JAIDS Journal of Acquired Immune Deficiency Syndromes
29:165-168 © 2002 Lippincott Williams & Wilkins, Inc., Philadelphia

Brief Report

Resistance to Antiretroviral Treatment in Gabon: Need for Implementation of Guidelines on Antiretroviral Therapy Use and HIV-1 Drug Resistance Monitoring in Developing Countries

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Summary: The protease and reverse transcriptase (RT) genes were studied in antiretroviral (ARV)-experienced and drug-naive HIV-1–infected individuals in Libreville, Gabon. We have shown, although on a limited number of samples that in 58% (11/19) of the patients, with a mean of 17.7 months of ARV drug experience, major mutations inevitably inducing resistances to ARV drugs were present. Resistance was mainly observed to the NRTIs (nucleoside analogue RT inhibitors). This high prevalence may reflect inappropriate ARV drug use. In order to avoid the rapid emergence of resistant viruses on a large scale in the developing world, it is important that the infrastructures necessary to monitor ARV treatment are also rapidly implemented in these countries and that clinicans are trained in the appropriate use of ARV drugs. A continuous surveillance of the circulation of ARV drug-resistant viruses must be organized to guide ARV treatment strategies and policies. **Key Words:** HIV-1—Drug resistance—Africa—AJ313390–AJ313421.

Drugs belonging to three different classes–nucleoside analog RT inhibitors (NRTIs), nonnucleoside RT inhibitors (NNRTIs), and protease inhibitors (PIs)–are currently used in various combinations to treat HIV-infected patients. These therapies have greatly reduced HIV/AIDS-related mortality and morbidity in developed countries but are not generally available in developing countries because of their very high cost. Only a limited number of patients have access to these drugs and in general, no official guidelines on antiretroviral (ARV)-drug use exist in many of these countries. It is not rare to observe that patients receive monotherapy, or various combinations of two or three different drugs, often with unadapted daily doses and on a discontinuous basis as a result of the lack of continuous supply of the drugs or to economic constraints. In addition, laboratory infrastructures necessary to monitor response to therapy, especially to measure changes in viral load and CD4 counts, are often not available. This inappropriate use of ARV and the absence of monitoring the efficacy of the treatment can rapidly lead to the development and spread of drug-resistant virus strains.

Here we describe the prevalence of genotypic ARV drug resistance among 22 ARV-experienced and 13 drug-therapy–naive HIV-1–infected people in Libreville, Gabon. The samples from drug-naive patients were obtained through a serosurveillance study conducted by the National AIDS Control Program, in November, 2000,

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Manuscript received July 16, 2001; accepted October 1, 2001.

among the general population in Libreville using the cluster-sampling technique previously described (1). In the same year, plasma samples were obtained from 22 ARV-experienced patients in whom CD4 counts were measured on an irregular basis and in whom viral RNA in plasma has never been analyzed. At the time of sampling 12 patients received a combination of two drugs (didanosine [ddI] + stavudine [d4T] [n = 9], zidovudine [AZT] + lamivudine [3TC] [n = 2], and AZT + ddI [n = 1]), 1 patient received a combination of three drugs (indinavir + 3TC + d4T) and 9 did not recall exactly which drugs they had received. Among the 22 patients, 7 patients did not remember exactly how long they received ARV-treatment, whereas for the 15 others, the mean duration of therapy was 17.7 months (range, 1–36 months) (Table 1).

Viral RNA was isolated from plasma and transcribed to cDNA. The protease and RT genes (1860-base pair [bp] fragments) were amplified from cDNA by a nested polymerase chain reaction (PCR) method and were directly sequenced as previously described (2). Reverse transcription (RT)-PCR was successful for all the 13

TABLE 1. *Genetic subtypes in the pol region and amino acid substitutions associated with drug resistance*

Patient number	Actual ARV treatment	Treatment start	Resistance mutations to PI (drugs)	Resistance mutations to NRTI (drugs)	Resistance mutations to NNRTI (drugs)	Resistance to following drugs	Subtype pol
1	ddI + d4T	5/99	L10V, M36I	**T69N,** K70N, **V75T**	—	d4T, ddC, (ddI)	A
2	ddI + d4T	4/00	M36I	V118I	—	—	D
3	?	6/97	M36I	—	—	—	A
4	3TC + d4T + IDV	6/99	M36I, L63P	K65R, K70R, V75I, F77L, F116Y, **Q151M**	—	multi-NRTI	A
5	AZT + 3TC	3/99	M36I	R211K	—	—	CRF02-AG
6	?	11/98	M36I	M41L, R211K, **T215Y**	—	ZDV	CRF02-AG
7	ddI + d4T	10/98	M36I	—	—	—	CRF02-AG
8	?	?	M36I, M46I, **V82I**	D67N, R221K	—	IDV, RTV, (NFV, SQV, AMP)	H
9	ddI + d4T	12/98	L10I, M36I, I93L	M41L, R211K, **T215Y**	—	ZDV	CRF11-cpx
10	?	?	M36I	K70R/K, **M184V,** R211K	—	3TC	G
11	ddI + d4T	1/98	K20R, M36I	R211K, **T215Y**	—	ZDV	CRF02-AG
12	?	?	M36I	M41L, R211K, **T215Y**	—	ZDV	CRF02-AG
13	ddI + d4T	10/98	NT	nt	NT	NT	Not amplified
I4	?	5/98	M36I, L63P	M41L, D67N, **T215Y**	—	ZDV, d4T	A
15	?	?	—	R211K	—	—	B
16	AZT + 3TC	2/99	L10V, M36I	**M184V**	—	3TC	CRF01-AE
17	AZT + DDI	11/99	nt	NT	NT	NT	A
18	?	?	L10V, M36I	—	—	—	A
19	ddI + d4T	6/99	M36I, L63P	V118I	—	—	D
20	?	?	L10V, K20R, M36I, I93L	R211K	—	—	K/?-recombinant
21	ddI + d4T	?	nt	NT	NT	NT	Not amplified
22	ddI + d4T	8/97	L10V, M36I, L63P	K70E, V75A, R221K	**Y181C**	NVP, DLV, (d4T)	K/D-recombinant
0202	naive	—	L10I, M36I				CRF11-cpx
0208	naive	—	K20R, M36I				A
0309	naive	—	M36I				CRF02-AG
0612	naive	—	M36I				G
1105	naive	—	L10I, K20R, M36I				J
1406	naive	—	M36I				CRF02-AG
1409	naive	—	M36I				CRF02-AG
1501	naive	—	M36I, L63P				CRF02-AG
1808	naive	—	K20R, M36I				A
2311	naive	—	M36I				CRF02-AG
2409	naive	—	M36I				CRF02-AG
2605	naive	—	M36I	V118I	A98G		G
3010	naive	—	M36I, L63P				CRF02-AG

Genetic subtypes in the pol region and the amino acid substitutions (major and minor) associated with drug resistance are shown for each patient. Major mutations are underlined and **in bold.** Patients 1 to 22 were receiving antiretroviral therapy (ARV); the actual drug regimen and the duration of treatment are shown, when available. Patients 0202 to 3010 were treatment naive. NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-NRTI; PI, protease inhibitors; d4T, stavudine, ddC, zalcitabine; ddI, didanosine; AZT, zidovudine; 3TC, lamivudine, IDV, indinavir; RTV, ritonavir; NFV, nelfinavir; SQV, saquinavir; AMP, amprenavir; DLV, delavirdine; NVP, nevirapine; NT, not tested; ?, not known.

drug-naive patients and also for 19 of the 22 (86.4%) drug-experienced patients, which suggests that viral suppression was already insufficient by the current treatments.

Phylogenetic analysis of the *pol* sequences (3,4), using the CLUSTAL W program (5), showed a high genetic diversity of HIV-1 strains in Gabon (Table 1). In decreasing order of importance, the following subtypes and circulating recombinant forms (CRFs) were observed: 12 CRF02-AG (38%), 7 A (22%), 3 G (10%), 2 D (6%), 2 CRF11-cpx (6%), 1 B (3%), 1 H (3%), 1 J (3%), 1 CRF01-AE (3%), and two strains (6%) that did not cluster with any of the known subtypes or CRFs. Further analyses with the Divert and Simplot programs (6,7), showed that these two strains were intersubtype recombinants with subtype K and D ($n = 1$), and with subtype K and an unknown subtype ($n = 1$).

The amino acid sequences of the protease and RT genes as determined were compared with a subtype B consensus sequence from the Stanford HIV RT and Protease Sequence database (8) to detect mutations associated with reduced sensitivity to ARV drugs (Table 1). In 11 of 19 (58%) patients who were receiving treatment, major mutations that induced inevitably resistances to ARV drugs were observed, in particularly to NRTIs. The following resistance patterns were observed: to AZT only ($n = 4$), to 3TC only ($n = 2$), to AZT and d4T ($n = 1$), to d4T and dideoxycytidine (ddC) ($n = 1$), to indinavir/saquinavir ($n = 1$), to nevirapine/delavirdine ($n = 1$) and in 1 patient we observed a multinucleoside resistance profile. Many accessory mutations, which did not result in a significant decrease in sensitivity, but which were associated with an increase in viral fitness (compensatory mutations) to NRTIs were also observed in the drug-experienced population, probably as determined by this drug class. Among the 10 patients whose therapy type was known, 3 were resistant to one ($n = 2$) or two ($n = 1$) of the prescribed molecules, and 2 had accessory mutations to one or two of the prescribed drugs. Some patients had mutations to other drugs than they are actually receiving, suggesting that their treatment schedule was not constant over time and that they had previously had therapy with several different drugs or drug classes.

None of the treatment-naive patients had major mutations in the protease and RT genes; accessory mutations were seen in the RT gene in only 2 patients. In both the drug-experienced and the drug-naive groups, many accessory mutations are observed in the protease gene: M36I (97%), L10I/V (25%), L63P (19%), K20R (16%), I93L (6%), and M46I (3%), and >50% of the strains carried two, or even more, mutations. Only the subtype B strain had no mutations. There were no significant differences between the two populations, confirming that many of these accessory mutations are naturally occurring in non-B HIV-1 strains (2,9). At present, the exact impact of the presence of preexisting accessory mutation is not completely understood, but it might lead to quicker development of viruses resistant to protease inhibitors (2,9,10). Preliminary data suggest that earlier M36I and L10I/V mutations are associated with a more rapid decrease in sensitivity during treatment (12), and it has also been shown that subtype A, C, and G viruses have decreased in vitro susceptibility to protease inhibitors (12,13). Given that most HIV-1 infections in the developing world are the result of non-B HIV-1 strains, large-scale introduction of protease inhibitors in this part of the world must be accompanied with clinical studies on the efficacy of protease inhibitors in vivo on non-B HIV-1 subtypes.

In this study, we have shown, although on a limited number of samples, that about 60% of patients with a mean 17.7 months' ARV drug experience have major mutations associated with drug resistance. Our data are comparable with those observed in Abidjan, Ivory Coast (14). In our study, no resistant strains were circulating in the treatment-naive population but Gabon is a country where antiretroviral therapies have only recently been introduced to a limited population of patients. The emergence of HIV-resistant variants, as a result of inappropriate ARV treatment practices, is a major public health issue. Owing to the absence in the health care infrastructure necessary to measure the efficiency of ARV drugs, treatment failure will not be rapidly detected and resistant viruses will be further transmitted. With the recent efforts to lower the price of ARV therapy, these drugs will now be widely introduced in many developing countries. To avoid the rapid emergence of resistant viruses on a large scale in the developing world, it is imperative that the structures necessary to monitor ARV treatment should also be rapidly implemented in these countries and that clinicians should be trained in the appropriate use of ARV drugs. Continuous surveillance of the circulation of ARV drug–resistant viruses must be in place to guide ARV treatment strategies and policies.

Acknowledgments: This work was financially supported by a grant from the Agence Nationale de Recherches sur le SIDA (ANRS, Projet Sidak), France and Foundation Leon MBA, France.

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