

SHORT REPORT

Antiviral efficacy and resistance in patients on antiretroviral therapy in Kigali, Rwanda: the real-life situation in 2002

A Fischer,[1] J-C Karasi,[2] D Kibibi,[2] C Omes,[2] C Lambert,[1] A Uwayitu,[2] R Hemmer,[1] J Van Den Ende,[3] J-C Schmit[1] and V Arendt[1]

[1]Centre Hospitalier de Luxembourg-Retrovirology Laboratory, CRP-Santé, Luxembourg, [2]Centre Hospitalier de Kigali, ESTHER programme, Kigali, Rwanda, and [3]Institute of Tropical Medicine, Antwerp, Belgium

Our study aimed to complete the published data on ARV therapy in Africa by describing the baseline situation in Rwanda before the launch of a large ARV programme (ESTHER). Prescription habits, frequency and reasons for treatment interruptions but also antiviral efficay, resistance to ARVs and genotypic variability of the viruses present in Rwanda were analysed. Among the 233 patients included in the study, it appeared that a vast majority (91%) were under triple therapy and that half of them had experienced at least one treatment interruption caused mainly by drug shortage or financial difficulties. Among 60 blood samples analysed, 26 were in virological failure with a viral load above 1000 RNA copies/ml and 11 presented major drug resistance mutations.

Finally, virological failure could mainly be explained by the high frequency of treatment interruptions but also by the emergence of drug resistance mutations. Consequently the major objective for the ESTHER programme to improve the situation in Rwanda will be to reduce the drug shortage and facilitate the financial accessibility of the treatments.

Received: 17 January 2005, accepted 30 May 2005

Access to antiretroviral therapy (ART) is increasing in developing countries. However, published experience with ART in Africa is limited and results obtained are variable: several studies [1,2] showed high levels of resistance whereas other prospective studies showed that excellent compliance is possible [3,4].

In Rwanda, among 500 000 people living with HIV/AIDS by the end of 2001 (UNAIDS Report, July 2002, 02-26E, ISBN 92-1973-18-54), less than 1000 patients were receiving ART. However, implementation of a large-scale ART programme, the ESTHER (Ensemble pour une Solidarité THérapeutique En Réseau) network, started at the Centre Hospitalier de Kigali (CHK) in October 2002 and aimed to treat 20 000 patients at six sites, through funding from the Global Fund against AIDS, TB and Malaria.

The aim of our study was to analyse the baseline situation in Rwanda (documenting current ART-prescribing habits, and the frequency of and reasons for treatment interruptions), before the launch of the ESTHER programme and before national treatment recommendations came into use. We also determined antiviral efficacy, resistance to antiretrovirals (ARVs) and genotypic variability of non-B viruses present in Rwanda.

Our study was a cross-sectional analysis of all adult patients on ART who attended the CHK between April and June 2002 for treatment follow up or hospitalization. The study should be representative of the situation regarding ART in Rwanda in the first half of 2002, as the majority of patients on ART in Rwanda were followed at the CHK.

Data were retrieved from patient charts and interviews and included demographic data, HIV-related clinical history, prior and current ART, treatment interruptions and their causes.

Biological follow up (CD4 count and viral load) was centralized at the national reference laboratory but was hindered by cost of tests and by occasional shortages of reagents. Consequently, treatment efficacy based on CD4 and viral load changes could not be evaluated because baseline CD4 counts and viral loads were frequently missing.

Blood samples from 60 patients on treatment for more than 3 months were collected and shipped to Luxembourg. HIV RNA levels were assayed there (Monitor Amplicor v1.5; Roche Molecular Systems, Branchburg, NJ, USA),

Correspondence: Aurelie Fischer, Retrovirology Laboratory, CRP-Santé, 84 rue Val Fleuri, L-1526 Luxembourg. Tel: (352) 26970 213; fax: (352) 26970 221; e-mail: aurelie.fischer@crp-sante.healthnet.lu

Table 1 Amino acid substitutions associated with drug resistance in relation to drug regimens used

Sample number	RT/protease subtype	Current regimen	Previous regimen	Treatment interruptions reported	Mutations associated with resistance to			
					NRTIs	NNRTIs	PIs	Resistance
116	C	ZDV/3TC/EFV	Unknown	No	K65R*			ddI
121	A	ZDV/3TC/IDV	No	No	D67N‡, K70R‡, M184V*		M46I, V82F*	3TC, ZDV, IDV
122	C	d4T/ddI/NFV	No	No	K65R*		L90M*	ddI, NFV, IDV†
132	A	d4T/ddI/IDV	ZDV/3TC/EFV	No	M184V*	K103N*		3TC, NVP, EFV
138	C	d4T/ddI/IDV	ZDV/3TC/d4T/ddI/IDV	No	M41L‡, D67N‡, T69D*, V75A/V‡, T215Y‡		L90M*	ZDV, d4T, ddI†, IDV†
145	A	ZDV/3TC/EFV	No	Yes§		K103N*		NVP, EFV
146	A	ZDV/3TC/EFV	No	No	K70R‡, M184V*			3TC, ZDV
148	Mosaïc	d4T/ddI/EFV	No	No		P236L*		EFV†, DLV
173	C	ZDV/3TC/EFV	ZDV/IDV	No		K103N*		NVP, EFV
176	C	ZDV/3TC/EFV	No	No		K101Q†		NNRTIs†
189	A	ZDV/3TC/EFV	No	No	M184V*	K103N*		3TC, EFV, NVP

*Major mutations; ‡NRTI-associated mutations (NAMs); †partial resistance; §treatment interrupted 1 month before blood collection. RT, reverse transcriptase; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor; ZDV, zidovudine; 3TC, lamivudine; EFV, efavirenz; IDV, indinavir; d4T, stavudine; ddI, didanosine.

and viral protease and reverse rranscriptase (RT) sequencing and genotypic resistance testing were performed with the Trugene HIV-1 sequencing kit (Visible Genetics Inc., Toronto, Ontario, Canada). Subtyping was performed using the National Centre for Biotechnology Information internet site.

A total of 233 patients were included in the 3-month study period, with a male/female ratio of 0.7, and 84% of patients being more than 35 years old.

Ninety-one per cent of patients were receiving triple therapy, 8% bitherapy and 1% monotherapy. The most commonly used regimens in 2002 were two nucleoside reverse transcriptase inhibitors (NRTIs) [zidovudine (ZDV) + lamivudine (3TC) or stavudine (d4T) + didanosine (ddI)] in association with one nonnucleoside reverse transcriptase inhibitor (NNRTI) [87.5% efavirenz (EFV)] or one protease inhibitor (PI) [12.5% indinavir (IDV)].

At least one treatment interruption had been experienced by 121 of the 233 patients (52%). The main reasons revealed by the patients were drug shortage (65 of 121 patients; 54%), financial accessibility (29 of 121 patients; 24%), drug intolerance (12 of 121 patients; 10%) and doubts about treatment efficacy (15 of 121 patients; 12%).

Among the 60 samples shipped to Luxembourg for plasma viral load measurements, 26 (43.3%) had a viral load above 1000 HIV-1 RNA copies/mL and were considered to be in virological failure.

RT and protease in the 26 patients with viral loads >1000 copies/mL were sequenced, of which 22 sequences could be analysed for subtyping, natural polymorphisms and resistance mutations.

The subtype distribution was as follows: 11 subtype A (50%), eight subtype C (36.4%) and three more complex strains, with high similarities to subtype A but also with other circulating recombinant forms such as CRF15 and CRF01_AE.

Analysis of sequences showed a V179I polymorphism in the RT gene in six of 22 patients (27.3%) and several polymorphisms in the protease gene: L10I/V/F (31.8%), K20R (13.6%), M36I (100%), L63P/T (45.4%) and I93L (36.4%). The protease I93L polymorphism was present in all subtype C strains.

Eleven of 26 patients (42.3%) in virological failure presented major drug resistance mutations. Nine of 11 (82%) showed resistance to at least one drug in their current regimen. Seven of them (63%) had NRTI-associated mutations, six (54.5%) had NNRTI-associated mutations and three (27.3%) had PI-associated mutations (Table 1).

In summary, most of treated patients were already under triple therapy in mid-2002.

Of the 233 patients included in the study, 60 had blood samples shipped to Luxembourg, but only 26 had a detectable viral load and could be used for resistance testing. However, despite the limited number of tests for viral load and drug resistance, it appeared that 43% of the tested patients were in virological failure. The main reason for this might be the high rate of treatment interruptions (52%), as resistance mutations explained only 11 of the 26 treatment failures (42%). Drug resistance rates may be underestimated as some mutations may have disappeared during treatment interruptions, but frequencies of resistance mutations according to the drug class are consistent with phenotypic resistance observed in Uganda [2].

The subtype distribution found here is similar to that found in our recent study on treatment-naïve patients [5],

with the majority of samples being subtype A but with an increasing proportion being subtype C. Further investigations on the three viral strains that could not be clearly subtyped would be of interest.

The natural polymorphisms observed in protease are similar to those previously described in non-B subtypes [1,6]. Interestingly, the major protease mutation L90 M and the polymorphism I93L were only present in subtype C strains. The association between the higher natural polymorphism in the protease gene of non-B strains and the lower possible response to PI regimens is still unclear [6,7].

To conclude, the major causes of virological failure in this setting seem to be treatment interruptions resulting from drug shortages and patients' financial constraints as most patients paid for their treatment out of their own pockets.

Standardized prescribing guidelines, uninterrupted procurement of affordable or free drugs and better counselling should improve these figures in the future. In fact, all ARV purchases are now centrally managed by the Centrale d'Achat des Medicaments Essentiels du Rwanda (CAMERWA) Central Pharmacy and major programmes have been set up to purchase large amounts of ARV in advance, assuring a security stock of ARV in the country. Moreover, a group of experts co-ordinated by the National Programme against AIDS produced a national ARV usage guide in the middle of 2002.

Today, the ESTHER program has been implemented and the situation has already improved: treatments have become free for the majority of patients, there are no longer any drug shortages, patient follow up is now standardized and data are collected in a computerized database. Evaluation of the ESTHER programme is ongoing and results will be published soon.

References

1 Vergne L, Malonga-Mouellet G, Mistoul I *et al.* Resistance to antiretroviral treatment in Gabon: need for implementation of guidelines on antiretroviral therapy use and HIV-1 drug resistance monitoring in developing countries. *J Acquir Immune Defic Syndr* 2002; **29**: 165–168.

2 Weidle P, Downing R, Sozi C *et al.* Development of phenotypic and genotypic resistance to antiretroviral therapy in the UNAIDS HIV drug access initiative – Uganda. *AIDS* 2003; **17** (Suppl. 3): S39–S48.

3 Landman R, Schiemann R, Thiam S *et al.* Once-a-day highly active antiretroviral therapy in treatment-naïve HIV-1-infected adults in Senegal. *AIDS* 2003; **17**: 1017–1022.

4 Laurent C, Diakhate N, Gueye NF *et al.* The Senegalese government's highly active antiretroviral therapy initiative: an 18-month follow-up study. *AIDS* 2002; **16**: 1363–1370.

5 Servais J, Lambert C, Karita E *et al.* HIV-1 Pol gene diversity and archived Nevirapine resistance mutations in pregnant women in Rwanda. *AIDS Res Hum Retroviruses* 2004; **20**: 279–283.

6 Frater AJ, Beardall A, Ariyoshi K *et al.* Impact of baseline polymorphisms in RT and protease on outcome of highly active antiretroviral therapy in HIV-1-infected African patients. *AIDS* 2001; **15**: 1493–1502.

7 Velazquez-Campoy A, Todd MJ, Vega S, Freire E. Catalytic efficiency and vitality of HIV-1 proteases from African viral subtypes. *Proc Natl Acad Sci USA* 2001; **98**: 6062–6067.