injecting drug users in developed countries. However, some determinants of unsafe sexual behaviours in HIV-infected persons that had been identified in studies carried out in the developed world [18], such as recent knowledge of HIV diagnosis, high alcohol consumption, and absence of episodes of acute morbidity, were also found to be present in Ivoirian patients. The well-established relationship between depressive moods and higher frequency of risky sexual practices among HIV-infected persons [40] was also found in this sample, although this was not confirmed by multivariate analysis.

In this survey, more than two-thirds of HIV-infected patients who lived in a stable relationship had no information about the serostatus of their partner and this absence of information was related to a greater likelihood of unsafe sex. This, and the additional fact that patients with a single partner were more likely than those with multiple partners to practise unprotected sex suggests the specific difficulties in negotiating condom use in the context of monogamous relationships that have already been documented in Africa [17,41].

Another finding of this study is that among sexually active HIV-infected patients, who were aware of their serostatus and in contact with the health care system in Côte d'Ivoire, access to antiretroviral treatment was associated with a lower likelihood of risky sexual behaviours in comparison with those who did not have access to ARVs. Of course, this finding must be interpreted with caution.

First, the observed lower frequency of risky behaviours among ARV-treated patients, in comparison with patients who did not get access to ARVs, may not be directly linked to ARV *per se*, but rather to the global improvement of care, support and counselling associated with prescription of ARVs in the context of the Ivoirian DAI. It has been recently argued that approaches to the prevention and control of the HIV epidemic in Africa have been too heavily based on early experiences and policies from industrialized countries, in which the disease only affects specific risk groups, and that it was urgent to redefine HIV/AIDS as 'a public health and infectious disease emergency' and to put a greater emphasis on HIV testing and counselling [42]. In comparison with other patients, ARV-treated individuals have certainly benefited from increased efforts for secondary prevention and promotion of safe sexual behaviours from various health care professionals. These include their prescribing physicians who may have given special attention to the potential risk of dissemination of resistant viral strains before initiating the use of antiretroviral drugs in these patients and during follow-up.

Second, in multivariate analysis, ARV was only one predictor of lower likelihood of risky sexual behaviour that appears to be more heavily influenced by other social and personal characteristics of respondents, including their interaction with their regular partner.

Third, some general limitations of this survey have to be acknowledged. Its design was cross-sectional whereas a longitudinal assessment, prior and after initiation of ARV, would have been more appropriate, especially to evaluate the potential impact of access to ARV on subsequent sexual behaviours. In addition, assessment of sexual behaviours was based on self-reports which may be biased by socially desirable responding [43], and we cannot exclude that these biases may have been more pronounced among patients who went through the complex process of getting access to ARV than in the rest of the sample. Moreover, this survey was carried out less than 18 months after the effective launching of the DAI and its design was cross-sectional. Therefore, treated patients in the sample had limited follow-up. We cannot exclude that the long-term impact of ARV on sexual and social life of patients may create new opportunities for risk behaviours. In comparison with current standards of care in developed countries, initiation of ARV in the Ivoirian DAI tended to happen at lower CD4 cell count levels and thus at lower levels of sexual activity. The relative improvement in quality of life, and consequently in willingness to have an active sexual life, associated with effective treatment, may therefore be more pronounced in this population. In such a context of later

initiation, the impact of ARV on the risk behaviours in the HIV-infected population may be quite different to that in the Western world and remains difficult to predict.

Making ARV more widely available in Africa certainly calls attention to the necessity of increasing parallel efforts for both primary and secondary prevention among patients who are already HIV infected. To date, the experience of the Ivoirian DAI does not support the a priori fears that access to ARVs may facilitate risk behaviours among African HIV-infected patients, although longer follow-up and further research are clearly needed to assess the impact of ARV on subsequent behaviours in these patients. In any case, it rather confirms the argument that prevention and access to care, including ARV, should mutually reinforce each other in resource-poor settings [44].

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Appendix

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Physicians' knowledge and attitudes toward HIV care in the context of the UNAIDS/Ministry of Health Drug Access Initiative in Côte d'Ivoire

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Objective: To evaluate the impact of the availabity of highly active antiretroviral therapies (HAART) in the context of the Drug Access Initiative (DAI) on physicians' knowledge, attitudes and practices toward HIV care in Côte d'Ivoire.

Design and methods: Cross-sectional survey using self-administered questionnaires among all consulting physicians in the six 'referral centers' of the DAI and five additional centers in charge of HIV care.

Results: Among the 123 respondents (response rate = 82.0%), 45.1% took care of more than 20 HIV-infected patients during the previous year. These physicians with the most experience in HIV care had a better knowledge than the rest of the sample about HIV disease, cotrimoxazole prophylaxis and antiretroviral treatment, and were more likely to declare that HIV-infected patients may be 'dangerous for others' (33.9 versus 17.9%; $P = 0.03$). Although 54.5% declared that the eligibility medical criteria for HAART 'should be the same in both developing and developed countries', only 30.9% adhered to the recently issued DAI guideline (October 1999) recommending initiation of HAART for patients with CD4 cell counts $< 500 \times 10^6$ cells/l.

Conclusion: Physicians involved in the DAI in Côte d'Ivoire have acquired appropriate expertise and knowledge about HAART, but dissemination of information about HAART must be extended to physicians with more limited experience in HIV care. Current international efforts to adapt HIV treatment guidelines for resource-limited settings may face difficulties for reaching consensus among the African health professionals in charge.

AIDS 2003, **17** (suppl 3):S79–S86

Keywords: Africa, antiretroviral treatment, attitudes, beliefs, HIV infection, knowledge, physicians, practices

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Sponsorship: This work was funded by the French Agency for AIDS Research (ANRS).

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Introduction

During the last 2 years, there has been a growing advocacy in favour of an improved access of HIV-infected patients from the developing world to effective care packages including antiretroviral treatments. Working independently, two expert consultation groups have recently assessed the obstacles standing in the way of such enlarged access to antiretroviral treatment in developing countries [1,2]. Lack of health care infrastructures, including lack of trained and experienced health care professionals, is often considered as a major barrier for rational prescription of antiretroviral drugs in developing countries and especially in Africa [3,4]. The Ministry of Health/UNAIDS Drug Access Initiative (DAI) in Côte d'Ivoire was officialy announced in November 1997 and effectively started in August 1998. Due to this early initiation, Ivoirian physicians and health care workers were among the first in Africa to be confronted with prescription and monitoring of antiretroviral therapies for a significant number of HIV-infected patients.

As soon as July 1997, a national consensus conference was held in Abidjan that issued guidelines for use of antiretroviral drugs (ARVs), which subsequently became the reference for the initial framework of the DAI. These guidelines established biological eligibility criteria for initiation of ARV treatment which were similar to those already recommended in developed countries at that time [5]: CD4 cell counts $< 500 \times 10^6$ cells/l and/or viral load $> 10\,000$ copies/ml. It must however be noted that these recommendations only referred to bitherapies (associating two nucleoside reverse transcriptase inhibitors, NRTIs) as the treatment of choice. Public subsidies covering part of the cost of ARVs were consequently restricted to NRTI drugs and excluded protease inhibitors (PIs). Following the experience accumulated with the first 15 months of the DAI, as well as the growing scientific evidence at international level that highly active antiretroviral therapies (HAART) including PIs were more effective, these national recommendations were ultimately modified in October 1999. HAART officially became the reference of the DAI for first line treatment and public subsidies were extended to coverage of PIs.

The evaluation of the socio-economic and behavioural impacts of the Ivoirian DAI included a cross-sectional survey among physicians working in hospital departments and medical centers in charge of HIV care in order to assess their knowledge, attitudes and practices toward HIV care. Because the survey took place between December 1999 and February 2000, it also gave us the opportunity to capture the immediate impact on physicians' attitudes of the change in DAI guidelines in favour of HAART.

Methods

A cross-sectional survey by anonymous self-administered questionnaires was carried out from December 1999 to February 2000 in 11 medical centers: the six referral centers, located in Abidjan, which were officialy accredited by the DAI for prescription of antiretroviral drugs and five additional hospital departments or antituberculous centers, in Abidjan and Bouaké, which took care of HIV-infected patients. The seven hospital departments involved in the UNAIDS DAI are the following: Service des maladies infectieuses du CHU de Treichville (SMIT), Unité de Soins Ambulatoires et de Conseil (USAC), Centre Anti-Tuberculeux d'Adjamé (CATA), Service de Pédiatrie du CHU de Yopougon, Hôpital Militaire d'Abidjan, CIRBA et Hôpital de Jour de Bouaké. The other departments are: Service de pneumophtisiologie du CHU de Treichville, Centres Anti-Tuberculeux de Bouaké et de Treichville, Service de médecine générale du CHU de Bouaké. Among these 11 centers, seven (four out of the six referral DAI centers and three out of the five others) could be considered as HIV specialized units to the extent that patients with known HIV diagnosis constituted 40% or more of their global clientele during the year 1999. Before the beginning of the survey, specific meetings were organized to make health care workers aware of the importance of the research, as a component of the evaluation of the DAI. The questionnaire was systematically proposed to all physicians practising in these 11 centers.

The questionnaire was derived from survey instruments previously used in research about HIV care among medical professionals in France [6,7]. It included a total of 88 questions and 300 items dealing with:

- socio-demographic, professional and personal characteristics of the respondents (14 questions);
- physician's experience about care of HIV-infected patients (22 questions);
- knowledge about HIV infection and care including specific questions about side effects of ARVs (21 questions);
- attitudes and practices toward HIV screening and counselling (12 questions);
- attitudes, knowledge and practices toward occupational exposure to blood injury (13 questions);
- attitudes toward HIV-infected patients including a shortened six-item version of the 'Prejudicial Evaluation Scale', which had been previously validated for measuring stigmatization toward AIDS patients among health care professionals [8] (3 questions); and
- ethical and public health issues associated with HIV care (3 questions).

The following two questions dealt with physicians' attitudes toward initiation of antiretroviral treatment in the

context of the recent evolution of DAI guidelines on that issue:

- 'at what CD4 cell count level would you consider appropriate to initiate bitherapy as antiretroviral therapy for an asymptomatic HIV-infected patient?' and;
- 'at what CD4 cell count level would you consider appropriate to initiate HAART as antiretroviral therapy?'

Answers to both questions allowed us to classify respondents in three differents groups. Group 1: physicians in favour of 'early' systematic prescription of HAART for patients with CD4 cell counts $< 500 \times 10^6$ cells/l. Group 2: physicians who would prescribe HAART only for patients with advanced disease (CD4 cell count $\leq 200 \times 10^6$ cells/l) while considering bitherapy for other patients with less advanced immunodepression. Group 3: physicians with no strictly defined prescription strategy.

Statistical analysis

Characteristics, attitudes and practices of physicians were first compared according to their degree of involvement in HIV care (whether or not they took care of 20 HIV-infected patients or more in the previous year), using a χ^2 test. In order to take into account potential cluster effect due to recruitment of respondents in different medical centers whether or not they belonged to the DAI as a referral center, crude odds ratios were calculated using a logistic regression based on generalized estimating equations [9].

A χ^2 test was also used to compare the three groups of physicians according to their attitudes toward initiation of HAART. Multiple comparisons were subsequently performed if the overall χ^2 was < 0.15 due to limited size of the subgroups.

Results

The total number of physicians consulting in the 11 participating centers was 150, and 123 out of these 150 physicians agreed to answer the questionnaire (response rate = 82.0%). The majority of respondents (n = 80, 65.0%) consulted in one of the DAI referral centers. However, less than half of the sample (n = 56, 45.5%) could be considered as having a high HIV-related medical practice by taking care of more than 20 HIV-infected patients during the previous 12 months. As shown in Table 1, this group of physicians with the highest experience in HIV care differed by some professionnal and personal characteristics from the rest of the sample. They were older, more likely to have graduated in pneumology or infectious diseases, to work in HIV care 'specialized' units, to have received a post-university medical training in HIV infection and to have experience of performing invasive procedures on HIV-infected patients. Contrary to the rest of the sample, the majority of them would personally undertake the post-test counselling following a positive HIV test result in one of their patients rather than relying on social workers or colleagues. They were also more likely to have been personally tested for HIV and hepatitis B and personally to know HIV-infected persons outside the context of their professional practice.

Table 2 clearly shows that this group of physicians with the highest activity in HIV care also had a significantly better knowledge of HIV disease and its treatments including the use of antiretroviral drugs.

Conversely, no differences were found between respondents according to their level of activity on HIV care for most issues related to attitudes toward HIV-infected patients and HIV care policies (Table 3). The sample expressed a strong consensus against discriminatory attitudes toward these patients and a commitment in favour of a larger free-of-charge access to ARV treatments going beyond the existing DAI rules, which only offered partial coverage of the costs of ARVs. It must however be noted that the minority of physicians who declared that HIV-infected patients sometimes may be 'dangerous for others' was significantly more numerous in the group with the highest activity in HIV care (Table 3). It must also be noted that the sample was nearly half split on the issues of considering that 'AIDS patients are expensive for society' and of applying the same criteria for initiation of ARV treatment in Côte d'Ivoire than in developed countries, although physicians' level of experience with HIV care did not interfere with their opinions on these issues.

In spite of DAI official guidelines which at that time recommended prescription of HAART for patients with lymphocytes CD4 cell counts $< 500 \times 10^6$ cells/l, attitudes toward initiation of antiretroviral treatment were variable according to the different professional and personal characteristics of the respondents (Table 4). Among the 123 practitioners, only approximately one-third (30.9%) were in complete agreement with this recent recommendation. Around another one-third (34.1%) tended to limit HAART prescription to patients with more advanced disease (CD4 cell count $< 200 \times 10^6$ cells/l) and the remaining third (35.0%) had no defined strategy.

Table 4 shows that the latter group, those who did not have a defined attitude towards HAART initiation, was made up of physicians with less experience and less appropriate knowledge in HIV care, and who were less likely to practice in medical centers with a high prevalence of HIV-infected patients among the clients. Conversely, the two other groups who expressed divergent opinions about HAART initiation had similar levels of experience and knowledge in HIV care.

Table 1. Characteristics of consulting physicians in the Drug Access Initiative referral and peripheral centers and experience with HIV care (December 1999–February 2000, n = 123).

	Took care of more than 20 HIV-infected patients during the last 12 months			
Physicians' characteristics	Yes n = 56 (%)	No n = 67 (%)	Level of sign (*P*)[a]	Crude odds ratios[b] (95% CI)
Age < 40 years (n = 87, 70.7%)	58.9	80.6	0.015	0.34 (0.15–0.76)
Men (n = 88, 71.5%)	75.0	68.7	0.565	1.41 (0.64–3.13)
Married (n = 70, 56.9%)	60.7	53.7	0.551	1.34 (0.65–2.76)
Has a regular religious practice (n = 25, 20.3%)	21.4	19.4	0.876	1.14 (0.47–2.75)
Has graduated in pneumology or infectiology (n = 25, 20.3%)	30.4	11.9	0.021	2.10 (1.15–3.82)
Belong to HIV specialized hospital department[c] (n = 58, 47.2 %)	73.2	25.4	< 0.001	6.79 (3.26–14.11)
Had post-university medical training on HIV infection (n = 69, 56.1%)	73.2	41.8	< 0.001	3.83 (1.78–8.23)
Had experience of invasive procedures with HIV-infected patients (n = 77, 62.6%)	80.4	47.8	< 0.001	4.49 (1.98–10.15)
Always personally does post test counselling following HIV+ diagnosis of patients (n = 55, 44.7%)	62.5	29.9	< 0.001	4.09 (1.93–8.67)
Has been personally tested for HIV (n = 57, 46.3%)	57.1	37.3	0.052	2.33 (1.13–4.80)
Has been personally tested for hepatitis (B) (n = 41, 33.3%)	42.9	25.4	0.063	2.25 (1.05–4.83)
Personally knows HIV-infected persons outside professional practice (n = 86, 69.9%)	85.7	56.7	< 0.001	4.93 (2.05–11.87)

[a]χ^2 test with Yates correction. [b]Odds ratios were calculated using a logistic regression based on generalised estimating equations to take into account the correlation existing among physicians consulting in the same center. [c]Hospital departments with high prevalence of HIV-infected patients in clientele (≥ 40%). CI, Confidence interval.

Table 2. Physicians' knowledge and experience with HIV care in referral and peripheral centers of the Drug Access Initiative in Côte d'Ivoire (December 1999–February 2000, n = 123).

	Took care of more than 20 HIV-infected patients during the last 12 months			
Percentage of physicians giving the correct answer[a]	Yes n = 56 (%)	No n = 67 (%)	Level of sign (*P*)[f]	Crude odds ratios (95% CI)[g]
• It's necessary to treat with cotrimoxazole all HIV-infected symptomatic patients or with CD4 cell count ≤ 200 × 10^6 cells/l (n = 67, 54.5%)[a]	71.4	40.3	0.001	3.88 (1.82–8.24)
• Cotrimoxazole dose for opportunistic infections prophylaxis in HIV-infected adult of average weight (n = 87, 70.7%)[b]	87.5	56.7	< 0.001	5.71 (2.28–14.27)
• Main side-effects of cotrimoxazole (n = 45, 36.6%)[c]	48.2	26.9	0.002	2.57 (1.21–5.47)
• It's necessary systematically to prescribe zidovudine to HIV-infected pregnant women (n = 83, 67.5%)[a]	78.6	58.2	0.027	2.64 (1.19–5.89)
• There is a risk of mother-to-child HIV transmission through breastfeeding (n = 100, 81.3%)	92.9	71.6	0.006	5.07 (2.58–8.30)
• With a patient with CD4 cell count 350 × 10^6 cells/l with a severe pneumonia, does not change the ARV treatment and treats with adapted antibiotics (n = 44, 35.8%)[a]	50.0	23.9	0.005	3.33 (1.55–7.18)
• Bitherapies allowed to initiate an ARV treatment (n = 50, 40.7%)[d]	58.9	25.4	< 0.001	4.65 (2.19–9.85)
• Main side-effects of zidovudine (n = 71, 57.7%)[e]	80.4	38.8	< 0.001	6.45 (2.82–14.72)

[a]On the basis of scientific information and medical guidelines used in the Drug Access Initiative at time of survey. [b]One dose of 800 mg/day or two doses of 400 mg/day. [c]Blood, skin and liver effects. [d]Zidovudine–didanosine or zidovudine–lamivudine were the two only good answers in the list of five proposed combinations. [e]At least three among the five following side-effects: anaemia, liver disorders, peripheral neuropathy; headache, nausea and vomiting (among 11 proposed items). [f]χ^2 test with Yates correction. [g]Odds ratios were calculated using a logistic regression based on generalised estimating equations to take into account the correlation existing among physicians consulting in the same center. CI, Confidence interval.

Table 3. Physicians' experience with HIV care and attitudes toward AIDS patients and public policies on HIV infection in referral and peripheral centers of the Drug Access Initiative in Côte d'Ivoire (December 1999–February 2000, n = 123).

Percentage of respondents who 'rather' or 'totally'agree with the following judgments about AIDS patients:	Took care of more than 20 HIV-infected patients during the last 12 months		
	Yes n = 56 (%)	No n = 67 (%)	Level of sign (*P*)[a]
• Deserve sympathy and solidarity (n = 116, 94.3%)	92.9	95.5	0.701[b]
• Endure a lot of suffering (n = 104, 84.6%)	82.1	86.6	0.670[b]
• Are responsible for their own disease (n = 7, 5.7%)	5.4	6.0	1.000[b]
• Dangerous for others (n = 31, 25.2%)	33.9	17.9	0.067[c]
• Are expensive for society (n = 61, 49.6%)	51.8	47.8	0.792[b]
• Much more HIV+ patients should have access to antiretroviral treatment (n = 94, 76.4%)	78.6	74.6	[b]
• Antiretroviral treatment should be free of charge for the more severely ill patients (n = 87, 70.7 %)	69.6	71.6	0.965[b]
• During pregnancy, antiretroviral treatment should be given freely to HIV-infected women (n = 107, 87.0%)	85.7	88.1	0.908[b]
• In Côte d'Ivoire, criteria for initiating antiretroviral treatment for HIV should be the same as in developed countries like France (n = 67, 54.5%)	55.4	53.7	1.000[b]

[a]χ^2 test or Fisher test when needed. [b]Absence of statistically significant difference was confirmed when computing odd-ratios (OR) using a logistic regression based on generalised estimating equations. [c]Logistic regression based on GEE OR = 2.37 (1.02–5.46).

They, however, did differ in some characteristics. Those who adhered to the recently issued DAI guidelines and supported early initiation of HAART tended to belong to medical specialties in infectious diseases and pneumology and had followed post-university medical training on HIV infection. In contrast, those who did not seem ready to approve the recent DAI guidelines and were in favour of reserving HAART initiation for patients with advanced immunodepression were mostly physicians consulting in DAI referral centers. They also tended to have a quite different style of practice from the rest of the sample in that they actively collaborated with non-governmaetal organizations and they expressed less concerns towards the risks of occupational exposure to HIV. They also seemed more conscious of the economic and resource allocation constraints for access to antiretroviral treatment (as suggested by the greater proportion in this group of those who agreed that 'AIDS patients are expensive for society', as well as the lower proportion of those who supported the idea of enlarged access to antiretroviral treatment).

Discussion

Although there have been numerous studies to investigate the knowledge, attitudes, beliefs and practices towards HIV care among medical professionals in the developed world [10,11], only limited data have been published about African physicians [12–16]. It is obvious that active involvement and adequate training of health care workers are a prerequisite for rational scaling up of access of HIV-infected patients to effective antiretroviral therapies in Africa [17]. Of course, this survey, carried out in the context of the evaluation of the Ivoirian DAI, was restricted to some of the hospitals and clinics in charge of HIV care in the two main cities of Côte d'Ivoire (Abidjan and Bouaké). It could not pretend to be representative of the totality of physicians in these two geographic areas and even less of the country as a whole. Nearly all consulting physicians in the DAI referral centers for prescription and surveillance of antiretroviral treatments were, however, included in the sample and the additional respondents were physicians who practised in medical centers that care for HIV-infected patients and were qualified for referring these patients to the DAI. At the time of our survey, more than 600 HIV-infected patients were already under ARV treatment through DAI referral centers. Therefore, this survey is the first attempt to document the impact of availability of antiretroviral treatment on the knowledge, attitudes and practices of medical practitioners who were among the most experienced in HIV care management in West Africa.

Results show that Ivoirian physicians who take care of a significant number of HIV-infected patients (> 20) are globally well informed about antiretroviral treatments, cotrimoxazole prophylaxis and prevention of mother-to-child transmission of HIV. As in most studies among health care professionals world-wide [6,10,11,18], a clear relationship was observed between a greater previous experience with management of these patients on the one hand, and a better knowledge of HIV disease as well as of recommended clinical practices in HIV care on the other. It must, however, be noted that almost one-third of physicians interviewed, although they were working in referral centers for the DAI or in other medical cen-

Table 4. Physicians' characteristics and attitudes toward initiation of highly active antiretroviral therapies (HAART) in referral and peripheral centers of the Drug Access Initiative in Côte d'Ivoire (December 1999–February 2000, n = 123).

	Attitudes toward systematic initiation of HAART in asymptomatic HIV+patients				
Physicians' characteristics	1 Early HAART (< 500 × 10^6 cells/l) (n = 38) (%)	2 Late HAART (≤ 200 × 10^6 cells/l) (n = 42) (%)	3 No defined attitude (n = 43) (%)	Level of sign (*P*)[a]	Level of sign 1 vs 2 (*P*)[b§]
Male (n = 88, 71.5)	84.2	64.3	67.4	0.11	0.08
Age < 40 years (n = 87, 70.7%)	65.8	69.0	76.7	0.53	–
Married (n = 70, 56.9%)	44.7	66.7	58.1	0.14	0.08
Belong to DAI referral centers	52.6	88.1	53.5	< 0.01	< 0.01
Belong to HIV specialized hospital department (n = 65, 58.3%)	47.4	64.3	30.2	< 0.01	0.19
Graduated in infectious diseases or pneumology (n = 25, 20.3%)	31.6	14.3	16.3	0.11	0.06
Had post-university medical training on HIV infection (n = 69, 56.1%)	73.7	54.8	41.9	0.02	0.01
Took care of more than 20 HIV-infected patients during the last 12 months (n = 66, 53.7%)	52.6	54.8	30.2	0.04	0.92
Adresses patients to spiritual or religious group (n = 22, 17.9%)	10.5	28.6	14.0	0.07	0.08
Adresses patients to HIV-NGOs (n = 41, 33.3%)	28.9	52.4	18.6	< 0.01	0.06
Systematically recommends sexual abstinence to HIV+ patients (n = 64, 52.0%)	42.1	64.3	48.8	0.12	0.08
Has been personally tested for HIV (n = 66, 53.7%)	47.4	54.8	37.2	0.26	–
Correct knowledge of bitherapies allowed to initiate an ARV treatment (n = 50, 40.7%)	50.0	52.3	25.6	0.02	0.94
Correct knowledge of cotrimoxazole prophylaxis (n = 87, 70.7%)	81.6	78.6	53.5	< 0.01	0.96
Systematically uses gloves with patients of unknown HIV serostatus (n = 89, 72.4%)	89.5	61.9	67.4	0.01	0.01
Would make official notification in case of needlestick injury with a patient of unkown HIV status (n = 39, 31.7%)	76.3	54.8	74.4	0.06	0.07
Agrees that 'much more HIV+ patients should have access to antiretroviral treatment' (n = 94, 76.4%)	86.8	64.3	79.1	0.05	0.04
Agrees that AIDS patient 'are expensive for society' (n = 61, 49.6%)	42.1	61.9	44.2	0.14	0.06

[a]χ^2 test on the 3 × 2 contingency tables. [b]χ^2 test comparing (1) versus (2) when overall $\chi^2 < 0.15$. ARV, antiretroviral

ters referring patients for antiretroviral therapy, were not really able to express a clear attitude about biological criteria for HAART initiation and had some important gaps of knowledge about these treatments.

Qualitative interviews, carried out among care-givers in parallel to this survey [19], confirmed the fact that information and training about antiretroviral therapies remained limited among health care professionals outside the group of practitioners 'highly specialized' in HIV care. Another survey, which was carried out during the same period as the present study and in the same two geographic areas, but among health professionals consulting in dispensaries not related to the DAI and with low HIV-related activities, clearly revealed a limited level of information about HIV care, including criteria for initiation and appropriate dosages for cotrimoxazole chemoprophylaxis [20]. The situation with regard to HIV/AIDS care is certainly not uniform among physicians and other health care professionals throughout the Côte d'Ivoire. As antiretroviral treatment will become more widely available, it would be essential, in Côte d'Ivoire as elsewhere in Africa, that information on management of these therapies be dissimenated more broadly among health care workers confronted with HIV-infected patients, even among those professionals who will not have a direct opportunity to prescribe ARVs.

Discriminatory and stigmatizing attitudes toward HIV-infected patients have been observed among health care professionals of both developed and developing coun-

tries, but have been shown to decrease as practical clinical experience with these patients increases [8,12,14]. Although most physicians in our sample expressed strong concerns about occupational risk of exposure to HIV transmission, very few expressed discriminatory and hostile attitudes toward HIV-infected patients, a fact that has to be related to their direct involvement in HIV care. It must however be noted that a significant minority of these physicians, even more numerous among those with the highest activity in HIV care, openly expressed concerns that HIV-infected patients may be 'dangerous for others'. Of course, this finding may reflect nothing more than the knowledge on the part of the most experienced clinicians that HIV-infected individuals are infectious for others, as well as the practical difficulties these clinicians have to face for preventive counseling of their patients who are already HIV infected. It is obvious in the Ivoirian context, which is similar to many other African countries, that patients often have difficulties in disclosing their serostatus to others in the community, and that consequently health practitioners are confronted with complex issues when counseling individuals in serodiscordant couples [19].

The survey also indicates that shortly after the decision of the DAI, in October 1999, to introduce HAART as the treatment of choice for initiation of antiretroviral therapy with identical biological eligibility criteria to those used in developed countries, such a recommendation had not yet reached a consensus among medical practitioners in charge of implementing them. Indeed, between and inside developed countries, a great variability of physicians' attitudes and clinical practices in HIV care has been documented [6,7,18,21]. In HIV, as in many other diseases, variability of clinical practice has been related to professional attitudes and personal characteristics of prescribing physicians, and to variations in the primary source of information about drugs and to the stage of diffusion of a medical innovation [22–24]. It is therefore not surprising to observe a similar variability of attitudes among Ivoirian physicians confronted with 'professional uncertainty' toward initiation of antiretroviral therapy at an early stage of diffusion of these treatments in their country.

The answers of these physicians to questions concerning access to ARVs, however, suggest that variability of clinical attitudes may be more difficult to manage due to the hardest constraints faced by health professionals in resource-limited settings where basic components of care, including drugs, are often 'not available'[25]. By tradition, Ivoirian physicians have a strong commitment to universal medical ethics and, similar to most of their colleagues in developed countries such as France, they claim the right to practice medicine 'free of financial constraints' [26]. From the beginning, the HIV epidemic has challenged the ethical and deontological principles universally recognized in medical practice [27]. Ethical dilemmas related to access to ARV treatment are unavoidingly exacerbated in the African context of absence of health insurance coverage for most patients and of limited availibility of resources for the health care system. Limited resources create strong pressures on care givers who have to decide which patients may have access to treatment and can hardly ignore the direct impact of socio-economic constraints on their practice [28–30]. The fact that some of the physicians consulting in the DAI referral centers, although they already knew that HAART was a more effective therapy and should be considered as the standard of care, were still considering bitherapy, at least for certain groups of patients with less advanced immunodepression, may indeed reflect a form of 'economic realism': in limited-resource settings, a more affordable less costly therapy, such as bi-therapy, although unsatisfactory from a pure medical point of view, remained far superior to no therapy at all.

In our survey, the Ivoirian physicians interviewed diverged about the necessity as to whether or not it is appropriate to adopt similar criteria for HAART initiation in their limited-resource context than in developed countries. The group of experienced physicians in HIV care who were the most reluctant toward the DAI guidelines for early initiation of HAART seemed to be the most aware about resource constraints which limit the universal practical implementation of this recommendation in their country. Of course, these differences of attitudes toward ARV treatment may have evolved since our survey was carried out and significant decreases in prices of antiretroviral drugs, including protease inhibitors and non-nucleoside reverse transcriptase inhibitors, have occurred in 2001 and 2002. Indeed, these differences may have limited impact in practice, to the extent that most ARV-treated patients in the DAI had CD4 cell counts $< 250 \times 10^6$ cells/l at initiation of HAART.

This survey however suggests that scaling up access to ARVs will certainly lead to difficult debates among African health care professionals about the most appropriate way to take into account resource constraints in their clinical practice. Current international efforts to adapt HIV treatment guidelines for resource-limited settings will certainly facilitate rational decision-making about antiretroviral treatment in Africa [17]. The experience of Ivoirian physicians in the context of the DAI shows that adoption of such guidelines will not be straightforward and will easily reach a consensus among the professionals in charge.

Acknowledgements

The authors thank M. Coulibaly (ONUSIDA, Abidjan), I. M. Coulibaly (PNLS/MST/TUB, Abidjan), R. Aka and A. Kadio (Service des Maladies infectieuses du CHU de Treichville, Abidjan) and all physicians who took part in the study.

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Appendix

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The most efficient use of resources to identify those in need of antiretroviral treatment in Africa: empirical data from Côte d'Ivoire's Drug Access Initiative

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Objective: To describe the cost and outcome associated with the use of CD4 cell count and viral load tests as part of screening strategies to identify persons eligible for subsidized antiretroviral therapy (ART) in Côte d'Ivoire.

Methods: Empirical data from the Drug Access Initiative in Côte d'Ivoire (DAI–CI) were used to describe the laboratory cost of patient screening using sequential clinical staging, CD4 cell count, and viral load and the proportion of screened patients identified as eligible for ART. We also estimated costs modelling a parallel screening algorithm, across a range of laboratory costs and with current international recommendations to assess treatment eligibility. Benefit was defined as being found eligible for ART.

Results: Of the 2138 HIV-positive, ART-naive, adults who presented to the DAI–CI between July 1998 and July 2000, median CD4 cell count was 172×10^6 cells/μl. DAI–CI criteria identified 2057 (96%) of these persons eligible for antiretroviral treatment. In a serial screening algorithm, 75% were eligible by CDC clinical stage B or C; 18% by CD4 cell count less than 500×10^6 cells/μl; and an estimated 3.9% by a viral load greater than 10 000 copies/ml. Use of the current US recommendations and a serial algorithm would have resulted in 1977 (92%) persons eligible for ART: 75% by CDC clinical stage B or C; 15% by CD4 cell count less than 350×10^6 cells/μl (including 8% < 200×10^6 cells/μl); and an estimated 3.6% due to viral load greater than 55 000 copies/ml. Using DAI–CI criteria and heavily subsidized laboratory test costs, the addition of CD4 cell count to clinical criteria cost US$50 (serial algorithm) and US$203 (parallel algorithm) to identify each additional eligible person. Modelling current recommendations with a serial algorithm, CD4 cell count cost an average US$62/eligible person (US recommendations) and US$109 (WHO recommendations). The addition of viral load cost between US$108 (serial algorithm DAI) to US$1700 (parallel algorithm DAI) to identify each additional eligible person.

Conclusion: In the African context of scarce resources and the huge unmet demands for voluntary HIV testing and for ART, simple screening strategies are needed to identify those most in need of ART. Health personnel should be trained to identify and refer clinically symptomatic persons. Viral load testing is of high cost and dubious benefit and should not be part of screening algorithms for initiating ART.

AIDS 2003, **17** (suppl 3):S87–S93

Keywords: Africa, AIDS, antiretroviral treatment, CD4 cell count, clinical, Côte d'Ivoire, eligibility, highly active antiretroviral therapy, HIV infection, Ivory Coast, viral load

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Conflict of interest: none declared.

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Introduction

With 42 million people currently living with HIV and 5 million new infections annually, in 2002 the HIV pandemic has surpassed the most pessimistic forecast [1]. Although a global problem, the brunt of the epidemic is borne in sub-Saharan Africa where 70% of all HIV-infected persons live among less than 10% of the world's population. In sub-Saharan Africa, AIDS is the leading cause of morbidity and mortality, and places an increasing burden on already overstretched social and health care systems which further reduces their capacities to respond to other public health and development challenges [2]. Expanded access to comprehensive, effective HIV prevention and care interventions are a prerequisite for African development and stability.

Côte d'Ivoire has a severe, mature and generalized HIV epidemic with an antenatal prevalence of approximately 10% [3]. In 1998, in recognition of this chronic humanitarian emergency, the national government partnered with UNAIDS to establish the 'Côte d'Ivoire Drug Access Initiative' (DAI–CI) to improve access to comprehensive HIV care including subsidized antiretroviral therapy (ART). The cost of treatment fell precipitously through DAI–CI price negotiations with the pharmaceutical industry, subsidies from national and international solidarity funds and the provision of free laboratory tests from the Centers for Disease Control and Prevention (CDC). The 2-year pilot programme was consistent with standards of care in industrialized countries including individualized regimens, sophisticated laboratory tests at baseline to assess eligibility for treatment, and ongoing clinical and laboratory monitoring to assess response to treatment [4]. Special measures were also put in place to increase access for women and children.

The evaluation of the 2-year DAI–CI pilot programme demonstrated a substantial proportion of persons with advanced disease had an initial visit but never began ART. Nevertheless by December 2001, 1011 men, 878 women and 126 children – a total of 2015 persons had commenced ART in the capital city, Abidjan. Overall, ART was successful; among those starting ART in the pilot period, toxicity, and immunologic and virologic outcomes were similar to those reported from populations in industrialized countries [5,6].

The government of Côte d'Ivoire and partners are now planning to decentralize antiretroviral services to increase access to care outside the capital city. The previous screening algorithm to define biologic eligibility for antiretroviral therapy included CD4 cell count and viral load quantification. However, if these sophisticated laboratory facilities and tests are prerequisites for care, they would represent a critical barrier to care for millions of HIV-infected Africans. As in other African countries, the current public health laboratory capacity in Côte d'Ivoire is grossly insufficient to process multiple samples from the millions of persons in need of life-saving treatment [7–12].

Our objective was to examine empirical data from the DAI–CI pilot programme in Côte d'Ivoire to describe the contribution of CD4 cell count and viral load screening tests as part of the screening strategy to identify persons eligible for ART. We also model current biologic eligibility recommendations and a range of test costs to determine if these would have a substantial impact on the cost per identified patient eligible for ART. These should be used to inform policy rapidly to expand access to life-saving antiretroviral therapy in resource-poor countries.

Methods

We used empirical data from the DAI–CI pilot programme to determine the direct costs of performing internationally accepted laboratory screening tests, as well as the proportion of clients identified to be eligible for subsidized ART by clinical staging, by CD4 cell count criteria, and by viral load criteria in a serial (sequential) screening algorithm.

Criteria for initiation of subsidized antiretroviral therapy in the DAI–CI programme

Eligibility criteria were set by a national advisory committee and were consistent with international standards of that time. These included:

- HIV infection (HIV-1, HIV-2 or dual HIV-1/HIV-2 infection)
- HIV-related symptoms – CDC stage B or C [13]
- CD4 cell count $< 500 \times 10^6$ cells μl (for asymptomatic patients)
- HIV-1 plasma viral load $> 10\,000$ copies/ml (for asymptomatic patients with CD4 cell count $> 500 \times 10^6$ cells μl)

We then used the same population to model costs with different eligibility criteria, with parallel as well as sequential testing, and with different test prices: we modeled current US and WHO treatment eligibility criteria [8,14] in place of the DAI–CI criteria; and we modeled costs across a range of CD4 cell count and viral load test prices. We considered 'benefit' to be the outcome of being defined 'eligible for subsidized ART' when meeting clinical criteria, CD4 cell count criteria in the absence of clinical eligibility or viral load criteria alone.

The data were analyzed from the project RETRO-CI maintained DAI–CI database. Project RETRO-CI is a collaboration between the Centers for Disease Control and Prevention (CDC) and the Ivorian Ministry of Health and provides free laboratory testing and data

management support to the clinical centers participating in the DAI–CI. The data are primarily collected for patient care and for programme evaluation, however the protocol and written consent forms have also been reviewed and approved by the CDC and Côte d'Ivoire ethics committees.

Patients were either self-referred or referred from other clinics and screened to determine if they were biologically eligible for subsidized ART and socio-economic criteria were used to determine the level of financial subsidy for drugs [4]. At the first screening visit a clinical examination was performed to categorize the stage of the patient's disease according to CDC staging criteria [13] and blood was drawn for CD4 cell count and hematological (hemoglobin, platelets, total white cell count and profile) and biochemical tests (liver function enzymes: alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransaminase (GGT); bilirubin, creatinine, urea, glucose and amylase). Patients were reviewed at a second visit, and if they were not eligible by clinical and/or CD4 cell count a further blood draw was performed to determine if the patient was eligible through viral load criteria. Eligible patients without significant hematological or biochemical abnormalities were prescribed subsidized ART. After ART initiation, patients were reviewed 1 month later and then quarterly for clinical and laboratory monitoring including CD4 cell count and viral load testing.

Definition of outcomes of interest and cost parameters

Patients enrolled in the DAI–CI were identified as eligible for subsidized ART if they met clinical or CD4 cell count criteria ($< 500 \times 10^6$ cells μl). If they did not meet either clinical or CD4 cell count criteria, they were also eligible if they had elevated viral loads (> 10 000 copies/ml). With the data collected, we were able to define each of the following parameters:

(a) Each eligible person as eligible for subsidized therapy in the Drugs Access Initiative through meeting:
   1. Clinical criteria
   2. CD4 cell count criteria (in absence of clinical criteria)
   3. Viral load criteria (in absence of clinical and CD4 cell count criteria)

   As 2–4% of clients were ineligible due to clinical or CD4 cell count criteria and did not have viral load results we also estimated the number of clients eligible through viral load criteria and calculated the minimum (actual) and maximum estimates.

(b) The programme cost reflects the costs associated with providing CD4 cell count and viral load tests for screening purposes. We used the Project RETRO-CI direct laboratory test costs to calculate the costs associated with performing these tests to identify eligible patients. The additional costs associated with providing CD4 cell count and viral load tests as part of the eligibility screening algorithm (serial or parallel) were calculated.

(c) The programme cost per eligible person identified reflects the direct laboratory costs required to identify an eligible person for each screening component (HIV, CD4 cell count and viral load tests, respectively).

All costs are shown in US dollars. Project RETRO-CI laboratory costs reflect only the direct costs for reagents, test kits, consumables, technician salaries and overheads. Shipping, supervision and infrastructures costs are not included and thus represent non-profit, partially subsidized costs. As all patients require confirmation of their HIV-infected status, HIV screening costs are included in the costs for clinical criteria alone. These estimates reflect current project RETRO-CI costs for HIV rapid tests able to differentiate HIV-1 and HIV-2, which can be used at decentralized sites. Direct project RETRO-CI costs were US$13.36 for HIV rapid tests, US$37.23 for CD4 cell count, and US$65.95 for viral load. Hematological and biochemical test costs have not been included.

As these costs do not reflect full market prices, we also modeled two other hypothetical test cost estimates:

1. High test costs (double RETRO-CI estimates) as these are more accurate reflections of current prices which might be available from a commercial laboratory
2. Low-test costs (half RETRO-CI estimates) as these might model what could be achieved if there are significant technological breakthroughs to identify low-cost alternative tests and/or if industry makes significant price reductions, as already demonstrated by many pharmaceutical companies for antiretroviral drugs.

Results

Population characteristics

Between 1 August 1998 and 31 July 2000, 2625 persons presented to the DAI–CI, of these 1.8 % were HIV negative, 90% were HIV-1 infected, 2.8% HIV-2 infected, and 5.3% dually infected with HIV-1 and HIV-2. Of the 2576 persons found to be HIV positive, 131 (5%) were under 18 years of age and 307 (12%) had previously received antiretroviral therapy. Of the remaining 2138 adult antiretroviral-naive patients, 1070 (50%) were female, the median age was 36 years, and 74% reported an income < US$ 200/month and 36% were unemployed. Patients generally presented with very advanced HIV disease and median CD4 cell count was 172×10^6 cells/μl.

Results of the serial screening algorithm to identify patients eligible for the DAI–CI

Of the 2138 HIV-positive, antiretroviral-naive adults who came for a screening visit, 2057 persons (96%) were

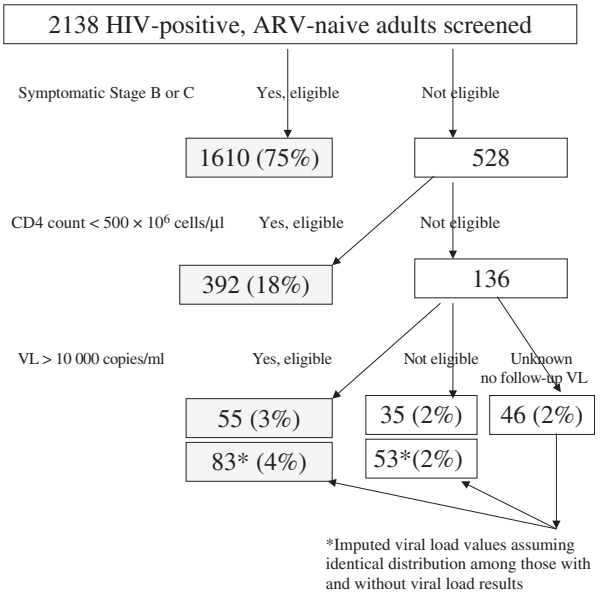

Fig. 1. DAI–CI screening results 8/1998–7/2000: DAI–CI screening criteria and serial algorithm.

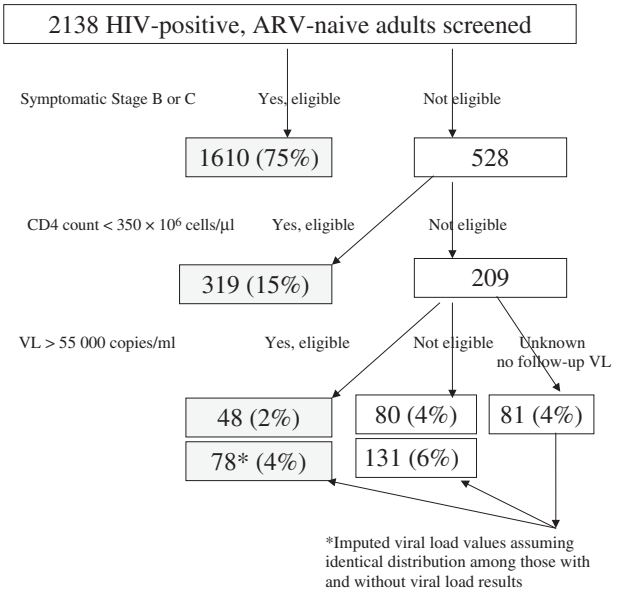

Fig. 2. DAI–CI screening results 8/1998–7/2000: current US criteria and serial algorithm.

eligible for antiretroviral treatment by DAI–CI criteria, 35 (2%) were ineligible by clinical, CD4 cell count and viral load criteria, and a further 46 (2%) were ineligible by clinical and CD4 cell count criteria but did not return for follow-up and/or had no viral load results (Fig. 1). Most persons had advanced CDC stage B or C at screening (1610 persons or 75% of those screened), a further 392 (18%) were eligible with a CD4 cell count less than 500×10^6 cells/µl in the absence of advanced clinical disease [including 180 (8%) with a CD4 cell count less than 200×10^6 cells/µl] and only 55 (2.6%) were eligible due to a viral load greater than 10 000 copies/ml alone.

Results of the serial screening algorithm modeling current US recommendations

Had the current US recommendations been used to define eligibility for subsidized ART in the same patient population, similar results would have been obtained (Fig. 2). Of the 2138 HIV-positive, antiretroviral-naive adults screened, 1977 persons (92%) would have been identified as eligible for antiretroviral treatment, 80 (4%) would have been ineligible by clinical, CD4 cell count and viral load criteria and a further 81 (4%) ineligible by clinical and CD4 cell count criteria but who did not return and/or had no viral load result. The same number of persons (1610) would have been identified through advanced disease at screening, a further 319 (15%) would have been eligible with a CD4 cell count less than 350×10^6 cells/µl and only 48 (2.2%) would have been eligible due to a viral load greater than 55 000 copies/ml alone.

Since not all the patients attended for a second visit and had the opportunity to receive viral loads, the proportion of patients identified as eligible through viral load testing represents a minimum estimate. Even in the worst-case scenario, when we assign all patients missing viral loads as 'eligible', only 6% of patients screened could be eligible using either the DAI–CI (5%) or current US criteria (6%). In the more likely scenario in which we assume that clients missing viral loads had similar viral load patterns to those tested, we estimated viral load screening would have identified 83 (3.9%) eligible patients in the DAI–CI (viral load > 10 000 copies/ml) and 78 (3.6%) with current US criteria (viral load > 55 000 copies/ml) (Fig. 1 and Fig. 2 and Table 1).

Programme costs for eligibility screening

The direct costs and benefits of the laboratory screening are shown in Table 1. Performing both HIV tests and CD4 cell count tests on all 2138 patients screened (the strategy used in the DAI–CI) cost approximately US$28 000 for HIV tests and US$80 000 for CD4 cell counts. Had viral loads been included in a parallel screening strategy it would have cost US$249 269 and resulted in expenditure of 57% of the laboratory screening budget in order to identify less than 4% of eligible clients. A serial screening strategy (total cost of US$57 197) was much cheaper than a parallel strategy (total cost of US$249 269). The use of clinical criteria with HIV testing cost an average of US$18 per eligible person identified. The addition of CD4 cell count in a serial algorithm cost an average of US$50 per eligible

Table 1. Costs associated with using HIV screening, CD4 cell count and viral load tests for eligibility screening.

	No. screened	No. eligible (adjusted)	% eligible for ART	Programme costs $	%	Cost to identify additional eligible person (US$)
Parallel algorithm: DAI (clinical, CD4 cell count and viral load)						
Clinical (HIV test)	2138	1610	75.3	28564	11	18
CD4 cell count < 500 × 10^6 cells/μl	2138	392	18.3	79598	32	203
VL > 10 000 copies/ml	2138	83*	3.9	141108	57	1700
Total	2138	2085	97.5	249269		
Serial algorithm: DAI (clinical, if not eligible CD4 cell count, if not eligible viral load)						
Clinical (HIV test)	2138	1610	75.3	28564	50	18
CD4 cell count < 500 × 10^6 cells/μl	528	392	18.3	19657	34	50
VL > 10 000 copies/ml	136	83*	3.9	8976	16	108
Total	2138	2085	97.5	57197		
Serial algorithm: current US recommendations modeled						
Clinical (HIV test)	2138	1610	75.3	28564	46	18
CD4 cell count < 350 cells/μl	528	319	14.9	19657	32	62
VL > 55 000 copies/ml	209	78*	3.6	13794	22	177
Total	2138	2007	93.9	62015		
Serial algorithm: 2002 WHO recommendations modeled						
Clinical (HIV test)	2138	1610	75.3	28564	46	18
CD4 cell count < 200 cells/μl	528	180	8.4	19657	32	109
Total	2138	1868	87.4	48221		

*Inputed vira load values assuming identical distribution among those with and without viral load results. ART, antiretroviral therapy; DAI, drug access initiative; VL, viral load.

person identified through meeting CD4 cell count criteria without clinical symptoms. The additional cost of a person identified through viral load criteria alone (as part of a serial algorithm) was US$108. Using the current US recommendations these additional costs increased to US$62 and 177 for CD4 cell count and viral load screening tests respectively. A WHO screening approach with sequential clinical evaluation and CD4 cell counts would have been the cheapest strategy (total cost US$48 221).

If we simulate more realistic commercial testing prices by doubling these test price estimates we double these additional costs. This suggests that with this patient population the use of viral load as a serial screening tool will identify persons eligible for ART at an average cost in excess of US$200. Even if test prices decreased dramatically, to less than half of our project RETRO-CI subsidized estimates, viral load testing would still cost more than US$50 for each additional patient identified to be eligible.

Discussion

Clinical screening with HIV confirmation identified the majority of patients eligible for ART at a low cost in the DAI–CI. A further 15–18% could be identified with CD4 cell count screening at a moderate cost. Current WHO recommendations would have identified 8% of asymptomatic persons with CD4 cell counts below 200 × 10^6 cells/μl in need of HAART. In contrast, the inclusion of quantitative viral load as a serial screening tool identified few additional persons eligible for ART and was extremely expensive. This result was robust across both the DAI–CI eligibility criteria and the current US recommendations: viral load screening identified 4% of persons screened to be eligible for ART, at a minimum cost in excess of US$100/eligible person. This result is primarily driven by the fact that most people seeking antiretroviral care in sub-Saharan Africa have very advanced HIV disease [5]. The profile of patients seeking care in Côte d'Ivoire DAI–CI is very similar to that described for other African countries including Uganda [15], Kenya [7] and South Africa [16].

While there is much discussion of expanding access to care [7–9,12], this is unlikely to change the profile of patients seeking antiretroviral care to any large extent in the near future for two inter-related reasons. First, there are vast numbers of untreated patients with advanced disease in Africa [1,2]. Second, there is limited access to counseling and voluntary HIV testing services in most African countries so that relatively few persons with asymptomatic disease know they are infected with HIV. Stigma and discrimination represent further substantial but not insurmountable barriers to HIV testing. In Côte d'Ivoire, there is a similarly elevated antenatal HIV prevalence at all major urban centers, yet, at the beginning of

2002, there were only four stand-alone Voluntary Counseling Testing (VCT) centers in a country of more than 16 million people. (There are plans to increase the number of VCT centers dramatically in the next 5 years).

In comparison with those with advanced disease, persons with elevated viral loads in the absence of symptomatic HIV disease or low CD4 cell counts have less to gain from commencing therapy [15]. There is ongoing debate about the benefit and harms of delaying treatment with CD4 cell counts in the range of $200–350 \times 10^6$ cells/μl [17,18]. The benefit of being identified as eligible with elevated viral load alone is of debatable clinical merit in the presence of an assured long-term supply of affordable drugs and of dubious benefit in their absence (as is the case in Côte d'Ivoire at present). It may be of both individual and public health benefit to delay commencing therapy until clinical or CD4 cell count criteria are met.

CD4 cell count and viral load baseline values may also have utility as part of monitoring the immunologic and virologic response to treatment. We did not consider this when defining 'benefit' as 'being assessed as eligible for subsidized ART'. However, the recent national recommendations in Côte d'Ivoire recommend viral load testing as part of monitoring only in the absence of a good clinical and/or CD4 cell count response. Baseline samples (including filter paper samples) could be stored prior to commencing therapy and tested if clinically indicated without the enormous expenditure required for systematic baseline viral loads.

We are also performing analyses to evaluate the utility of other parameters or combinations of parameters (such as total lymphocyte count, hemoglobin, p24 antigen levels, nutritional indices and client age) for client screening as well as therapeutic response. Ongoing evaluations to define the predictive values of clinical screening, CD4 cell count, viral load and other parameters in various patient populations and contexts are needed to inform optimal screening and monitoring practices in a dynamic environment. In the not too distant future, we hope that sustained international commitment will result in simpler less-expensive laboratory screening tools and widespread affordable VCT and care services in many heavily affected countries such as Côte d'Ivoire.

Our data suggest that viral load testing should not be included as a mandatory part of eligibility screening as it is not an effective use of scarce public health resources. Health personnel should be trained to identify and refer clinically symptomatic persons for HIV care with ART. Overall, in sub-Saharan Africa, there are an estimated 28 million persons infected with HIV, and the first public health and humanitarian imperative must be to expand access to life-saving treatments to the millions of people with the most advanced disease.

Acknowledgements

All those involved in the design, analysis and support of the Drug Access Initiative, Côte d'Ivoire (DAI–CI) including: all the patients attending the DAI–CI; clinicians and staff at the DAI–CI accredited centers; the RETRO-CI staff : Gaston Djomand, Thierry Roels, Terry Chorba, Madeleine Sassan-Morokro, Rene Ekpini, Marie-Yolande Borget, Babette Gbaie and the clinical, data and laboratory support staff; the CDC Global AIDS Program: Stephan Wiktor, Eve Lackritz and Tedd Ellerbrock, the UNAIDS: Badara Samb and Joseph Saba and the staff from the National AIDS Control Program including Makan Coulibaly (former DAI–CI coordinator). The authors are also grateful for insightful review and comments from: CDC DHAP, Epidemiology Branch: Stefanie Sansom and Paul Weidle.

Contributors: Fabien Diomandé and Monica Nolan contributed to all phases of the study including the study concept, design, analysis, writing the first draft and subsequent revisions. Emmanuel Bissagnéné, John Nkengasong, Chantal Maurice, Ben Monga and Marie Laga contributed to the analysis and revisions of the manuscript for this nested study.

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Access to antiretroviral drugs and AIDS management in Senegal

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Objectives: Description and analysis of the Senegalese Antiretroviral Drug Access Initiative (ISAARV), the first governmental highly active antiretroviral therapy (HAART) treatment programme in Africa, launched in 1998.

Methods and results: ISAARV was initially an experimental project designed to evaluate the feasibility, efficacy and acceptability of HAART in an African context. It was based on four principles: collective definition of the strategy, with involvement of the health professionals who would be called on to execute the programme; matching the objectives to available means (gradual enrollment according to drug availability); monitoring by several research programmes; and ongoing adaptation of treatment and follow-up according to the latest international recommendations.

Persons qualifying for antiretroviral (ARV) therapy are selected on the basis of immunological and clinical criteria, regardless of economic and social considerations. A system of subsidies was created to favor access to ARV. Following the ARV price reductions that occurred in November 2000, 100% subsidies were created for the poorest participants. Optimal adherence was ensured by monthly follow-up by pharmacists and support groups held by social workers and patient associations. The chosen supply and distribution system allowed drug dispensing to be strictly controlled.

Conclusion: The ISAARV programme demonstrates that HAART can be successfully prescribed in Africa. This experience has served as the basis for the creation of a national treatment programme in Senegal planned to treat 7000 patients by 2006.

AIDS 2003, **17** (suppl 3):S95–S101

Keywords: Access, Africa, antiretroviral, highly active antiretroviral therapy, HIV, public health, Senegal

Introduction

In discussions held since 1996 on access to antiretroviral therapy in Africa, it has clearly emerged that provision of drugs alone will not solve the crisis [1].

The principal obstacles so far identified include:

- the lack of health infrastructures required to ensure treatment follow-up;
- high drug costs relative to national health budgets in low-income countries;

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Sponsorship: This work was partially supported by a grant of the French National Agency for Research on AIDS (ANRS), by European Union (contract B7-6211/99/005) and by Institut de Recherche pour le Développement (IRD).

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- concerns over adherence to treatment;
- the risk of accentuating inequalities between rich and poor; and
- the emergence of viral resistance.

Several consensus conferences have concluded that these obstacles must not be used as an excuse to delay implementation of life-saving antiretroviral therapy programmes [1]. However, these arguments correspond to real public health challenges, as stated in international expert recommendations [1–6].

When the National Program Against AIDS in Senegal (PNLS) decided in 1998 to launch a pilot project – the Senegalese Initiative for Access to Antiretroviral Drugs (ISAARV) – its strategic choices were determined by the desire to create an infrastructure capable of avoiding these pitfalls. This infrastructure needed to be capable of being integrated into the existing care system and not require major supplementary resources that would threaten its sustainability.

After 3 years of operation, these strategic choices can be debated in the light of results of research programmes [7]. This critical analysis is necessary before extrapolating the pilot project into a national scale programme.

This article describes the organization of ISAARV, including drug access and distribution. The results of the pilot project are interpreted in terms of the above-mentioned public health challenges. Questions concerning adherence and resistance are dealt with in two other articles published in this issue [8,9].

The context of ISAARV

The seroprevalence of HIV in Senegalese adults remains below 2%, and is not currently increasing. This relatively low prevalence is partly due to early implementation of well-designed national prevention campaigns [10]. Mobilization of health authorities and political decision-makers aimed at facilitating the management of HIV-infected patients in Senegal was accompanied by debates on the use of antiretroviral drugs.

Strategic choices

The initial strategic choices for the ISAARV programme were prudent, given uncertainties over the feasibility, efficacy and acceptability of antiretroviral therapies, and the material and financial constraints existing at that time. ISAARV was designed as a pilot project linked institutionally to PNLS, under the authority of the Ministry of Health. The project was designed to function integrally within hospital services, with four overriding principles: collective definition of the strategy; objectives in keeping with available means; monitoring through research programmes; and the choice of appropriate treatment and follow-up protocols.

Collective definition of the strategy

The health professionals who would be responsible for executing the strategic choices (physicians, biologists, virologists, pharmacists and welfare assistants) were invited to participate in the definition and planning of the programme. This choice, necessitated by financial constraints, had two merits: it ensured first that the design of the project took into account existing difficulties, and second that management modalities were adaptable to rapid changes in scientific knowledge, available drugs, and treatment costs. Four committees were created to ensure the institutional organization of the project.

The first committee to be created defines the main orientations of the project and is responsible for control and monitoring; decisions are taken collectively, after discussion among the members. Known as the Eligibility Committee, this committee has overall responsibility for patient recruitment. It counts 15 statutory members (physicians, biologists, pharmacists, persons living with HIV/AIDS, and administrators).

The Medical Committee defines and periodically revises the medical aspects of the programme (inclusion criteria, therapeutic protocols, clinical follow-up, etc). Monthly meetings are held to examine the medical files of candidates for treatment, and to assess the appropriateness of the therapeutic regimens chosen by clinicians. The committee includes PNLS prescribers and physicians.

The Welfare Committee is responsible for decisions concerning non-medical aspects of the project and provides support measures aimed at optimizing adherence. In particular, it coordinates social surveys of candidates for treatment. It is composed of PNLS health professionals (pharmacists, psychiatrists, etc.) and social workers.

The Drugs and Reagents Management and Supply Committee is charged with managing drug supplies, organizing dispensing sites, and managing relations with private wholesalers.

Matching the objectives with available means

ISAARV objectives, in terms of the numbers of prescribing and dispensing sites and the number of patients to be treated, were defined in keeping with available means. In 1998, only Dakar, the capital, had an adequate technical infrastructure and sufficient personnel trained in the use of antiretroviral drugs. Three consulting sites were opened (Infectious Diseases Department and Ambulatory Treatment Center of Fann Hospital, and the Internal Medicine Department of the Principal Hospital), with nine prescribing physicians. It was decided that 60 patients could be enrolled in ISAARV with the budget initially available. The number of available treatments increased gradually, following the reduction in drug prices, the launch of clinical trials, and new grants.

Monitoring through research programmes

Several basic and operational research programmes were associated with ISAARV from the outset. These research projects provided a technical accompaniment in virological, bioclinical and social science fields, and their results help in the evaluation of the pilot project.

Choice of treatment and follow-up protocols

The medical criteria for treatment initiation were chosen on the basis of the 1997 Dakar consensus, as revised in October 2000 [2,3].

Similarly, protocols chosen to treat children, to prevent mother–child transmission, and for post-exposure prophylaxis, initiated in June and July 2000, were based on international recommendations adapted to African countries (according to the drugs available in Senegal), and were revised in October 2000 [2,3].

Patients are seen on day 1, day 7, day 14 and day 30, and then on a monthly basis. At each visit, the physician writes a prescription and keeps a copy. The drugs are dispensed by a pharmacist, who gives advice on adherence and records the drugs dispensed. The patient gives his/her financial contribution towards the treatment directly to the pharmacist.

Welfare follow-up was intended to include several interviews, but this could no longer be done regularly after the first 180 patients had been enrolled. The welfare interventions consist mainly of monthly group discussion and information sessions held at one of the sites and aimed at optimizing adherence; follow-up social surveys are only done if requested by the patient, the prescriber or the pharmacist.

The strategic choices and organization of ISAARV were efficient for the management of the pilot project. Clinical evaluation after 18 months has shown that the treatment of 58 adult patients was efficient [11]. However, with the increase in the number of patients the limits of the system began to emerge, particularly the time-consuming nature of the data collection and co-ordination systems. The institutional organization was maintained during the transition from the pilot project to the national programme in 2001, but 'light' patient follow-up procedures were implemented, based on monthly visits and data recording. In February 2002, 400 adult patients and 19 children are under highly active antiretroviral therapy (HAART), 17 HIV-positive pregnant women have been included in the (prevention of mother to child transmission (PMTCT) programme and received single dose nevirapine or short course zidovudine according to timing, nine health professionals had a post-exposure prophylaxis.

Access to antiretroviral drugs

ISAARV was designed to be accessible to all persons requiring treatment with antiretroviral drugs, whatever their nationality or socio-economic status, provided they were resident in Senegal. Residence was preferred over nationality, which has inherent political implications. This ensured optimal medical follow-up, and avoided attracting patients from neighboring countries. In 1998 it was decided to accept the principle of patients' financial participation, the amount paid being calculated according to individual resources.

Access modalities

Patients selected by a physician on the basis of immunovirologic and clinical criteria (see above) underwent an 'inclusion' social survey by a social worker, with the aims of assessing the patient's economic resources and social support network, identifying other HIV-infected persons in the domestic group, and checking that the patient had correctly understood the constraints of three-drug regimens. The results of the survey were discussed by the Eligibility Committee, which endorsed the decision to treat and determined the patient's financial participation from a table. This procedure was applied throughout the pilot project (1998–2001). Children, health personnel, and active members of people living with HIV/AIDS (PLWA) self-help groups were exempted from financial participation.

The patients' contributions to the cost of their treatment evolved according to the prices imposed by pharmaceutical firms [12]. Prices were reduced after November 2000, and a government subsidy of 100% was introduced.

During the first period (August 1998 to October 2000), patients were required to be able to pay a minimal monthly sum of 21 000 FCFA (1 US$ = 640 FCFA; 1000 FCFA = 1.56 US$) in order to be enrolled in the project (Table 1). To avoid raising false hopes, clinicians generally conducted an informal selection, prior to the social survey, on the basis of their patients' presumed ability to pay.

In November 2000 the reduction in prices of antiretroviral drugs was immediately translated into a four-fold reduction in patients' financial participation. The following year, 44% of newly enrolled patients received a grant covering 100% of the drug costs (Table 2).

The income-based calculation of patients' financial contributions was impractical, mainly owing to the difficulty of assessing an individual's available resources. In addition, the social surveys were lengthy, difficult to conduct, and imprecise. They came up against the pitfalls already known to economists working in Africa when determining the income of a person or a domestic group, as most of them have no declared salary and hold precarious and casual jobs.

During the first 9 months of treatment, the estimated mean direct medical costs paid for by the patient, includ-

Table 1. Changes in tariffs prevailing in the Initiative during its first 3 years (FCFA).

ISAARV period	August 1998 to October 2000	November 2000	February 2001	July 2001
Minimum cost of a three-drug regimen	320 000	100 000	100 000	60 000
Tariffs (patients' participation)				
Maximum	198 000			
	150 000			
		100 000	100 000	
	64 000			
		60 000	60 000	60 000
	50 000			
	40 000		40 000	40 000
	21 000[a]			
		20 000	20 000	20 000
			10 000	10 000
		5000	5000	5000
Minimum		0	0	0

[a]Free access was granted to children, health professionals and active members of PLWA self-help groups. 1 US$ = 640 FCFA; 1000 FCFA = 1.56 US$. ISAARV, Senegalese Antiretroviral Drug Access Initiative.

Table 2. Changes in patients' financial participation at day 1 following the fall in drug prices (FCFA).

ISAARV period		August 1998 to October 2000	November 2000 to November 2001
Participation at day 1	Median	21 000	5000
	Mean	27 800	4350
	Maximum	198 000	60 000
Number of patients receiving a 100% grant at day 1		10 (11.5%)	64 (44%)
Number of enrollments (excluding clinical trials)		87	146

ISAARV, Senegalese Antiretroviral Drug Access Initiative.

ing costs of medical visits, biological and radiological examinations, hospitalizations, travel and medicines but excluding the purchase of antiretroviral drugs, were 5200 FCFA/month, a sum representing nearly 15% of the minimum wage in Senegal [13]. This sum was far beyond the capacities of the bulk of the Senegalese population: in Dakar, a relatively well-off region compared with the rest of the country, 60% of the population have no permanent job or regular income, nearly 60% of households live below the poverty threshold, and 83% of the population have no welfare protection [14]. Thus, the pilot project quantified the weaknesses of an antiretroviral access programme based on patients' financial contribution towards the cost of their treatment and provided an estimate of the amount needed to support patient access to antiretroviral therapy in addition to the cost of drugs.

Drug distribution

Context

Before and during the first period of the ISAARV project, antiretroviral drugs were available in Dakar from three wholesalers and a few private pharmacies, which supplied between 20 and 30 wealthy persons at a monthly cost sometimes exceeding 300 000 FCFA. Some of these patients could not be enrolled in ISAARV owing to their place of residence, whereas others preferred the perceived anonymity of this type of access.

After November 2000, in order to ensure that all patients benefited from the lower drug prices, an agreement was reached between PNLS and wholesalers, whereby the latter could obtain antiretroviral drugs at reduced rates from Fann Hospital pharmacy. The wholesalers thus became secondary dispensing sites (while sales of antiretroviral drugs were forbidden in pharmacies) but, in January 2002, only a dozen patients continued to use them.

Organization

In Senegal, drugs for the public and semi-public sectors are supplied by the National Supply Pharmacy (PNA). Antiretroviral drugs are delivered to and stored at the Supply Pharmacy, and are dispensed according to the needs of Fann Hospital pharmacy, which was the only

antiretroviral dispensing site for the ISAARV project until January 2001.

In February 2001 a second site was opened at the Social Hygiene Institute (IHS), and secondary sites were chosen as the different components of the ISAARV programme were launched (management of children treatment and mother-to-child prevention).

Fann pharmacy continues to control the distribution of antiretroviral drugs to dispensing sites, including wholesalers. This has the advantage of permitting strict monitoring of stocks and their distribution. However, the increase in drug throughput and in the number of dispensing sites planned to take place when the programme is extended will probably create a major increase in workload in this service that has no mandate to do so.

Available drugs

In 1998, the ISAARV programme started with five drugs (zidovudine, lamivudine, stavudine, didanosine and indinavir). In early 2002, eight drugs were available, with nine different preparations and a total of 23 different combinations (including 20 three-drug regimens).

The informal drug market

In Senegal, as elsewhere in Africa, the sale of drugs, including the most recent, also occurs through an informal market, developed by street vendors and shopkeepers [15–17]. It was thus to be expected that antiretroviral drugs would be sold through this channel, although their supply was legally restricted to hospital pharmacies designated by PNLS or to wholesalers, and necessitated medical prescription by selected doctors.

The supply of antiretroviral drugs through the informal market gradually diversified, and prices fell in similar proportions to the ISAARV programme. Injectable Retrovir®, sold for 125 000 FCFA per box, was the first product to be identified on the informal market, in early 2000. The same year Retrovir® capsules, Videx®, and Zerit 40® also became available. The latter was offered for 120 000 FCFA per box in July 2000 and only 12 000 FCFA in January 2002. In early 2002, 12 drugs had been identified, comprising 13 proprietary preparations and including three preparations at two different dose strengths (Epivir® 150, Combivir®, Crixivan® 400, Éfavirenz, Invirase®, Norvir®, Rétrovir® 100 and 250, Trizivir®, Videx® 100 and 200, Viracept®, Viramune®, Zérit® 30 and 40, Ziagen®).

Two years of market observation show that sales volumes of antiretroviral drugs are low (less than five boxes per product) and clients infrequent. But, since late 2001, attempts have been made to develop sales: some merchants now clearly identify antiretroviral drugs as 'drugs against AIDS', whereas others are testing the market. In January 2002, one merchant had a stock of 20 boxes of Zerit® 40; this clearly required a major investment and suggested that a guarantee of sale had been obtained. Finally, some merchants are specializing in antiretroviral drugs, and are writing (inappropriate) prescriptions themselves, such as monthly or weekly injection of Retrovir®, two-drug regimens (zidovudine + didanosine), and Zerit 40® monotherapy (touted as being more effective than a three-drug regimen).

The lot numbers reveal that most of these products come from the northern hemisphere, in the form of donations (by individuals or associations), or health structures supplied by donations, or more elaborate strategies in which drugs are specifically collected in the northern hemisphere for sale in Senegal. Only 13 boxes with lot numbers corresponding to those used in the ISAARV programme have been found, pointing to occasional illicit sale in designated dispensing centers, retrieval of unused drugs after treatment switches, or, more likely, resale by a very small number of patients [18].

Surveys thus confirm that antiretroviral drugs are available on the informal market but suggest that the phenomenon is limited. This is no doubt partly explained by the strict control of drug distribution in the ISAARV programme, and the close follow-up of treated patients.

Discussion

The strategic choices on which the ISAARV programme was based have been more or less validated in the light of the public health challenges raised by access to antiretroviral drugs in Africa.

The ISAARV programme was set up in a small number of highly competent, specialized sites. The capacities of the health care facilities and the committees managing patient access to ISAARV proved adequate to meet demands, albeit thanks to a pre-selection process that was not initially planned and that, considering access to care, was a failure. Not all patients for whom antiretroviral treatment was medically indicated had access to the programmes, for reasons relating both to AIDS itself (diagnostic errors, difficult access to screening, fear of condemnation, etc.) and available health services in a low-income country (few specialized consultations, high cost of care); the number of medically eligible patients that did not have access to the programme could not be estimated. Social accompaniment ensured through the health care system could not be as sustained as it was planned; it requires the involvement of community-based organizations.

The results of the ISAARV programme show that drug costs alone are not a valid reason for withholding antiretroviral treatment in poor communities. Antiretroviral therapy was funded through specific budgets, thus ensur-

ing that resources allocated to other health problems were not 'hijacked'. The spectacular drug price reductions that occurred in 2000 transformed the financial situation. The ISAARV programme facilitated the emergence of an antiretroviral drug market, thus allowing other patients to benefit from the lower cost of treatment.

The risk of accentuating inequalities between rich and poor can currently only be overcome by granting subsidies that cover the entire cost of treatment for a large proportion of the patient population. Indeed, the economic situation in African countries, the impact of AIDS on household budgets, and the cost of treatments other than antiretroviral drugs mean that most patients, despite being able to return to paid employment while on treatment, still cannot pay for their drugs.

Access to strongly subsidized or free treatment permitted providing rational treatment to patients; it also contributed to reducing the demand for antiretroviral drugs on the informal market, and therefore reduced the risk of emergence of viral resistance due to inappropriate use.

Conclusion

The ISAARV programme demonstrates the feasibility and efficacy of a pilot project of antiretroviral drug access in Africa, with about 400 patients treated in a small number of sites. Senegal's Strategic Plan Against AIDS for 2002–2006 is intended to treat 7000 patients with antiretroviral drugs. Maintaining a high level of care is the main challenge for this programme, as treatment protocols will be managed by less specialized care centers and the programme management will be decentralized. The new sites will be opened gradually, and their performance will be evaluated.

The ISAARV programme has been hailed as a model of AIDS management in Africa [19]. However, this initiative cannot be directly transplanted to other African settings, mainly because part of the information infrastructure was set up in a research perspective. This infrastructure must now be adapted to a public health programme.

Finally, the ISAARV programme demonstrates that the social, health and economic situation that prevails in most sub-Saharan countries is not an insurmountable obstacle to the creation of a sound and effective programme of antiretroviral therapy. The need to favor non-discriminatory access to antiretroviral therapies in response to the state of emergency created by AIDS in Africa was underlined at the general assembly of the United Nations on 27 June 2001 [20]. The creation of national antiretroviral programmes is not only the affair of African policy-makers: it must also be based on an international strategic response proportional to the world AIDS crisis.

Acknowledgements

The authors are grateful to Professor Salif Badiane (deceased), Dr Ngagne Mbaye, the members of the Eligibility Committee, the Medical Committee, the Welfare Committee, and the Drugs and Reagents Management and Supply Committee.

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Adherence to HAART and its principal determinants in a cohort of Senegalese adults

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Background: Access to programmes providing highly active antiretroviral therapy (HAART) is recent in Africa. In Senegal, a national initiative was launched in 1998. The capacity of African patients to adhere to complex antiretroviral treatments (ARV) is largely unknown.

Methods: We assessed adherence and identified the main reasons for treatment interruption in a prospective observational cohort of patients participating in an ARV access programme in Dakar, Senegal. Adherence was estimated each month on the basis of the patients' stated consumption and on the proportion of the prescribed dose returned unused to the dispensing pharmacy. A total of 158 patients were studied between November 1999 and October 2001.

Results: A cross-section analysis showed that the stated level of adherence was high: on average, over the study period, the patients said they had taken 91% of each monthly dose and that they had taken the full monthly dose during two-thirds of the months studied. Adherence tended to be better among patients who were required to make little or no contribution to the cost of their treatment, through an appropriate pricing structure. Adherence was also better with efavirenz-containing regimens than with indinavir-containing regimens.

Conclusion: These results show that adherence to HAART can be as high in Africa as that generally observed in industrialized countries, and that the cost and type of drug regimen must be taken into account when designing ARV access programmes for poor communities.

AIDS 2003, **17** (suppl 3):S103–S108

Keywords: Adherence, Africa, antiretroviral, epidemiology, highly active antiretroviral therapy, HIV, Senegal

Introduction

Adherence to multi-drug antiretroviral regimens has been a focus of attention since their introduction, owing to their complexity, frequent adverse effects, and chronic nature. Recent cohort follow-up studies in industrialized countries have identified several obstacles to adherence, and shown that a high level of adherence is required to delay disease progression effectively [1–3]. However, adherence is difficult to measure, and is known to vary over time [4]. Few data have been published on adherence to multi-drug antiretroviral therapy (ARV) in the context of African drug access programmes [5].

In industrialized countries, where treatments are usually provided free of charge, measures adopted to improve adherence to treatment include counseling, patient edu-

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Sponsorship: This work was partially supported by a grant of the French National Agency for Research on AIDS (ANRS) and by European Union (contract B7-6211/99/005)

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cation programmes and telephone hot-lines. These measures are seldom feasible on a large scale in poor countries [6]. Studies are therefore needed to determine the level of adherence and to identify the determinants of high adherence in the African context. This knowledge would help to define relevant, efficient and acceptable adherence support measures for patients within the African health system.

In November 1999, we started a pilot project designed to assess adherence and causes of treatment interruption among patients treated through the Senegalese Antiretroviral Access Initiative (ISAARV). This programme had set up access to treatment conditions and adherence support measures fitted to low-income countries [7].

The project comprised both a quantitative study of descriptive and analytical epidemiology, first results of which are reported here and a qualitative socio-anthropological survey, the preliminary results of which have been published elsewhere [8,9].

Patients and methods

Patients

The first 180 adults enrolled in the ISAARV prospective observational cohort were eligible for this study if they had been monitored medically for at least 30 days, during the 24 months of observation (November 1999 to October 2001). Eighty patients were receiving ARV within clinical trials [40 patients in French National Agency for Research on AIDS (ANRS) 12-04 project and 40 patients in ANRS 12-06 project] and the remaining 100 patients were enrolled in the ARV access programme. The two groups differed principally by their date of enrollment, their clinical stage at enrollment, their previous exposure to ARV, the type of ARV regimen prescribed, and their contribution to the cost of their treatment.

The patients were monitored in three Dakar health structures (Internal Medicine Unit of Hôpital Principal, and the Infectious Diseases Unit and Ambulatory Treatment Center of Fann Hospital). They all obtained their drugs from a single dispensing site.

Adherence assessment

Adherence to treatment was assessed during regular monthly visits to the dispensing pharmacy. The dispensing procedures and support measures (counseling, access to discussion groups, social and financial support) were the same for all the patients.

Adherence data were collected by the dispensing pharmacist, who interviewed each patient before dispensing each month's treatment, based on a questionnaire containing mainly closed questions. The pharmacist also discussed with the patient any discrepancy between declared adherence and the number of doses returned unused. Adherence was calculated as the ratio between the stated number of tablets taken and the number of tablets prescribed, expressed as a mean percentage for the different components of each multi-drug regimen.

Treatment interruptions for medical reasons were excluded from the definition of non-adherence.

Some missing information due to irregular visits to the pharmacy was subsequently obtained by the prescriber or a social worker, who contacted the patient concerned.

Statistical analysis

Epi Info 6.04 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA) and Stata 6.0 (Stata Corporation, College Station, Texas, USA) were used to record and analyze the data. Adherence between different subgroups (defined by enrollment in a clinical trial, cost or type of regimen, etc.) at a given time point of follow-up was compared by using the Kruskal–Wallis test for quantitative variables. Adherence between two time points was compared by using the Wilcoxon test for paired data. The level of significance was set at $P < 0.05$.

Ethical considerations

Ethical approval was received from the Committee of the AIDS Control National Program. Patients with poor adherence were referred to the Welfare Committee, and were offered appropriate support.

Results

Patients

A total of 167 patients met the eligibility criteria. Adherence data were collected among 158 of them (94.6%) as one patient refused to participate, two did not collect their treatment personally and four did not provide any data due to a short follow-up. Follow-up of these 158 patients during the 24-month study period yielded a total of 2752 patient-months of observation; adherence data were available for 2389 patient-months (86.8%); the total treatment period was 2471 patient-months. Eleven deaths (7%) and three drop-outs (2%) occurred during the study period. The median length of follow-up in the ISAARV programme was 21 months for the 158 patients of whom 80 were included in clinical trials

Baseline characteristics

The study population consisted of 84 men and 74 women (M : F sex ratio 1.1 : 1), with a mean age of 38 years. The CDC stage distribution at the outset of antiretroviral treatment in the ISAARV programme (155 patients) was as follows: 6% stage A, 39% stage B, and 55% stage C. The infection was due to HIV-1 in 97% of cases, HIV-2 in 1% of cases, and HIV-1+2 in 2% of cases.

At enrollment, the mean viral load (log_{10}) was 5.34 copies/ml (n = 154) and the mean CD4 cell count was 156×10^6 cells/l (n = 154).

Forty-four per cent of the patients were married and 15% were widowed. The mean number of children per patient was 2.6. Thirty-two per cent of the patients had never been to school, and 41% were not in paid employment. The median monthly income was 15 000 FCFA (20 US$).

Ninety-three per cent of patients were antiretroviral-naive at inclusion. The intent to treat regimen was a dual therapy comprising two nucleoside reverse transcriptase inhibitors (NRTI) in 4% of cases and a three-drug regimen in 96% of cases, including two NRTI and one protease inhibitor (PI) in 43% of cases, and two NRTI and one non-nucleoside reverse transcriptase inhibitor (NNRTI) in 53% of cases. The intent to treat regimen prescribed to the patients participating in clinical trials consisted of two NRTI and one NNRTI [efavirenz (EFZ)].

Treatment regimens prescribed during the study period

Most antiretroviral treatments prescribed during the 24-month study period were multi-drug combinations, including stavudine (D4T)/didanosine (ddI)/indinavir (IDV) (26%) and lamivudine (3TC)/ddI/EFZ (30%). Treatment switches were sometimes necessitated by concurrent antituberculous treatment, an adverse effect, or, in rare cases, by temporary unavailability of a low-dose formulation of stavudine (Zerit® 15 or 20).

Adherence

Mean adherence rate

The mean adherence among the 158 patients during the 24-month study period was 91% [median, 100%; interquartile range (IQR), 97–100%]. The patients stated that they had taken the entire monthly dose during 69% of the months covered by the study period.

Changes in adherence during the study period

Mean adherence was 90% during the first year (median, 100%; IQR, 97–100%) and 92% in the second year (median, 100%; IQR, 98–100%). Mean adherence always remained above 80%, oscillating between 83 and 95% according to the month.

Adherence tended to be better, with a smaller dispersion of values, among the 80 patients included in the clinical trials than among the remaining 78 patients (97 versus 87%). This difference diminished with time (Fig. 1), being statistically significant every month during 17 of the 24 study months.

Adherence improved between October 2000 and April 2001 among patients not included in clinical trials ($P = 0.02$) and declined between October 2000 and October 2001 among patients participating in trial ANRS 12-04 ($P = 0.03$).

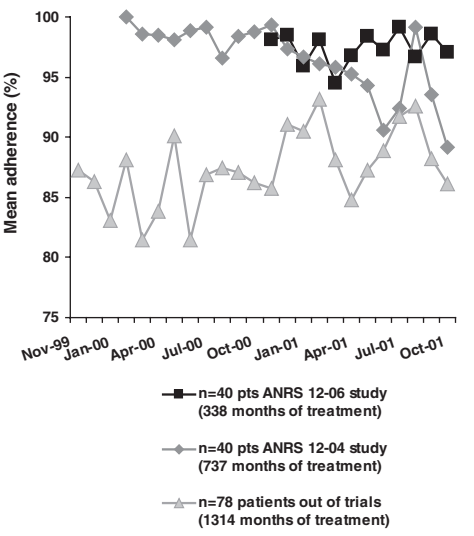

Fig. 1. Changes in mean adherence between November 1999 and October 2001 in the different patients' categories.

Determinants of adherence

This analysis of factors influencing adherence focused on treatment-related factors, namely the duration of antiretroviral treatment, the patient participation towards the cost of treatment, and the treatment regimen. This choice was guided by the strategic decisions to be taken in the process of generalizing access to HAART in Senegal.

Treatment duration

A difference in adherence emerged between patients participating in clinical trials and other patients at month 6 ($P = 0.001$) and remained at months 12 and 18 ($P = 0.04$ and $P = 0.003$, respectively). It was difficult to identify overall temporal trends, owing to the different follow-up periods among the three patient subpopulations related to a 33-month inclusion period, leading to few observations at certain months of treatment.

Patients' financial participation

Patients enrolled in the two clinical trials were treated free of charge, whereas the other patients paid monthly between 0 and 198 000 FCFA (264 US$) towards the cost of their treatment.

Mean adherence among patients receiving D4T/ddI/IDV outside the clinical trial setting decreased as their financial participation increased. This trend was noted during the first year of the study and, albeit less so, during the second year (Table 1). During year 1, most patients made monthly a substantial minimum payment of about 21 000 FCFA (28 US$), while the legal minimum wage in Senegal at that time was 36 250 FCFA (48.3 US$) a month. Thus a

Table 1. Mean adherence according to patient's monthly financial contribution among patients receiving a three-drugs regimen including stavudine/didanosine/indinavir.

Patient's monthly contribution (FCFA[a])	Whole study period (24 months) (n = 619 person-months)	First year (n = 301 person-months)	Second year (n = 318 person-months)
Free	92.2% (n = 144)	90.8% (n = 69)	93.4% (n = 75)
From 1 to 20 000	88.4% (n = 203)	97.3% (n = 3)	88.3% (n = 200)
From 20 000 to 49 999	85.,3% (n = 244)	83.7% (n = 207)	94.1% (n = 37)
50 000 and more	66.4% (n = 28)	60.1% (n = 22)	89.8% (n = 6)

[a] 750 FCFA = 1 US$.

large proportion of the patients encountered financial difficulties. Following the antiretroviral drug price reduction that occurred early in year 2, the mean contribution made by patients already on treatment was cut four-fold, and the minimum participation was cancelled.

This sharp decrease in the sum the patients who were not participating in clinical trials had to pay towards their treatment probably contributed to the improvement in adherence during year 2 (83% in year 1 versus 90% in year 2). This is supported by the statements made by these patients: financial difficulties were reported as the leading cause of treatment interruption during the first year, and as only the fifth cause during the second year.

Treatment regimen

IDV was the most widely prescribed PI in our cohort, being the only low-cost PI available in Africa; EFZ was the most widely prescribed NNRTI. Among the patients who were receiving their treatment free of charge, mean adherence was 89% with IDV and 97% with EFZ during the 24-month study period. The differences at months 6, 12 and 18 of treatment were close to statistical significance ($P = 0.09$, $P = 0.17$ and $P = 0.05$, respectively).

Relationship between adherence and virologic efficacy

At months 6, 12, 18 and 24, adherence during the previous month was compared to contemporary viral load values (logarithmic scale).

In the group of patients who were not participating in clinical trials and who were receiving a PI-containing, three-drug regimen, we compared mean viral load values between those with stated adherence of 90% or more and those with poorer adherence. As expected, viral load was higher in the less adherent patients (mean differences of 1.7 and 1.8 $\log_{10}$ copies/ml at months 18 and 24, respectively; $P < 0.05$).

Discussion

Methodological considerations

The method and the unit period (30 days) chosen to assess adherence in this study require discussion.

There is no reference method to quantify adherence [10]. The context of a resource-limited setting adds limitations in the choice of adherence measurements' methods: for example, plasma drug assays and electronic pill boxes are unavailable and illiteracy rules out the use of self-rating questionnaires. In this African setting, it seems reasonable to estimate adherence on the basis of stated drug intakes and/or unused tablet counts, as these parameters are simple, inexpensive to assess and recognized as valid estimates. It is known that patients tend to overestimate their adherence but counts of unused tablets, an objective measure, helps to refine this subjective estimate [11,12]. The unavoidable approximations inherent in all procedures used to assess adherence, given the lack of a reference tool, have been widely described in the literature [10,13,14]. It is generally agreed that the correlation between adherence and the virologic response observed by some authors (and confirmed in this study) tends to validate the subjective assessment by patients receiving their first antiretroviral treatment [1,2,10,14–16]. The high stated level of adherence is also in keeping with the good immunovirological efficacy observed in this cohort [17–20]. Moreover, adherence data gathered by the prescribers were very consistent with those collected by the dispensing pharmacist [20].

The unit period of recall chosen here is relatively long (30 days). Stated adherence during the last 3 days of each 1-month period in this survey tended to be slightly poorer than the corresponding 30-day estimate (89 versus 91% during the 24-month study period). However, we felt that the use of a 30-day unit assessment period was more likely to reflect the distribution of causes for non-adherence, notably illness and travel.

Adherence data were unavailable for 386 patient-months of follow-up (14%). In nearly half these cases the patients had received their drugs but the corresponding datasheet had not been filled out (pharmacist or patient unavailable, or dispensing of more than 1 month of treatment). In these cases we postulated that the treatment had not been taken differently than in the documented months. During the remaining months (7% of all months of follow-up), the patients had not received the drugs from the pharmacy but may have had sufficient personal stocks.

Further analysis taking into account the correlation structure of the data generated by the repetition of the observations within each patient as well as multivariate methods to assess more potential determinants of adherence will deepen these first results.

Results

The main result of this study is the high stated level of adherence in each patient category. Indeed, the patients declared that they had taken, on average, 91% of their dose during each month of follow-up, and the entire dose during nearly 70% of months. These results are similar to those obtained in follow-up cohorts in industrialized countries; for example, on the basis of self-rating questionnaires, 73.3% of respondents in the Aproco cohort stated that they had taken the entire dose during the previous 4 days at month 4; and 67% of respondents in the Ciel Bleu trial stated that they had taken 100% of their doses) [16,21]. A recent study conducted in an industrialized country suggests a lower adherence level among African patients [22]. Several factors may explain the good adherence observed in this Senegalese study, such as the experimental nature of the programme, recent inclusions in a small cohort, and the fact that most of the patients had never previously received ARV and were highly motivated (a large proportion of patients were symptomatic) [23].

The size of the patients' contribution to their treatment costs had a major impact on adherence. Treatment interruptions because of financial problems had been reported in other African studies [5,24–26]. In our study, financial obstacles led to lengthy treatment discontinuations until a more appropriate pricing structure was adopted. The increase in adherence between October 2000 and April 2001 among patients not included in clinical trials was probably largely due to the ARV cost reduction.

Adherence tended to be better with EFZ- than with IDV-containing regimens, and experience in industrialized countries suggests that this difference will persist in the post-trial period [27].

Adherence support programmes have to be extended in low income countries while remaining affordable, feasible and acceptable. In our experience, pharmacist counseling was a major measure, complementary to prescribers' counseling. Discussion groups, support from other persons living with AIDS were under implementation during the study period. Patients often expressed a fear of lack of confidentiality regarding treatment delivery and uptake. Some of them wished to get treatment for 2 months or more in order to reduce the frequency of contacts with the medical infrastructures, sometimes far from their homes. The high mean level of adherence observed along with patients' fears and wishes suggest that a programme proposing DOTS (directly observed treatment strategy) may be inadequate and may not be useful for a majority of patients in this context [28].

In conclusion, adherence to three-drug regimens was consistently high in this 24-month follow-up cohort study conducted in Senegal. These results provide evidence of high adherence capacities in people living with HIV/AIDS in Africa.

Two main factors were found to influence adherence, namely the cost of treatment and the type of drug combination. As in industrialized countries, simplified treatments (especially with NNRTI) appear to be better managed and better accepted. The cost of treatment must be adapted to the individual patient's financial resources (which are often nil) if the continuity of treatment is to be assured. These two points, and the measures set up to support adherence, should receive particular attention in the design of future ARV access programmes in Africa.

Acknowledgements

The authors are grateful to the multidisciplinary team involved in the Senegalese ART Access Program. They also thank the social workers of the Infectious Diseases Unit of Fann Hospital for their active collaboration, and the members of the Welfare Committee (especially Dr Khoudia Sow and Dr Bernard Taverne) for their technical advice.

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Antiretroviral use in Ouagadougou, Burkina Faso

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Burkina Faso has the second highest seroprevalence rate for HIV in West Africa, estimated at 6.5% of the population. Although it is one of the poorest countries in the world, antiretrovirals have been used on an extremely limited basis in Burkina Faso since at least the early 1990s. In this article we will review the evolution of antiretroviral availability in this country, describe the mechanisms by which drugs are being accessed, and review our experience with expanding antiretroviral access through drug donations in community-based settings. Finally, we will discuss some of the implications for future attempts to expand access to treatment for people living with HIV in Africa.

AIDS 2003, **17** (suppl 3):S109–S111

Keywords: Africa, antiretrovirals, Burkina Faso, community groups

Introduction

The data presented here derive from the authors' involvement in efforts to expand access to antiretroviral drugs in Burkina since 1995, as well as interviews conducted in December 2001*. Although community groups now have considerable experience with antiretroviral drugs (ARVs), little has been published to date [1].

Evolution of availability of antiretroviral drugs in Burkina Faso

The advent of highly active antiretroviral therapy (HAART), and the ensuing therapeutic optimism that culminated at the XIth International AIDS Conference in Vancouver in 1996, focused attention on access to antiretroviral treatment for Africans living with HIV. At that time, the number of Burkinabè who had been tested for HIV, found to be positive, and informed, were few, but there were a sufficient number to constitute a market for these drugs. The first ARVs available for purchase in private pharmacies were brand-name zidovudine, didanosine, stavudine and indinavir, which were found in local private pharmacies from 1996, although the high cost of these drugs was a significant barrier to their proper use. Nonetheless, many use their savings and mobilized resources to purchase limited quantities of ARVs for personal use. This led to irrational use of the drugs, with most patients only able to afford intermittent monotherapy or, at best, dual therapy. A study conducted in 1998 also found that a number of practitioners were prescribing and administering antiretroviral 'cocktails' in

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dosages that were of unproven benefit and unlikely to be effective – for instance, monthly treatments cycling zidovudine, ciprofloxacin and gancyclovir were reported [2]. This shows that 'antiretroviral anarchy' [3] occurs in the absence of programmes to expand and rationalize, access to treatment.

In 1999, the Burkinabè government moved to regulate procurement of ARVs by simultaneously licensing these drugs and limiting authorization to import the drugs to the CAMEG, a para-statal company that procures, distributes and promotes essential generic medicines at cost. However, little was done to rationalize their use. The CAMEG began importing ARVs in April 1999, in the process halving the price of triple therapy, which went from an average 600 000 CFA (roughly US$ 800) per month of treatment to 300 000 CFA (roughly US$ 400). Within a month, pledges were made by pharmaceuticals manufacturers to reduce the prices of ARVs, largely as a result of international lobbying on the part of AIDS activist groups. When this political pressure was joined by competition from generic ARVs manufactured in India and elsewhere, the prices of ARVs dropped much more significantly [4]. As a result, the cost per month of the cheapest triple therapy has dropped from over 600 000 CFA per month at the end of 1998 (US$ 798) to 77 125 CFA (US$ 103) in June 2001 and prices have held at that level since.

How individuals obtain antiretroviral drugs

For ARVs the CAMEG exceptionally sells directly to individuals who have a prescription from a physician who has been approved to prescribe these drugs. In the first year that it sold ARVs to individuals, the CAMEG furnished treatment to 135 patients. The subsequent decrease in price is cited as the principal reason for the increased uptake of ARVs: in 2000, the year of the price reductions, CAMEG supplied 165 patients. This increased to 528 in 2001, and currently the number of patients purchasing ARVs through the CAMEG has increased to over 700 [5].

In addition to private purchase through the CAMEG, ARVs drugs are also available through drug donations managed by local organizations. These take two forms. In the first, grants are made specifically for the purchase of drugs to treat people living with HIV. This is a little-used mechanism, as the vast majority of donor agencies do not give grants for purchases of drugs, referring to their inability to commit to sustaining such funding. However this picture may be changing. In Ouagadougou, the *Centre de traitement ambulatoire*, (CTA) an ambulatory care centre established through a collaboration between the French Red Cross and the Burkinabè Ministry of Health, has received a grant ensuring a lifetime supply of HAART for 300 individuals.

Second, drug recycling programs furnish in-kind donations. These are collected in northern countries by health care workers and patient groups, and then sent to southern correspondents for use by patients there. A small but growing number of Burkinabè with HIV, whose membership in patient groups gives them contacts with northern activists and organizations, have access to these recycled drugs. In Burkina as elsewhere, drug recycling programs are proliferating and have been the vanguard of subsequent expanded ARV access programs [6, Tavi-Ouattara Y, personal communication, 2002].

These donations occur either on an individual-to-individual basis, as in the case of Burkinabè living abroad who collect drugs for afflicted relatives, or as group-to-group donations. Groups involved in AIDS care in France and Canada recycle unused drugs by shipping them to a number of Burkinabè non-governmental organizations (NGOs) that have established programs to care for people living with HIV. Currently, at least three local groups are able to offer some treatment support in the form of donated ARVs for an estimated 100 persons with HIV, most of whom also purchase part of their treatments using their own funds, or receive direct donations from friends and relatives abroad. Less than 20 of these are entirely dependent on drug recycling program donations for their treatment. As individuals treated through donations may occasionally purchase ARVs from the CAMEG, it is difficult to establish a total number of patients on treatment with certainty, as patients move from being able to purchase drugs to relying increasingly on donations as funds dry up, to being able to move back to purchasing drugs again if the prices drop.

One community-based experience with donated drugs

One grassroots community group in Ouagadougou, the *Association African Solidarité*, has stocked recycled drugs for the last 3 years. Cost was not used to ration the drugs. Rather, informal criteria were employed to use the drugs as effectively as possible given their limited supply. In the first 2 years of use, the drugs were mostly used to tide over patients who were already paying for treatment and who otherwise might have had to interrupt their treatments, either because drugs were unavailable for purchase or they did not have enough funds. In addition, some very ill patients were treated 'on humanitarian grounds'.

The outcomes of the first 50 patients treated in this manner between June 1998 and August 2000 were examined in a review of the organization's records. Twenty-eight patients were able to afford CD4 cell counts, and the average CD4 cell count was

146×10^6 cells/l (median 89×10^6 cells/l). Three were treated with monotherapy, 34 with double nucleoside reverse transcriptase inhibitors (NRTI) therapy, two with double NRTI therapy plus hydroxyurea, and 11 with triple therapy. During this period, 15 patients died, 20 did not return regularly for follow-up and 15 are still followed regularly. Most of those who did not return for regular follow-up withdrew because they could no longer afford to pay for drugs and there was an insufficient stock of recycled drugs to keep them on treatment. In other words, while they had been offered recycled drugs as a temporary measure with the understanding that they would be able to resume paying for their treatments, this did not turn out to be the case. Having exhausted their personal finances and/or their ability to mobilize resources from family members and friends, most of these patients were unable to resume purchasing ARVs and, as a result, did not return for follow-up.

Observations and lessons learned

Four observations from the Burkinabè experience with ARVs merit consideration. First, cost has been, and remains, the main barrier to increased access and adherence to treatment. This mirrors experience in other African countries [7]. Second, as prices have gone down, access has increased and some infrastructure has followed, largely in the form of community organizations' attempting to respond to the needs of people living with HIV. Further investments in infrastructure and, more importantly, human resources to support optimal adherence are urgently needed. Third, NGOs have been the most responsive to the need to expand access to treatment. However, and this is our fourth observation, the dynamism of the community response resulted in synergies with the public health care system. For instance, institutions such as CAMEG can help decrease costs through rationalized procurement, thereby increasing access, and new models of NGO-type governance can be experimented with within the public health care system, as in the case of the CTA. Over the long term, we believe that these new models of community-driven health care advocacy and private–public synergies will, if given adequate financial support, help to change the culture of public health and increase health equity in countries like Burkina Faso.

This experience parallels that of other groups in Africa that have had access to ARVs. Most of these groups had not thought out beforehand how to use recycled drugs, but used informal criteria to make the best use of them. These criteria have at times conflicted with western donors' priorities for treatment. Donor agencies may prefer to target the groups they perceive to be most vulnerable (women and children, for instance) with an eye to reducing inequities in health care access. However, treating certain categories preferentially may undermine solidarities within groups or lead to perverse effects, such as patients sharing their drugs with those who do not have access to treatments. Relying on 'objective' medical criteria, such as CD4 cell counts, may also be inequitable. The poor, for instance, access health care later and often present with significant comorbidity that may label them as being at a higher risk for treatment failure, either because of 'non-adherence' or rapidity of disease progression. Alternatively, clinical criteria that are too broad allow social biases to creep into decision-making concerning who should have access [8].

Despite expanded access to ARVs in Africa, rationing will always be a reality, posing a challenge for health equity. Although all agree that equity is the goal, strategies for achieving equity may differ. This experience shows that local groups may chose to ration access differently than donor organizations. It will be important to establish a process that respects local views with how to use ARVs most effectively and equitably, as well as establishing selection criteria that are clear, identify those who need treatment most urgently, and are transparent and accountable to local decision-making processes.

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